Health Evidence Review Commission's Value-based Benefits Subcommittee

Part 1

November 13, 2014

8:30 AM

Meridian Park Hospital
Community Health Education Center, Room 117B&C
19300 SW 65th Avenue, Tualatin, OR 97062

Section 1.0

Call to Order

Section 1.0

Staff Report

AGENDA
VALUE-BASED BENEFITS SUBCOMMITTEE
November 13, 2014
8:30am - 1:00pm
Meridian Park Room 117B&C
Community Health Education Center
Tualatin, OR 97062
A working lunch will be served at approximately 12:00 PM
All times are approximate

I. Call to Order, Roll Call, Approval of Minutes – Kevin Olson 8:30 AM

II. Staff report – Ariel Smits, Cat Livingston, Darren Coffman 8:35 AM
A. Update on the non-covered table

III. Biennial Review – Staff 8:40 AM
A. Report on the Back Pain Line Reorganization Task Force
B. Staff report on other biennial review updates/items

IV. Straightforward/Consent Agenda – Ariel Smits 8:50 AM
A. Straightforward table
B. Hip fracture treatment codes
C. Arthropod-borne viral fevers and viral hemorrhagic fevers
D. Determination of refraction
E. Diagnosis codes to be added to the Prioritized List from DMAP Excluded list review

V. 2015 CPT codes – Ariel Smits 9:00 AM
A. 2015 CPT codes
    A. Arthrocentesis
    B. 2015 CPT code issues
    C. Negative pressure wound therapy
    D. Genetic testing CPT codes
        1. High risk for breast cancer guideline
B. 2015 CDT codes
    A. Denture guideline

VI. New discussion items – Ariel Smits 11:00 AM
A. Unilateral tonsillar hypertrophy
B. Hemangiomas
C. Intraocular steroid implants

VII. Coverage Guidances for review – Ariel Smits 11:30 AM
A. Percutaneous interventions for cervical spine pain
B. Ablation for atrial fibrillation

C. Nuclear cardiac imaging

VIII. Previous Discussion Items – Cat Livingston, Ariel Smits 12:15 PM

A. Surgical treatment of sleep apnea in children with craniofacial anomalies

IX. Guidelines – Ariel Smits, Cat Livingston 12:25 PM

A. Adenoidectomy – revisions to the obstructive sleep apnea guideline and sinusitis surgery guideline
B. Retained tympanostomy tube guideline
C. Breastfeeding support and pumping supplies

X. Public comment 12:55 PM

XI. Adjournment – Kevin Olson 1:00 PM

Value-based Benefits Subcommittee Recommendations Summary
For Presentation to:
Health Evidence Review Commission in August 2014

For specific coding recommendations and guideline wording, please see the text of the 8-14-14 VbBS minutes.

RECOMMENDED CODE MOVEMENT (effective 10/1/14 unless otherwise noted)

- Make various straightforward coding changes
- Remove various nerve block procedure codes from lines on the Prioritized List and recommend they be returned to the Ancillary List
- Add diagnosis codes for diabetic retinopathy to the diabetic retinopathy line
- Add ICD-10 diagnosis codes for certain types of diabetes to the appropriate diabetes lines (effective 1/1/15)
- Remove diagnosis codes for chiropractic and osteopathic manipulation from the migraine headache line
- Add a new coding specification to the tension headache line specifying that chiropractic and osteopathic manipulation are on this line only for pairing with cervicogenic headache
- Move the diagnostic code for unspecified myopathy from the covered dysfunction lines to the new fibromyalgia line (effective 1/1/16)
- Add the diagnostic code for chronic fatigue syndrome to the new fibromyalgia line (effective 1/1/16)
- Remove diagnosis codes for osteomyelitis from the hyperbaric oxygen therapy line (to be reviewed by HERC in November, 2014)
- Add chemodenervation of the anal sphincter to the uncovered chronic anal fissure line
- Remove chemodenervation of extraocular muscles from the covered amblyopia line but leave on two covered strabismus lines
- Add various surgical codes for sex reassignment surgery to the gender dysphoria line (effective 1/1/15)

ITEMS CONSIDERED BUT NO RECOMMENDATIONS FOR CHANGES MADE

- Did not change the placement of tympanic membrane perforation diagnosis codes
- Leave diagnosis codes for unspecified rheumatism and fasciitis on a very low priority line rather than moving them to the new fibromyalgia line
- Did not change placement for botulinum toxin injections for treatment of neck or lower back pain or chronic daily headaches

RECOMMENDED GUIDELINE CHANGES (effective 10/1/14 unless otherwise noted)

- Eliminate the proposed guideline for treatments for Hepatitis C (which had been approved at the August 8, 2014 meeting)
- Modify the nerve block guideline to include the CPT codes for these procedures and make into an ancillary guideline

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- Add a new guideline which specifies that removal of tympanostomy tubes is a covered service
- Modify the hyperbaric oxygen therapy guideline to add diabetic wounds as an indication, with specifics about when hyperbaric oxygen therapy would be appropriate (to be reviewed by HERC in November)
- Modify the spinal disorders guideline to require objective evidence of neurologic injury or radiculopathy
- Modify the rehabilitation guideline to limit the total number of therapy visits for all conditions to 30 per year, except for certain neurological conditions or when in an inpatient rehabilitation facility
- Modify the lymphedema guideline to specify that compression dressings/garments are covered for treatment of lymphedema even in the absence of complications
- Delete the Synagis guideline for RSV prophylaxis
- Add several new coding specifications regarding use of botulinum toxin and delete the guideline note regarding botulinum toxin use for bladder indications
- Modify the gender dysphoria guideline to add specifications for when cross-sex hormone therapy and sex reassignment surgery are appropriate (effective 1/1/15)

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VAL□E-BASED BENEFITS S□BCOMMITTEE
Meridian Park Health
Community Health Education Center, Room 117B□C
Tualatin, OR
August 14, 2014
8:30 AM – 1:00 PM

Members Present: □evin Olson, MD, Chair; James Tyack, DMD; David Pollack, MD (left at 12:35 PM); Susan Williams, MD; Mark Gibson; Irene Croswell, RPh; Holly Jo Hodges, MD.

Members Absent: Laura Ocker, LAc.

Staff Present: Darren Coffman; Ariel Smits, MD, MPH; Cat Livingston, MD, MPH; Jason Gingerich; Denise Taray, RN; Daphne Peck.

Also Attending: Jesse Little, Actuarial Services Unit of DMAP; Laura Hill and Becky Reynolds, Abbvie; Sarah Schmidt, □oll; Camille □err and Deirdre Monroe, Allergan; Amy Burns and Mark Bradshaw, AllCare CCO; Josh Balloch, Pac/West Communications; Seth Adams, WVP Health Authority; □im Blood, WVCH; Jim Gardner, PhRMA; Bill Struyk, Johnson □Johnson; Paul Neilsen, Astra □eneca (Medimmune); Lisa Valaika, Genzyme; Rachel Seltzer, OHSU/OHA; Brian Neiubuurt, OHA; John Beckwith and Debbie Christensen, Sacred Heart Hospital; Aubrey Harrison, Danielle Askini, and Alex Lausen, Basic Rights Oregon; Megan Bird, MD, Legacy Health Systems; Ann Murray, BMS; Shannon Beatty, Medimmune; □athleen □lemann, FamilyCare; Lorren Sandt, Caring Ambassadors; Seth Johnstone and Jenn Burleton, TransActive; Eric Larsson, Lovaas Institute; Jim Murray, Hill-Ray; BJ Cavnor, One in Four Chronic Health; □ent Benner, MD, Oregon Clinic.

- **Roll Call□Minutes Approval□Staff Report**

  The meeting was called to order at 8:35 am and roll was called. Minutes from the June, 2014 VbBS meeting were previously reviewed and approved at the August 8th meeting. The minutes from the August 8th meeting are not yet available and will be reviewed and approved at the November, 2014 VBBS meeting.

  Coffman reviewed the timing of upcoming Prioritized Lists. The HERC retreat has been set for October 30, 2014. There was some discussion about whether this was a public meeting; staff will review the agenda and public meetings law and advise the commission.

  Note: line numbers referenced in these minutes are in the format of *October 1, 2014 line/January 1, 2015 line*

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- **Topic: Straightforward Consent Agenda**

  Discussion: There was no discussion about the consent agenda items.

MOTION: To approve the consent agenda as presented CARRIES 7-0

Recommended Actions:

1) Add 40530 (Resection of lip, more than 1/4, without reconstruction) to line 292/279 CANCER OF S IN, E CLUDING MALIGNANT MELANOMA
2) Add 77418 (Intensity modulated treatment delivery, single or multiple fields/arcs, via narrow spatially and temporally modulated beams, binary, dynamic MLC, per treatment session) and 77421 (Stereoscopic -ray guidance for localization of target volume for the delivery of radiation therapy**)** to lines 252/242 CANCER OF OVAR and 459/439 CANCER OF GALLBLADDER AND OTHER BILIAR
3) Add 35606 (Bypass graft, with other than vein; carotid-subclavian) to line 440/419 TRANSIENT CEREBRAL ISCHEMIA; OCCLUSION/STENOSIS OF PRECEREBRAL ARTERIES WITHOUT OCCLUSION
4) Add 35452 (Transluminal balloon angioplasty, open; aortic) to line 472/452 ATHEROSCLEROSIS, AORTIC AND RENAL
5) Add 15120 (Split-thickness autograft, face, scalp, eyelids, mouth, neck, ears, orbits, genitalia, hands, feet, and/or multiple digits; first 100 sq cm or less, or 1% of body area of infants and children) and 15121 (each additional 100 sq cm, or each additional 1% of body area of infants and children, or part thereof) to line 448/427 COMPLICATIONS OF A PROCEDURE USUALL RE UIRING TREATMENT
6) Add 14040 (Adjacent tissue transfer or rearrangement, forehead, cheeks, chin, mouth, neck, axillae, genitalia, hands and/or feet; defect 10 sq cm or less) to line 459/438 H POSPADIAS AND EPISPADIAS
7) Add 62311 (Injection(s), of diagnostic or therapeutic substance(s),including anesthetic, antispasmodic, opioid, steroid, other solution, not including neurolytic substances, including needle or catheter placement, includes contrast for localization when performed, epidural or subarachnoid; lumbar or sacral) to lines 78/75 NEUROLOGICAL D SFUNCTION IN BREATHING, EATING, SWALLOWING, BOWEL, OR BLADDER CONTROL CAUSED B CHRONIC CONDITIONS; ATTENTION TO OSTOMIES and 318/297 NEUROLOGICAL D SFUNCTION IN POSTURE AND MOVEMENT CAUSED B CHRONIC CONDITIONS
8) Add 90670 (Pneumococcal conjugate vaccine, 13 valent) to line 3 PREVENTIVE SERVICES, BIRTH TO 10 EARS OF AGE and 4 PREVENTIVE SERVICES, OVER AGE OF 10 of the October 1, 2014 list and line 3 PREVENTION SERVICES WITH EVIDENCE OF EFFECTIVENESS of January 1, 2015 list
    a. Advise DMAP to remove 90670 from the Excluded List.

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- **Topic: Nerve blocks**

  Discussion: Smits introduced the summary document regarding nerve blocks. The subcommittee felt that the return of these codes to the Ancillary List was the better of the two proposed staff recommendations. This will allow the nerve blocks to continue to be treated as anesthesia for the peri-operative uses. Additionally, HERC staff could not find previous discussion/rationale for moving these codes from Ancillary to the Prioritized List. The Subcommittee felt that this was likely a mistake and should be corrected. The guideline note is recommended to be changed to an Ancillary Guideline.

MOTION: To recommend the code and guideline note changes as presented CARRIES 7-0

Recommended Actions:

1) Remove all nerve block CPT codes (64400-64450) from lines on the Prioritized List
2) DMAP was advices to place CPT codes (64400-64450) on the Ancillary List
3) Make the current nerve block guideline into an Ancillary Guideline and modify to add the CPT codes. See Appendix A for final approved wording.
4) Note: The meeting materials mistakenly listed the code range as 64400-64550

- **Topic: Diabetic retinopathy codes**

  Discussion: Smits presented the staff summary for this topic. Allergan representatives informed staff that GN 116 needs to be considered for modification to allow pairing of intraocular steroids with the new FDA approved indication for limited use in diabetic retinopathy. Staff agreed to look into this possible GN change and bring back for further discussion at the November, 2014 VbBS meeting.

MOTION: To recommend the code changes as presented CARRIES 7-0

Recommended Actions:

1) Add the 362.0x codes (diabetic retinopathy, mild/moderate/severe, with or without proliferative retinopathy, and diabetic macular edema) to line 106/100 DIABETIC AND OTHER RETINOPATH
   a. Advise DMAP to remove the 362.0x codes from the Excluded List
   b. Note: there was a typographical error in the meeting materials. The correct ICD-9 code is 362.0x, not 367.0x
2) Add all codes in the E10.3xx family (Type 1 diabetes mellitus with ophthalmologic complications) to line 100 DIABETIC AND OTHER RETINOPATH for the January 1, 2015 Prioritized List

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3) Add all codes in the E08.3xx family (diabetes due to underlying condition with retinopathy), E09.3xx family (drug or chemical induced diabetes with retinopathy), E11.3xx family (Type 2 diabetes mellitus with ophthalmologic complications), and E13.3xx family (other specified diabetes with retinopathy) to line 30 T□PE 2 DIABETES MELLITUS for the January 1, 2015 Prioritized List
4) Staff will review possible changes to GN116 for the November, 2014 VbBS meeting

- **Topic: Tympanostomy tube removal**

Discussion: Livingston reviewed the material in the packet for this topic. There was minimal discussion.

MOTION: To recommend the guideline note changes as presented□CARRIES 7-0□

Recommended Actions:
1) Make no changes to the placement of tympanic membrane perforation diagnosis codes
2) Add a new guideline specifying that retained tympanostomy tube removal is a covered procedure even if the underlying diagnosis for placement of these tubes was a non-covered diagnosis. See Appendix B for guideline wording.

- **Topic: Spinal manipulation for tension and migraine headaches**

Discussion: Smits reviewed the materials in the meeting packet for this topic. The subcommittee discussed that the evidence for treatment of migraine headache and tension headache showed moderate to good evidence of lack of effectiveness, which is generally a rationale for removing a service from a Prioritized List pairing. There was some discussion about adding a guideline note specifying what is meant by "cervicogenic headache." The group decided that such a guideline note could be added later if the health plans required assistance with this definition.

MOTION: To approve the code changes and coding specification as presented□ CARRIES 6-1 (Opposed: Hodges)□

Recommended Actions:
1) Remove osteopathic and chiropractic manipulation (CPT 98926- 98929, 98940-98943) from line 435/414 MIGRAINE HEADACHES
2) Add the following coding specifications to line 563/546 TENSION HEADACHES
    i. "OMT and CMT (CPT 98926- 98929, 98940-98943) pair on this line only with cervicogenic headache (R51)."

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- **Topic: Wearable cardiac defibrillators**

  Discussion: Smits reviewed the packet materials regarding wearable cardiac defibrillators. Sarah Schmidt, representing □oll, the company which manufactures Life Vest, gave testimony that the purpose of this device is a temporary use until ICD placement or until the patient has his or her medications optimized and no longer will need an ICD. Ms. Schmidt noted that the studies regarding the effectiveness of Life Vest have difficulties in their methodology, although she was unsure what the issues with the methodology actually were. These methodology issues are the reason that no mortality benefit was seen in these studies. Ms. Schmidt stated that there are studies which show what the therapeutic shock rates were, and noted that she can provide these studies to HERC staff. She noted that in the □oll studies, 74% of patients who received a shock were alive 6 months later. She noted that the Life Vest allows discharge from hospital sooner. □oll is currently conducting an RCT of the Life Vest with planned release of the results in fall 2015.

  Subcommittee discussion centered around how successfully the vests stop ventricular fibrillation or ventricular tachycardia, and how often the shocks given by the vests are for these arrhythmias. It was noted that the lethal rhythms treated by this device generally have no symptoms other than sudden death, and so cannot be adequately treated by other means besides a bedside defibrillator. There is increasing numbers of requests for these devices. Hospitals and physicians are hesitant to discharge patients without these devices, as there is not another method in place to treat any fatal arrhythmias which might occur at home. Olson requested data on how often these devices are successfully converting serious arrhythmias. If was noted that the lack of evidence of effective reduction in mortality might stem from lack of patient compliance with wearing the device. Hodges noted that Life Vest use can be monitored, and that her health plan takes back the device if it is not being worn 21 or more hours a day.

  The decision was made to table this topic and have HERC staff find more information on the rate of successful shock of such lethal rhythms as ventricular fibrillation or ventricular tachycardia.

  Action:
  1) This topic was tabled until the November, 2014 VBBS meeting.

- **Topic: Fibromyalgia related diagnoses (general conditions and chronic fatigue syndrome)**

  Discussion: Smits reviewed the materials of general conditions related to fibromyalgia. The subcommittee felt that Rheumatism, unspecified and Fasciitis,

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unspecified should not be moved to the fibromyalgia line as this would raise their priority on the Prioritized List.

Smits also reviewed the materials summarizing the research and recommendations regarding chronic fatigue syndrome. There was minimal discussion.

MOTION: To approve the code changes and line name change as amended CARRIES 7-0

Recommended Actions:

1) Move ICD-9 359.9 (Myopathy, unspecified) from lines 75,297,349,381 to the new fibromyalgia line
2) eep ICD-10 G72.9 (Myopathy, unspecified) on the Excluded List.
3) Add chronic fatigue syndrome (ICD-9 780.71/ICD-10 R53.82) to the new fibromyalgia line
    a. Advise DMAP to remove ICD-9 780.71/ICD-10 R53.82 from the Excluded List
4) Change line name to "Fibromyalgia, Chronic Fatigue Syndrome, and Related Disorders"

- **Topic: Coverage guidance—Hyperbaric Oxygen**

Discussion: Smits reviewed the packet materials regarding hyperbaric oxygen coverage. There was minimal discussion.

MOTION: To approve the code and guideline note change as presented CARRIES 7-0

Recommended Actions:

For the biennial review (to be reviewed by HERC later in the day)

1) Combine the two hyperbaric oxygen lines (lines 336 and 373) into one line placed at line 336 for the next biennial Prioritized List.

For review by HERC in November, along with the coverage guidance, for potential inclusion in January 1, 2015 list:

1) Add ICD-9 250.7, 250.8 and ICD-10 E11.5x,E11.621,E11.622,E11.628, S07.xxx,S17.xxx,S38.xxx, S57.xxx,S67.xxx, S77.xxx,S87.xxx,S97.xxx,and T79.Axx, to line 336 TO IC EFFECT OF GASES, FUMES, AND VAPORS RE UIRING H PERBARIC O GEN
2) Remove ICD-9 526.4, 686.00-686.09,709.3 and ICD-10 M46.20-M46.39 and M86.9 from line 336
3) Modify GN 107 as shown in Appendix A

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- **Topic: Coverage guidance—percutaneous interventions for cervical spine pain**

  Discussion: The summary document was reviewed. A mistake in the recommendations was found: CPT 64490-64495 was proposed for exclusion as well as for addition to 3 lines on the Prioritized List. The proposed placement on the Excluded list was a mistake.

  Hodges cautioned that there would be a large demand for these types of procedures is coverage is added. Gibson expressed concern that the recommendations from HTAS to cover cervical epidural steroid injections and facet joint neurotomy were weak and based on a weak level of evidence. Shaffer commented that HTAS was led to recommend coverage based on the evidence plus clinical expertise and a desire to avoid more costly alternatives, such as spinal surgery. Gibson noted that the CCOs could elect to cover these procedures for cases in which they would help avoid a surgery, without adding these services to the Prioritized List and requiring coverage in all cases. He was concerned about adding these procedures to the Prioritized List based on weak evidence and provider testimony. The HTAS report was reviewed. The rational for recommending adding coverage for epidural spinal steroid injections was found to be "Though quality of evidence from trusted sources is very low, there is evidence from additional sources, namely a retrospective single cohort study, of some benefit when the recommended criteria are met. Additionally, other payer policies include a similar recommendation, namely Medicare and Washington State's payer policies." Williams expressed concern over using a retrospective cohort study as the basis for the decision. There was general concern over the low level of evidence and over using other payer policies as a deciding factor for adding a service to the Prioritized List. However, some members noted that these types of procedures may prevent surgery, reduce ER visits, lower narcotic use, and have other beneficial outcomes.

  HERC staff clarified that currently, epidural steroid injections are ancillary. This was felt to give these procedures the same status as procedures already included for coverage on the Prioritized List—such procedures must have evidence of ineffectiveness or evidence of harm or lower cost effectiveness compared to other procedures in order to be taken off the Prioritized List and therefore take away existing coverage. Procedures considered for initial addition to the list have a higher bar—they must have good evidence of effectiveness. The group initially felt that ancillary procedures should have the lower bar of evidence for inclusion on the List. However, this topic was readdressed later in the meeting, and several subcommittee members expressed concern with this policy. It was felt that many ancillary procedures were never formally reviewed and had a formal placement decision. Therefore, these procedures should be fully reviewed before addition to the List, just like new procedures. HERC staff agreed that this policy needed to be discussed further and placed it on the agenda for the VBBS/HERC retreat in October.

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Hodges noted that clarification of when epidural steroids should be covered, such as in the proposed guideline, would be very helpful for the CCOs as these procedures are currently covered as ancillary. Shaffer noted that these injections are treatments and do not belong on the Ancillary List. Therefore, some placement decision needs to be made.

The subcommittee members noted that facet joint neurotomy was currently excluded. The evidence summary in the HTAS report stated that the recommendation for addition to coverage was based on the following: "Though quality of evidence from trusted sources is very low, the evidence shows there is some benefit when the recommended criteria are met. Coverage with these criteria was recommended by appointed expert." There was general agreement that expert opinion was not good enough evidence to justify coverage when the quality of evidence from trusted sources was very low. The group felt that facet joint neurotomy should not be added to the List.

Initially, the subcommittee voted to approve the addition of epidural steroid injections with the appropriate guideline and to not approve the addition of facet joint injections (Vote 6-0 with Pollack abstaining). However, this decision was reopened at a later point in the meeting and voided (Vote 7-0). The subcommittee wished to have this topic returned for further discussion to their November meeting.

Actions:
1) HERC staff will add a discussion about the level of evidence required for adding ancillary procedures to the Prioritized List to the VBBS/HERC agenda for the October retreat
2) This topic was tabled for further discussion in November.

- **Topic: Guideline Note 37, Disorders Of Spine With Neurologic Impairment**

Discussion: Smits introduced the summary document on this topic. There was no discussion.

MOTION: To approve the guideline note changes as presented CARRIES 7-0

Recommended Action:
1) GN 37 was modified as shown in Appendix A

- **Topic: Rehabilitation guideline**

Discussion: Smits reviewed the packet materials regarding suggested changes to the rehabilitation guideline. Olson expressed frustration with the medical directors requesting changes to VbBS decisions when they are not coming to

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VbBS meetings. Smits noted that there has not been a medical director representative on the VbBS for over a year, since Chris □irk left. This meeting is the first official meeting for the new medical director representative, Holly Jo Hodges, who promised to bring the medical directors input to the VbBS meetings going forward.

The subcommittee felt that the staff recommended wording, including the suggesting additional wording, for the rehabilitation guideline was acceptable.

MOTION: To approve the guideline note changes as presented with additional optional language in the meeting materials□CARRIES 7-0□

Recommended Action:
1) Modify the rehabilitation guideline wording as shown in Appendix A

- **Topic: Lymphedema guideline**

Discussion: Smits presented the summary on proposed changes to the lymphedema guideline. John Beckwith, PT, testified that he was very concerned that the language in the guideline be very clear that compression dressings/garments were covered even when no complications were present. The subcommittee felt that the wording was sufficient. Hodges requested that clarification be made to the guideline to specify whether compression dressings included compression garments. Smits offered amended wording, adding in the term "garments." Beckwith requested that chronic venous insufficiency (CVI) be covered for compression dressings when appropriate. Smits reviewed her previous findings that CVI includes a range of conditions, from very mild to quite severe. It would be difficult to write a guideline specifying at what stage CVI was covered. The subcommittee requested that HERC staff work with experts to see if some coverage of compression dressings/garments should be done for CVI.

MOTION: To approve the guideline note changes as amended□CARRIES 7-0□

Recommended Actions:
1) GN 43 was modified as shown in Appendix A
2) HERC staff to evaluate potential coverage of compression dressings/garments for chronic venous insufficiency and bring back for subcommittee review at a future date

- **Topic: Denture guideline**

Discussion: This topic was tabled until the November, 2014 VbBS meeting.

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- **Topic: Adenoidectomy—revisions to the obstructive sleep apnea guideline and sinusitis surgery guideline**

  Discussion: This topic was tabled until the November, 2014 VbBS meeting.

- **Topic: RSV guideline**

  Discussion: Livingston reviewed the summary document for proposed revisions to the RSV guideline. The CCO representatives testified that referring to P□T criteria would not be acceptable to them. The CCOs have their own committees and develop their own coverage criteria. They do not attend or participate in DMAP's P□T Committee meetings and do not feel that their preferences or opinions are taken into account in the P□T decision making process. If no guideline referring to P□T was adopted, then coverage of Synagis would revert to the individual CCO P□T committee decisions (or DMAP P□T coverage guidance for fee-for-service patients). This was felt to be the preferable alternative.

  There was also public comment received from Paul Nielson, Astra□eneca, who raised concerns about the quality of the new American Academy of Pediatrics guideline on RSV prophylaxis.

  The subcommittee decided to not adopt a new guideline referring to DMAP P□T criteria. However, it was recognized that the current Synagis guideline was out of compliance with national coverage recommendations by the American Academy of Pediatrics. Because DMAP and CCO P□T committee decisions can be much more timely than VbBS/HERC guideline note changes, the subcommittee decided to remove the Synagis guideline and similar drug related guidelines and not add any guidelines referring to DMAP P□T decisions.

MOTION: To approve the guideline note deletion but reject the proposed new guideline note□CARRIES 7-0□

Recommended Action:
1) Delete GN 69

- **Topic: Botulinum toxin**

  Discussion: Smits reviewed the summary document regarding Botox. The VbBS had previously indicated that they did not wish to have guidelines referring to the DMAP P&T coverage criteria (see "RSV guideline" discussion above). The proposed new guideline referring to P□T criteria was therefore not accepted.

MOTION: To approve the coding changes, coding specifications and guideline note deletion but not the proposed new guideline note□CARRIES 7-0□

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Recommended Actions:

1) Add 46505 (Chemodenervation of internal anal sphincter) to line 506/532 CHRONIC ANAL FISSURE
2) Remove 67345 (Chemodenervation of extraocular muscle) from line 397/372 AMBL□OPIA
3) Do not add coverage for botulinum toxin injections for treatment of neck or lower back pain or chronic daily headaches
4) □eep the coding specification shown below on line 388/364 D□STONIA (UNCONTROLLABLE); LAR□NGEAL SPASM AND STENOSIS
   a. "Chemodenervation with botulinum toxin injection (CPT 64612, 64616) is included on this line only for treatment of blepharospasm (ICD-9 333.81/ICD-10-CM G24.5), spasmodic torticollis (ICD-9 333.83/ICD-10-CM G24.3), and other fragments of torsion dystonia (ICD-9 333.89/ICD-10-CM G24.9)."
5) Add the coding specification shown below to line 318/297 NEUROLOGICAL D□SFUNCTION IN POSTURE AND MOVEMENT CAUSED B□ CHRONIC CONDITIONS
   a. "Chemodenervation with botulinum toxin injection (CPT 64642-64647) is included on this line for treatment of upper and lower limb spasticity (ICD-9 333.6x, 333.7x, 340.xx, 341.0, 342.xx, 343.xx, 344.0x, 344.1, 344.2, 344.3x, 344.4x, 344.5, 344.89, 344.9, 359.0-359.2, 438.2x-438.5x/ICD-10 G24.02, G24.1, G35, G36.0, G71.xx, G80-G83, I69.03-I69.06).
6) Add the coding specification shown below to lines 452 STRABISMUS WITHOUT AMBL□OPIA AND OTHER DISORDERS OF BINOCULAR E□E MOVEMENTS; CONGENITAL ANOMALIES OF E□E/354 STRABISMUS DUE TO NEUROLOGIC DISORDER and 398 STRABISMUS WITHOUT AMBL□OPIA AND OTHER DISORDERS OF BINOCULAR E□E MOVEMENTS; CONGENITAL ANOMALIES OF E□E; LACRIMAL DUCT OBSTRUCTION IN CHILDREN
   a. "Chemodenervation with botulinum toxin injection (CPT 67345) is included on this line for the treatment of strabismus due to other neurological disorders (ICD-9 378.73 /ICD-10 H50.89)."
7) Add the coding specification shown below to line 421 ACHALASIA, NON-NEONATAL/382 ESOPHAGEAL STRICTURE; ACHALASIA
   a. "Chemodenervation with botulinum toxin injection (CPT 43201) is included on this line for treatment of achalasia (ICD-9 530.0/ICD-10 K22.0)."
8) Add the coding specification shown below to line 542/523 DISORDERS OF SWEAT GLANDS
   b. "Chemodenervation with botulinum toxin injection (CPT 64650, 64653) is included on this line for the treatment of axillary hyperhidrosis and palmar hyperhidrosis (ICD-9 705.2 and 780.8/ICD-10 L74.52, R61).
9) Delete GUIDELINE NOTE 103, CHEMODENERVATION OF THE BLADDER

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➢ **Topic: Gender dysphoria**

Discussion: Smits reviewed the summary document in the meeting packet. There was considerable discussion regarding concerns for lack of an age restriction for surgical procedures. Smits reviewed that the Oregon age of consent for surgical procedures is age 15. Olson felt that these types of procedures should not be singled out for restriction until age 18 or even higher, as many other procedures which have life-long impact can be consented to at age 15. Expert testimony by Danielle Askini from Basic Rights Oregon and Jenn Burleton from TransActive indicated that youth are required to have extensive evaluations by qualified mental health professionals and that the year of hormone therapy required in the guideline acts as another safeguard against rash decisions by youth.

There was a minor edit to the proposed guideline to add in a missing word.

MOTION: To approve the line rescoring, updated treatment description, and the addition of cross-sex hormone therapy and various surgical procedures related to sex reassignment to the gender dysphoria line, and the new guideline as amended CARRIES 5-1 (Opposed: Tyack, Absent: Pollack)

Recommended Actions:

1) Change the treatment description of the gender dysphoria line to ~~MEDICAL/PS CHOTHERAP~~ MEDICAL AND SURGICAL TREATMENT; PS CHOTHERAP
2) Rescore the gender dysphoria line as shown below:
    a. Scoring proposal
    Category: 6
    HL: 6
    Suffering: 4
    Population effects: 0
    Vulnerable population: 0
    Tertiary prevention: 3
    Effectiveness: 2
    Need for service: 1
    Net cost: 2
    Score: 1040
    Approximate line placement: 312
3) Add cross-sex hormone therapy to the new gender dysphoria line
4) Add CPT 19301-19304, 53430, 54125, 54400-54417, 54520, 54660, 54690, 55175-55180, 55970, 55980, 56625, 56800, 56805, 56810, 57106-57107, 57110-57111, 57291-57292, 57335, 58150, 58180, 58260-58262, 58275-58291, 58541-58544, 58550-58554, 58570-58573, 58661, 58720 to the new gender dysphoria line
    a. Advise DMAP to remove CPT 55970 and 55980 from the Excluded List

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5) Modify the guideline for the new gender dysphoria line as shown in Appendix A
6) Note that all of these recommended actions would take effect on January 1, 2015 when the new gender dysphoria line appears on the Prioritized List

- **Topic: Hepatitis C**

Discussion: Livingston reviewed the summary document regarding treatments for hepatitis C in the meeting packet. Written testimony from CCOs, legislators and concerned citizens was received.

The following public testimony was received verbally:

Amy Burns and Mark Bradshaw from AllCare presented a collaborative statement from 9 CCOs. The collective strongly discouraged a guideline note requiring CCOs to adhere to the P□T Committee's drug use criteria. They strongly felt that each CCO manages their own drug use and formularies and this drug class should be no different. They also had specific concerns about the criteria including:

1) Allowing off-label use of simeprevir and sofosburiv
2) Allowing for off-label use for patients who are post transplant. This goes against OARs that do not allow for investigational or experimental practices. There is no outcome data to support this. The liver transplant population has not been studied and is not recommended in the Sovaldi package insert.
3) Subjective language around who can prescribe
4) Important psychosocial and medical factors around treatment that aren't addressed

Jim Gardner, Oregon Council for PhMRA, a paid advocate, recommended not looking at single therapeutic products and raised legal concerns with this approach.

Ann Murray, Bristol Myers Squibb, stated that she thinks this would be the first time HERC would have taken this approach, in which a treatments for a condition appear both on a funded and unfunded line.

Subcommittee members made a request to PhRMA to decrease the cost of the drug, with the answer that they appreciated the comment.

□imberly Wyatt, Willamette Valley Community Health CCO and Dr. Seth Adams, pharmacist with WVCH, offered public comment. They stated they have their own pharmacy and therapeutics committee and have already created PA criteria for approval of these medications. It is important to the CCOS to have freedom to develop their own criteria. If there was going to be

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accountability to another organization (DMAP's P□T Committee) then CCOs would have wanted to be responsible in the development of these criteria.

Subcommittee members asked about the CCOs concerns regarding criteria in the proposed guideline note for drug and alcohol testing. It was clarified that they felt 6 months sobriety was too short of a time. They also require a mental health evaluation to assess readiness to treat and ascertain whether patients are compliant with prescription drugs for other diseases, such as diabetes and hypertension.

Lorren Sandt, from Caring Ambassadors, and BJ Cavnor, 1 in 4 Chronic Disease, testified as well, both stating pharmaceutical funding for their organizations. Sandt requested hepatitis C drug treatments should remain above the funding line and that everyone with hepatitis C should be treated with these drugs. She also pointed to the AASLD/IDSA guideline not requiring abstinence or sobriety. Cavnor requested a workgroup that would follow national guidelines and demanded that all people be allowed to come to the table.

Dr. □ent Benner offered testimony with no current conflicts of interest. He spoke about the community standard that was developed by local hepatologists, and discussed the rationale for this.

Subcommitee members discussed the significant impact of these medications on the budget. There was a proposal to table the decision. Given the concerns CCOs have about the reference to P□T criteria, the need for ongoing dialogue, the new AASLD guidelines, it was felt that a final decision on this policy should be tabled until a future meeting.

It was clarified that there will be a pharmacoeconomic anlaysis available by the November meeting and that an updated DERP report and NICE guidelines may be available later this year or early next year as well.

MOTION: To reject the proposed new guideline regarding treatment of hepatitis C and table the topic until a future meeting□CARRIES 7-0□

Recommended Actions:

1) None

- **Topic: Proposed "Non covered" section of the Prioritized List**

Discussion: Smits introduced this topic and noted that the new "non-covered" table was presented for approval in concept only. There was minimal discussion.

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Actions:

1) HERC staff will continue to work on developing the "non-covered" table and will publish it as a new portion of the January 1, 2015 Prioritized List.

- **Topic: Applied behavioral analysis for treatment of non-autistic self-injurious behavior**

  Discussion: This topic was tabled to the August HERC meeting.

  Actions: See minutes from the August HERC meeting.

- **Topic: Standardized assessment tools for evaluating progress for autism spectrum disorder**

  Discussion: This topic was tabled to the August HERC meeting.

  Actions: See minutes from the August HERC meeting.

- **Public Comment:**

  No additional public comment was received.

- **Issues for next meeting:**
  - Wearable cardiac defibrillators
  - Denture guideline
  - Adenoidectomy—revisions to the obstructive sleep apnea guideline and the sinusitis surgery guideline
  - Coverage guidance—percutaneous interventions for cervical spine pain
  - A new breastfeeding supplies guideline
  - Unilateral hearing loss in adults
  - OMT/CMT and the dysfunction lines
  - Tobacco cessation coverage
  - Tobacco smoking and procedures
  - PET scan for fever of unknown origin
  - Unilateral tonsillar hypertrophy
  - Various straightforward coding changes

- **Next meeting:**

  November 13, 2014 at Meridian Park Health Education Center, Tualatin, OR Room 117B□C.

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- **Adjournment:**

  The meeting was adjourned at 1:30 PM.

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Appendix A

Revised Guideline Notes

~~G□IDELINE NOTE 76~~ ANCILLARY G□IDELINE A3, NERVE BLOCKS *(Effective October 1, 2014)*

~~Lines 1,3,6,8,9,11,20,24,25,28,30,32,37-41,46-55,59-63,65,67,68,70-76,81-100,102-105,107,109-112,115-121,123-125,129-132,134-145,147,148,154,156-164,166-170,172-175,178,180,183-195,197-201,203,204,206,208,209,211-214,216-221,223,225-228,230,232-239,241-251,253-255,257-267,269,273-275,277-280,283-286,289-294,297-306,308-312,314-317,319-324,327,328,330-335,337-341,343,344,346,349,350,352,354-360,362-370,372,374-376,378-384,386,389,391,392,394,397-402,404-408,410,412,417,419-424,426-428,430-436,438-441,443-447,450,452,453,456,458,459,464-466,468-472,474,476,477,480-484,486,487,496,504,519,526,527,532,535,549,568,630~~

The Health Evidence Review Commission intends that single injection and continuous nerve blocks (CPT 64400-64550) should be covered services if they are required for successful completion of, perioperative pain control for, or post-operative recovery from a covered operative procedure when the diagnosis requiring the operative procedure is also covered. Additionally, nerve blocks are covered services for patients hospitalized with trauma, cancer, or intractable pain conditions, if the underlying condition is a covered diagnosis.

G□IDELINE NOTE 6, REHABILITATIVE THERAPIES *(Effective October 1, 2014)*

Lines 37,50-52,64,74-76,78,80,85,89,90,94,95,98-101, 108, 109, 115, 116, 122, 129, 139, 141-143,145,146,158,161,167,179,184,185,189, 190, 192, 194, 195, 201, 202, 208,209,216,226,237,239,270,271,273,274,279,288,289,293,297,302,304,307-309, 318, 336,342,349, 350, 363, 367, 369, 375,376,378, 382,384,385,387, 400,406, 407, 434, 441,443,448,455,467,478,489,493,507,516,535,549,562,580, 597,619,638

A total of 30 visits per year of rehabilitative therapy (physical, occupational and speech therapy, and cardiac and vascular rehabilitation) are included on these lines when medically appropriate. Additional visits, not to exceed 30 visits per year, may be authorized in exceptional circumstances, such as in cases of rapid growth/development. Physical, occupational and speech therapy, and cardiac and vascular rehabilitation are only included on these lines when the following criteria are met:

1) therapy is provided by a licensed physical therapist, occupational therapist, speech language pathologist, physician, or other practitioner licensed to provide the therapy,
2) there is objective, measurable documentation of clinically significant progress toward the therapy plan of care goals and objectives,
3) the therapy plan of care requires the skills of a ~~therapist~~ medical provider, and
4) the client and/or caregiver cannot be taught to carry out the therapy regimen independently.

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Appendix A

No limits apply while in a skilled nursing facility for the primary purpose of rehabilitation, an inpatient hospital or an inpatient rehabilitation unit.

Spinal cord injuries, traumatic brain injuries, or cerebral vascular accidents are not subject to the visit limitations during the first year after an acute injury.

G□IDELINE NOTE 37, DISORDERS OF SPINE WITH NE□ROLOGIC IMPAIRMENT *(Effective October 1, 2014)*

Lines 374,545

Diagnoses are included on Line 374 when objective evidence of neurologic impairment or radiculopathy is present, as defined as:

A) Markedly abnormal reflexes
B) Segmental muscle weakness
C) Segmental sensory loss
D) EMG or NCV evidence of nerve root impingement
E) Cauda equina syndrome,
F) Neurogenic bowel or bladder
G) Long tract abnormalities

Otherwise, disorders of spine not meeting these criteria (e.g. pain alone) fall on Line 545.

G□IDELINE NOTE 43, LYMPHEDEMA *(Effective October 1, 2014)*

Lines 448,597,598

Lymphedema treatments are included on these lines when medically appropriate. These services are to be provided by a licensed practitioner who is certified by one of the accepted lymphedema training certifying organizations or a graduate of one of the National Lymphedema Network accepted training courses within the past two years. The only accepted certifying organization at this time is LANA (Lymphology Association of North America; http://www.clt-lana.org). Treatments for lymphedema are not subject to the visit number restrictions found in Guideline Note 6 REHABILITATIVE THERAPIES. It is the intent of the HERC that compression dressings/garments and other medical equipment needed for the treatment of lymphedema be covered even in the absence of ulcers or other complications.

G□IDELINE NOTE 43, LYMPHEDEMA *(Effective January 1, 2016)*

Line~~s 427,577,579~~ XXX (new lymphedema line ~427)

Lymphedema treatments are included on this line ~~these lines~~ when medically appropriate. These services are to be provided by a licensed practitioner who is certified by one of the accepted lymphedema training certifying organizations or a graduate of one of the National Lymphedema Network accepted training courses within the past two years. The only accepted certifying organization at this time is LANA (Lymphology

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Appendix A

Association of North America; http://www.clt-lana.org). Treatments for lymphedema are not subject to the visit number restrictions found in Guideline Note 6 REHABILITATIVE THERAPIES.-It is the intent of the HERC that compression dressings/garments and other medical equipment needed for the treatment of lymphedema be covered even in the absence of ulcers or other complications.

G□IDELINE NOTE 107, HYPERBARIC O□YGEN *(Effective date pending HERC review of coverage guidance, not to be prior to January 1, 2015)*

Lines 336~~,373~~ (delete only for biennial review List)

Hyperbaric oxygen is a covered service only under the following circumstances:

- ~~when paired with ICD-9-CM code 526.4 for osteomyelitis of the jaw only~~
- when paired with ICD-9-CM codes 250.7x and 250.8x/ICD-10-CM E11.5x and E11.621,E11.622,E11.623 for diabetic wounds with gangrene OR diabetic wounds of the lower extremities in patients who meet the all of the following criteria:
    - Patient has Type 1 or Type 2 diabetes and has a lower extremity wound that is due to diabetes, AND
    - Patient has a wound classified as Wagner grade III or higher, AND
    - Patient has failed an adequate course of standard wound therapy including arterial assessment, with no measurable signs of healing after at least thirty days, AND
    - Wounds must be evaluated at least every 30 days during administration of hyperbaric oxygen therapy. Continued treatment with hyperbaric oxygen therapy is not covered if measurable signs of healing have not been demonstrated within any 30-day period of treatment.
- when paired with ICD-9-CM codes 526.89/ ICD-10--CM codes M27.8 for osteoradionecrosis of the jaw only
- when paired with ICD-9-CM codes 639.0, 670.02, and 670.04/ ICD-10--CM codes O08.0, M60.000-M60.09 only if the infection is a necrotizing soft-tissue infection
- ~~when paired with ICD-9-CM codes 730.10-730.99/ICD-10-CM M46.20-M46.39, M86.9 only for chronic refractory osteomyelitis unresponsive to conventional medical and surgical management~~
- when paired with ICD-9-CM codes 927-929/ICD-10 CM codes S07.xxx,S17.xxx,S38.xxx,S47.1xxA-S47.1xxD,S47.2xxA-S47.2xxD,S47.9xxA-S47.9xxD, S57.xxx,S67.xxx, S77.xxx,S87.xxx,S97.xxx, T79.Axx, only for posttraumatic crush injury of Gustilo type III B and C
- when paired with ICD-9-CM codes 990/ ICD-10--CM codes T66.xxxA only for osteoradionecrosis and soft tissue radiation injury
- when paired with ICD-9-CM codes 996.52, 996.7/ ICD-10--CM codes T86.820-T86.829,T82.898A, T82.898D, T82.9xxA, T82.9xxD, T83.89xA, T83.89xD, T83.9xxA, T83.9xxD, T84.89xA, T84.89xD, T84.9xxA, T84.9xxD, T85.89xA, T85.89xD, T859xxA, T859xxD only for compromised myocutaneous flaps.

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Appendix A

G□IDELINE □□□ GENDER DYSPHORIA *(Effective January 1, 2015)*

Line 413

Hormone treatment is included on this line for use in delaying the onset of puberty and/or continued pubertal development with GnRH analogues for gender questioning children and adolescents. This therapy should be initiated at the first physical changes of puberty, confirmed by pubertal levels of estradiol or testosterone, but no earlier than Tanner stages 2-3. Prior to initiation of puberty suppression therapy, adolescents must fulfill eligibility and readiness criteria, and must have a comprehensive mental health evaluation. Ongoing psychological care is strongly encouraged for continued puberty suppression therapy.

Cross-sex hormone therapy is included on this line for treatment of adolescents and adults with gender dysphoria who meet appropriate eligibility and readiness criteria. To qualify for cross-sex hormone therapy, the patient must:

1) have persistent, well-documented gender dysphoria
2) have the capacity to make a fully informed decision and to give consent for treatment
3) have any significant medical or mental health concerns reasonably well controlled
4) have a thorough psychosocial assessment by a qualified mental health professional with experience in working with patients with gender dysphoria

Sex reassignment surgery is included for patients who are sufficiently physically fit and meet eligibility criteria. To qualify for surgery, the patient must:

1) have persistent, well documented gender dysphoria
2) have completed twelve months of continuous hormone therapy as appropriate to the member's gender goals unless hormones are not clinically indicated for the individual
3) have completed twelve months of living in a gender role that is congruent with their gender identity unless a medical and a mental health professional both determine that this requirement is not safe for the patient
4) have the capacity to make a fully informed decision and to give consent for treatment
5) have any significant medical or mental health concerns reasonably well controlled
6) have two referrals from qualified mental health professionals with experience in working with patients with gender dysphoria who have independently assessed the patient. Such an assessment should include the clinical rationale supporting the patient's request for surgery, as well as the rationale for the procedure(s)

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Appendix B

New Guideline Notes

GUIDELINE NOTE XXX RETAINED TYMPANOSTOMY TUBES *(Effective October 1, 2014)*

Lines 178, 308, 405, 418, 502

Removal of retained tympanostomy tubes under anesthesia, if indicated (CPT code 69424 Ventilating tube removal requiring general anesthesia) or as part of an office visit, are intended to be covered for Line 502 diagnoses with the Line 405 ICD-9 code 383.83 (Retained foreign body of middle ear*).*

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Section 2.0

Consent Agenda-

Straightforward Items

Straightforward Issues—November, 2014

Code	Code Description	Line(s) Involved	Issue	Recommendation(s)
61782	Stereotactic computer-assisted (navigational) procedure; cranial, extradural	**391** ACUTE SINUSITIS **498** CHRONIC SINUSITIS **532** NASAL POLYPS, OTHER DISORDERS OF NASAL CAVITY AND SINUSES **548** BENIGN NEOPLASM OF NASAL CAVITIES, MIDDLE EAR AND ACCESSORY SINUSES **600** DEVIATED NASAL SEPTUM, ACQUIRED DEFORMITY OF NOSE, OTHER DISEASES OF UPPER RESPIRATORY TRACT	OHP medical director has requested that 61782 be paired with sinus surgery. This code is a standard add-on code when there is complex anatomy, previous surgery in the sinus area, scarring, etc. 61782 is currently on lines 137, 162, 201, 266, 320, 342, 359.	Add 61782 to lines 391, 498, 532, 54, and 600
		360 DISEASES OF PULMONARY ARTERY	Line 360 includes both injury and disease diagnosis codes. HERC staff suggest renaming line to reflect this range of diagnoses.	Rename Line 360 ~~DISEASES~~ CONDITIONS OF PULMONARY ARTERY
44620-44626	Closure of enterostomy, large or small intestine	**75** NEUROLOGICAL DYSFUNCTION IN BREATHING, EATING, SWALLOWING, BOWEL, OR BLADDER CONTROL CAUSED BY CHRONIC CONDITIONS; ATTENTION TO OSTOMIES	DMAP requested that 44620 pair with V55.2 Attention to ileostomy. 44620 is currently on 10 lines on the Prioritized List.	Add 44620-44626 to line 75
61500	Craniectomy; with excision of tumor or other bone lesion of skull	**204** CANCER OF BONES	DMAP requested that 61500 pair with 238.0 Neoplasm of uncertain behavior of bone and articular cartilage. 61500 is currently on lines 130,299,600.	Add 61500 to line 204
32673	Thoracoscopy, surgical; with resection of thymus, unilateral or bilateral	**266** CANCER OF LUNG, BRONCHUS, PLEURA, TRACHEA, MEDIASTINUM AND OTHER RESPIRATORY ORGANS	DMAP requested that 32673 pair with 235.8 Neoplasm of uncertain behavior of pleura, thymus, and mediastinum. 32673 is currently on lines 144, 263, 376.	Add 32673 to line 266

Straightforward Issues—November, 2014

Code	Code Description	Line(s) Involved	Issue	Recommendation(s)
32663	Thoracoscopy, surgical; with lobectomy (single lobe)	**51** DEEP ABSCESSES, INCLUDING APPENDICITIS AND PERIORBITAL ABSCESS **62** BRONCHIECTASIS **266** CANCER OF LUNG, BRONCHUS, PLEURA, TRACHEA, MEDIASTINUM AND OTHER RESPIRATORY ORGANS **288** CHRONIC OBSTRUCTIVE PULMONARY DISEASE; CHRONIC RESPIRATORY FAILURE **360** DISEASES OF PULMONARY ARTERY **376** BENIGN NEOPLASM OF RESPIRATORY AND INTRATHORACIC ORGANS	DMAP requested that 32663 be added to line 266 to pair with 162.3 Malignant neoplasm of upper lobe, bronchus, or lung. On OHP medical director also requested additional pairings. 32663 is current on line 201 CONGENITAL LUNG ANOMALIES. The CPT code for the equivalent open procedure (32480) is on 7 lines	Add 32663 to lines 51, 62, 266, 288, 360, and 376
31645 31646	Bronchoscopy, rigid or flexible, including fluoroscopic guidance, when performed; with therapeutic aspiration of tracheobronchial tree, initial (eg, drainage of lung abscess) Subsequent	**62** BRONCHIECTASIS	DMAP requested that 31645 be paired with 494.1 Bronchiectasis with acute exacerbation. 31645 is currently on lines 51,208,237,266,469.	Add 31645 and 31646 to line 62
64455	Injection(s), anesthetic agent and/or steroid, plantar common digital nerve(s) (eg, Morton's neuroma)	**544** LESION OF PLANTAR NERVE; PLANTAR FASCIAL FIBROMATOSIS	64455 is currently on 350+ lines. Morton's neuroma (ICD-9 355.6) is on line 544. 64455 is not on line 544	Add 64455 to line 544 and remove from all other lines on the Prioritized List.

Straightforward Issues—November, 2014

Code	Code Description	Line(s) Involved	Issue	Recommendation(s)
99363-99364	Anticoagulant management for an outpatient taking warfarin,	**579** LYMPHEDEMA	99363-4 are on the current low lymphedema line, but are scheduled to be removed from the biennial review lymphedema line. HERC staff requests that they be removed from the January 1, 2014 List line as well.	Remove 99363-4 from line 579
69717, 69718	Replacement (including removal of existing device), osseointegrated implant, with or without mastoidectomy	**283** SENSORINEURAL HEARING LOSS - AGE 5 OR UNDER; COCHLEAR IMPLANT **317** HEARING LOSS - AGE 5 OR UNDER; MEDICAL THERAPY INCLUDING HEARING AIDS **423** SENSORINEURAL HEARING LOSS – OVER AGE 5; COCHLEAR IMPLANT **450** HEARING LOSS – OVER AGE 5; MEDICAL THERAPY INCLUDING HEARING AIDS	CPT 69717 and 69718 code for replacement of BAHA type hearing aids. They are incorrectly on the cochlear implant lines and should be on the hearing aid lines with other codes for placement of BAHA type units.	Add 69717 and 69718 to lines 317 and 450 Remove 69717 and 69718 from lines 283 and 423
			The following coding specification is on line 564 for the January 1 ICD-10 List. It is not needed. “R49.0 is located on the Diagnostic List for use for the work-up of hoarseness.”	Delete the coding specification regarding R49.0 from line 564.

Straightforward Issues—November, 2014

Code	Code Description	Line(s) Involved	Issue	Recommendation(s)
26426	Repair of extensor tendon, central slip, secondary (eg, boutonniere deformity); using local tissue(s), including lateral band(s), each finger	**211** DEEP OPEN WOUND, WITH OR WITHOUT TENDON OR NERVE INVOLVEMENT **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **380** DISRUPTIONS OF THE LIGAMENTS AND TENDONS OF THE ARMS AND LEGS, EXCLUDING THE KNEE, RESULTING IN SIGNIFICANT INJURY/IMPAIRMENT **391** DEFORMITY/CLOSED DISLOCATION OF MINOR JOINT AND RECURRENT JOINT DISLOCATIONS	DMAP requested that 26426 pair with 736.21 (boutonniere deformity), which is on lines 381 (dysfunction line) and 391. 26426 is on lines 211,290,380. Boutonniere deformity is a condition of the finger. 26426 involves surgery on a finger, which is inappropriate for line 380	Remove 26426 from line 380 Add 26426 to line 391
64721	Neuroplasty and/or transposition; median nerve at carpal tunnel	**211** DEEP OPEN WOUND, WITH OR WITHOUT TENDON OR NERVE INVOLVEMENT	DMAP requested that 64721 pair with 955.1 (Injury to median nerve). 64721 is on lines 420,511,534,430.	Add 64721 to line 211
57287	Removal or revision of sling for stress incontinence (eg, fascia or synthetic)	290 COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT	DMAP requested that 57287 pair with 996.39 (Other mechanical complication of genitourinary device, implant, and graft). 57287 is currently on lines 331,459,471.	Add 57287 to line 290
34203	Embolectomy or thrombectomy, with or without catheter; popliteal-tibio-peroneal artery, by leg incision	**290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT	DMAP requested that 34203 pair with 996.74 (Other complications due to other vascular device, implant, and graft). 34203 is on lines 239,257,352.	Add 34203 to line 290

Straightforward Issues—November, 2014

Code	Code Description	Line(s) Involved	Issue	Recommendation(s)
10061	Incision and drainage of abscess (eg, carbuncle, suppurative hidradenitis, cutaneous or subcutaneous abscess, cyst, furuncle, or paronychia); complicated or multiple	**290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT	DMAP requested that 10061 pair with 998.59 (Other postoperative infection). 10061 is on lines 51, 209, 383, 405, 487, 528, 632, 377.	Add 10061 to line 290
43229	Esophagoscopy, flexible, transoral; with ablation of tumor(s), polyp(s), or other lesion(s) (includes pre- and post-dilation and guide wire passage, when performed)	**519** ESOPHAGITIS AND GERD; ESOPHAGEAL SPASM; ASYMPTOMATIC DIAPHRAGMATIC HERNIA	DMAP requested that 43229 pair with 530.82 (Barrett's esophagus). 43229 is on lines 319,382,600,647.	Add 43229 to line 519
		300 APLASTIC ANEMIAS	GN7 ERYTHROPOIESIS-STIMULATING AGENT (ESA) GUIDELINE is not referenced on line 300, which contains the diagnosis for anemia due to chronic kidney disease.	Add line 300 to GN7

Hip Fracture Treatment Codes

Issue: Hip fractures were consolidated on one line in May, 2014. All hip fracture diagnosis codes were moved to the new hip fracture line. HERC staff have identified certain CPT codes which related to hip fractures that need to be taken off other lines as the relevant paired diagnoses are no longer present on those lines.

CPT code	**Code description**	**Current Lines**	**Appropriate Lines**
27236	Open treatment of femoral fracture, proximal end, neck, internal fixation or prosthetic replacement	**85** FRACTURE OF HIP **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **358** CLOSED FRACTURE OF EXTREMITIES (EXCEPT MINOR TOES)	85, 290
27267	Closed treatment of femoral fracture, proximal end, head; without manipulation	**85, 358** **362** DEFORMITY/CLOSED DISLOCATION OF MAJOR JOINT AND RECURRENT JOINT DISLOCATIONS **391** DEFORMITY/CLOSED DISLOCATION OF MINOR JOINT AND RECURRENT JOINT DISLOCATIONS	85
27268	with manipulation	**85, 358, 362, 391**	85

HERC staff recommendations:

1) Remove 27236 from line 358
2) Remove 27267 and 27268 from lines 358, 362, and 391

Arthropod-borne Viral Fevers and Viral Hemorrhagic Fevers

Question: where should hemorrhagic fevers and certain arthropod borne diseases be located on the Prioritized List?

Question source: HERC staff

Issue: The ICD-10 codes for hemorrhagic fevers, such as Ebola, are currently located on one of two unfunded lines--623 OTHER VIRAL INFECTIONS or 660 INFECTIOUS DISEASES WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY. These infections require hospitalization and may require various intensive care treatments. The ICD-9 codes for these diseases are codes grouped with the arthropod borne diseases in the ICD-9 coding range, and are located on line 271 RICKETTSIAL AND OTHER ARTHROPOD-BORNE DISEASES. For example, Ebola is coded as 065.8 (Other specified arthropod-borne hemorrhagic fever). Line 271 will allow these diagnoses to have hospital and intensive care services. In the ICD-10 A98 family of codes, some diagnoses are arthropod-borne and some are not.

Dengue and yellow fever and West Nile are mosquito borne diseases which require medical care, and may require hospitalization and/or intensive care. The ICD-9 codes for these diagnoses are on line 271, but the ICD-10 code are inappropriately placed on line 660 INFECTIOUS DISEASES WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY.

HERC staff recommendation:

1) Move ICD-10 codes for arthropod-borne and hemorrhagic fever diagnoses as shown in the table below

ICD-10 Code	**Code description**	**Current Line**	**Recommended Line**
A90	Dengue fever	**660** INFECTIOUS DISEASES WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY	**271** RICKETTSIAL AND OTHER ARTHROPOD-BORNE DISEASES
A91	Dengue hemorrhagic fever	**660**	**271**
A92	Other mosquito-borne viral fevers	**660**	**271**
A93	Other arthropod-borne viral fevers, not elsewhere classified	**660**	**271**
A94	Unspecified arthropod-borne viral fever	**660**	**271**
A95	Yellow fever	**660**	**271**
A96	Arenaviral hemorrhagic fever	**623** OTHER VIRAL INFECTIONS	**196** SEPTICEMIA
A98.0	Crimean-Congo hemorrhagic fever	**660**	**271**
A98.1	Omsk hemorrhagic fever	**660**	**271**
A98.2	Kyasanur Forest disease	**660**	**271**
A98.3	Marburg virus disease	**623**	**196**
A98.4	Ebola virus disease	**623**	**196**
A98.5	Hemorrhagic fever with renal syndrome	**623**	**196**
A98.8	Other specified viral hemorrhagic fevers	**660**	**196**
A99	Unspecified viral hemorrhagic fever	**660**	**196**

Determination of Refraction Code 92015

Question: Where should eye exams for determination of refraction (CPT 92015) be located on the Prioritized List?

Question source: DMAP, HERC staff

Issue: 92015 (DETERMINATION OF REFRACTIVE STATE) is included on 55+ lines of the prioritized list. However, this service is intended to be used for the purpose of prescribing lenses/spectacles. While there is value and medical justification for an ophthalmological exam for medical conditions such as diabetes and MS, the medical conditions other than visual acuity disorders do not support the need for the determination of refractory state. The prioritized list has line 455 DISORDERS OF REFRACTION AND ACCOMODATION which includes 92015.

Despite the funded condition on line 455 for the treatment of disorders of refraction, the OHP benefit plan limits the coverage of lenses and spectacles as follows:

> Current OAR:
> 410-140-0140(4)(d)Visual services for the purpose of prescribing glasses/contact lenses, fitting fees, or glasses or contact lenses for:
> (A) Pregnant adult women are covered, and a complete examination and determination of refractive state is limited to once every 24 months;
> (B) Non-pregnant adults are not covered, except when the client:
> (i) Has a medical diagnoses of aphakia, pseudoaphakia, congenital aphakia, keratoconus; or
> (ii) Lacks the natural lenses of the eye due to surgical removal (e.g. cataract extraction) or congenital absence; or
> (iii) Has had a keratoplasty surgical procedure (e.g. corneal transplant) with limitations described in OAR 410-140-0160 Contact Lens Services and Supplies; and
> (iv) Is limited to one complete examination and determination of refractive state once every 24 months.

HERC staff recommendation:

1) Remove 92015 from all lines except line 455 DISORDERS OF REFRACTION AND ACCOMODATION

Diagnosis Codes on Current DMAP Excluded List Suggested for Placement on the Prioritized List

Question: Should certain codes now in the DMAP excluded file be placed on the Prioritized List?

Question source: DMAP and HERC staff

Issue: Coverage for services in OHP benefit plan is define by either prioritized funded or non-funded condition/ treatment pairs or non-prioritized conditions managed by DMAP Lists. HERC makes recommendations for placement of non-prioritized diagnosis codes into any one of the four DMAP Lists (Excluded, Exempt, Ancillary, Diagnostic Workup).

Historically, the DMAP lists have not been published, are poorly defined, and inconsistently utilized to the intent of the Oregon Administrative Rules. Those lists that include diagnosis codes have been revised in an effort to improve access to healthcare by making transparent the management of those diagnosis codes not included on the Prioritized List, provide more precise and useful information to providers about benefit coverage, and improve the accuracy of claims processing.

Old DMAP groups:
Excluded = never covered/ not eligible for exception
Exempt = Always covered
Ancillary = always covered when the CPT code is above the funded line (could not find any definition of an ancillary diagnosis code)
Diagnostic Workup File = always paid when paired with procedure codes above the funded line

The new DMAP lists replace the use of the Excluded, Exempt, and Ancillary lines for diagnosis codes and are defined as follows:

1. Conditions Not Covered (never covered/no exceptions) – This list is for the diagnosis codes that are specifically excluded by Oregon Administrative Rule and not eligible to be reviewed for coverage;
    a. Infertility
    b. Cosmetic
    c. Unrelated to improving health status
    d. Participation in experimental/ research programs

2. Undefined (usually not covered but eligible for review) – This list includes diagnosis codes that are unspecified. Some unspecified diagnosis codes are appropriately placed on the Prioritized List. Some unspecified codes may be used for both covered and uncovered diagnoses and are not placed on the List; these codes are eligible for review and for coverage if a more definitive diagnosis is not known. This list includes diagnosis codes that are

a. not definitive enough to determine line placement and ranking of the condition on the Prioritized List, or
b. other coding is available to more specifically define and support treatment.

3. Informational (not covered/informational only) – This list is for diagnosis codes that are not valid to determine reimbursement or benefit plan coverage and may include diagnosis codes that are
    a. External Causes of Mortality
    b. Personal/ Family History not relevant to covered preventative services
    c. Health status codes
    d. Health Care Encounter codes (non-specific or redundant)
    e. Not eligible as a primary diagnosis

4. Diagnostic Workup File (payable when paired with procedure codes)– Diagnosis codes included on this list are only reimbursable with non-prioritized procedure codes that are in the Diagnostic Procedure List. It is intended to allow for diagnostic services to more definitively diagnose
    a. Signs & symptoms
    b. Conditions requiring further evaluation to better define the primary diagnosis

In reviewing these lists, DMAP and HERC staff have identified ICD-9 diagnosis codes that can appropriately be placed on the Prioritized List.

HERC Staff Recommendations:

1) Add the ICD-9 diagnosis codes on the following spreadsheet to the indicated lines on the Prioritized List and advise DMAP to remove them from their current DMAP lists

ICD-9	Diagnosis Description	Current file	Recommended Placement
051.1	Pseudocowpox	EXCLUDED FILE	623 OTHER VIRAL INFECTIONS
051.2	Contagious pustular dermatitis	EXCLUDED FILE	623
051.9	Paravaccinia, unspecified	EXCLUDED FILE	623
173.00	Unspecified malignant neoplasm of skin of lip	EXCLUDED FILE	279 CANCER OF SKIN, EXCLUDING MALIGNANT MELANOMA
173.10	Unspecified malignant neoplasm of eyelid, including canthus	EXCLUDED FILE	279
173.20	Unspecified malignant neoplasm of skin of ear and external auditory canal	EXCLUDED FILE	279
173.30	Unspecified malignant neoplasm of skin of other and unspecified parts of face	EXCLUDED FILE	279
173.40	Unspecified malignant neoplasm of scalp and skin of neck	EXCLUDED FILE	279
173.50	Unspecified malignant neoplasm of skin of trunk, except scrotum	EXCLUDED FILE	279
173.60	Unspecified malignant neoplasm of skin of upper limb, including shoulder	EXCLUDED FILE	279
173.70	Unspecified malignant neoplasm of skin of lower limb, including hip	EXCLUDED FILE	279
173.80	Unspecified malignant neoplasm of other specified sites of skin	EXCLUDED FILE	279
173.90	Unspecified malignant neoplasm of skin, site unspecified	EXCLUDED FILE	279
228.09	Hemangioma of other sites	ANCILLARY CODES	636
278.02	Overweight	EXCLUDED FILE	661 ENDOCRINE AND METABOLIC CONDITIONS WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY

ICD-9	Diagnosis Description	Current file	Recommended Placement
278.8	Other hyperalimentation	EXCLUDED FILE	661
284.2	Myelophthisis	EXCLUDED FILE	118 APLASTIC ANEMIAS; AGRANULOCYTOSIS 300 APLASTIC ANEMIAS
300.20	Phobia, unspecified	EXCLUDED FILE	463 SIMPLE PHOBIAS AND SOCIAL ANXIETY DISORDER
300.89	Other somatoform disorders	EXCLUDED FILE	497 SOMATIZATION DISORDER, SOMATOFORM PAIN DISORDER, CONVERSION DISORDER
302.51	Trans-sexualism with asexual history	EXCLUDED FILE	413 GENDER DYSPHORIA
302.52	Trans-sexualism with homosexual history	EXCLUDED FILE	413
302.53	Trans-sexualism with heterosexual history	EXCLUDED FILE	413
302.81	Fetishism	EXCLUDED FILE	501 PARAPHILIAS AND OTHER PSYCHOSEXUAL DISORDERS
302.82	Voyeurism	EXCLUDED FILE	501
302.83	Sexual masochism	EXCLUDED FILE	501
302.84	Sexual sadism	EXCLUDED FILE	501
302.89	Other specified psychosexual disorders	EXCLUDED FILE	501
307.40	Nonorganic sleep disorder, unspecified	EXCLUDED FILE	614 DISORDERS OF SLEEP WITHOUT SLEEP APNEA
312.30	Impulse control disorder, unspecified	EXCLUDED FILE	467 OBSESSIVE-COMPULSIVE DISORDERS

ICD-9	Diagnosis Description	Current file	Recommended Placement
315.4	Developmental coordination disorder	EXCLUDED FILE	297 NEUROLOGICAL DYSFUNCTION IN POSTURE AND MOVEMENT CAUSED BY CHRONIC CONDITIONS 381 DYSFUNCTION RESULTING IN LOSS OF ABILITY TO MAXIMIZE LEVEL OF INDEPENDENCE IN SELF-DIRECTED CARE CAUSED BY CHRONIC CONDITIONS THAT CAUSE NEUROLOGICAL DYSFUNCTION
315.5	Mixed development disorder	EXCLUDED FILE	297,381
315.8	Other specified delays in development	EXCLUDED FILE	297,381
315.9	Unspecified delay in development	EXCLUDED FILE	297,381
321.0	Cryptococcal meningitis	EXCLUDED FILE	113 SUBACUTE MENINGITIS (EG. TUBERCULOSIS, CRYPTOCOCCOSIS)
321.1	Meningitis in other fungal diseases	EXCLUDED FILE	113
321.2	Meningitis due to viruses not elsewhere classified	EXCLUDED FILE	554 ASEPTIC MENINGITIS
321.3	Meningitis due to trypanosomiasis	EXCLUDED FILE	113
321.4	Meningitis in sarcoidosis	EXCLUDED FILE	113
321.8	Meningitis due to other nonbacterial organisms classified elsewhere	EXCLUDED FILE	113,554
323.01	Encephalitis and encephalomyelitis in viral diseases classified elsewhere	EXCLUDED FILE	540 VIRAL, SELF-LIMITING ENCEPHALITIS, MYELITIS AND ENCEPHALOMYELITIS
323.02	Myelitis in viral diseases classified elsewhere	EXCLUDED FILE	540

ICD-9	Diagnosis Description	Current file	Recommended Placement
323.1	Encephalitis, myelitis, and encephalomyelitis in rickettsial diseases classified elsewhere	EXCLUDED FILE	540
323.2	Encephalitis, myelitis, and encephalomyelitis in protozoal diseases classified elsewhere	EXCLUDED FILE	540
323.41	Other encephalitis and encephalomyelitis due to other infections classified elsewhere	EXCLUDED FILE	540
323.42	Other myelitis due to other infections classified elsewhere	EXCLUDED FILE	540
323.61	Infectious acute disseminated encephalomyelitis (ADEM)	EXCLUDED FILE	540
323.62	Other postinfectious encephalitis and encephalomyelitis	EXCLUDED FILE	540
323.63	Postinfectious myelitis	EXCLUDED FILE	540
323.71	Toxic encephalitis and encephalomyelitis	EXCLUDED FILE	540
323.72	Toxic myelitis	EXCLUDED FILE	540
327.01	Insomnia due to medical condition classified elsewhere	EXCLUDED FILE	614
327.02	Insomnia due to mental disorder	EXCLUDED FILE	614
327.14	Hypersomnia due to medical condition classified elsewhere	EXCLUDED FILE	614
327.15	Hypersomnia due to mental disorder	EXCLUDED FILE	614
330.2	Cerebral degeneration in generalized lipidoses	EXCLUDED FILE	75,297,349,381

ICD-9	Diagnosis Description	Current file	Recommended Placement
330.3	Cerebral degeneration of childhood in other diseases classified elsewhere	EXCLUDED FILE	75,297,349,381
336.2	Subacute combined degeneration of spinal cord in diseases classified elsewhere	EXCLUDED FILE	75,297,349,381
336.3	Myelopathy in other diseases classified elsewhere	EXCLUDED FILE	75,297,349,381
337.1	Peripheral autonomic neuropathy in disorders classified elsewhere	EXCLUDED FILE	75,297,349,381
347.10	Narcolepsy in conditions classified elsewhere, without cataplexy	EXCLUDED FILE	614
347.11	Narcolepsy in conditions classified elsewhere, with cataplexy	EXCLUDED FILE	614
357.1	Polyneuropathy in collagen vascular disease	EXCLUDED FILE	515 PERIPHERAL NERVE DISORDERS
357.3	Polyneuropathy in malignant disease	EXCLUDED FILE	515
357.4	Polyneuropathy in other diseases classified elsewhere	EXCLUDED FILE	515
366.41	Diabetic cataract	EXCLUDED FILE	301 CATARACT
366.42	Tetanic cataract	EXCLUDED FILE	301
366.43	Myotonic cataract	EXCLUDED FILE	301
366.44	Cataract associated with other syndromes	EXCLUDED FILE	301
372.15	Parasitic conjunctivitis	EXCLUDED FILE	508 CHRONIC CONJUNCTIVITIS, BLEPHAROCONJUNCTIVITIS
373.4	Infective dermatitis of eyelid of types resulting in deformity	EXCLUDED FILE	575 BLEPHARITIS

ICD-9	Diagnosis Description	Current file	Recommended Placement
373.5	Other infective dermatitis of eyelid	EXCLUDED FILE	575
373.6	Parasitic infestation of eyelid	EXCLUDED FILE	575
377.00	Papilledema, unspecified	ANCILLARY CODES	659 INTRACRANIAL CONDITIONS WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY
377.9	Unspecified disorder of optic nerve and visual pathways	EXCLUDED FILE	659
378.9	Unspecified disorder of eye movements	EXCLUDED FILE	398 STRABISMUS WITHOUT AMBLYOPIA AND OTHER DISORDERS OF BINOCULAR EYE MOVEMENTS; CONGENITAL ANOMALIES OF EYE; LACRIMAL DUCT OBSTRUCTION IN CHILDREN
420.0	Acute pericarditis in diseases classified elsewhere	EXCLUDED FILE	86 MYOCARDITIS, PERICARDITIS, AND ENDOCARDITIS
421.1	Acute and subacute infective endocarditis in diseases classified elsewhere	EXCLUDED FILE	86
484.1	Pneumonia in cytomegalic inclusion disease	EXCLUDED FILE	208 PNEUMOCOCCAL PNEUMONIA, OTHER BACTERIAL PNEUMONIA, BRONCHOPNEUMONIA
484.3	Pneumonia in whooping cough	EXCLUDED FILE	208
484.5	Pneumonia in anthrax	EXCLUDED FILE	208
484.6	Pneumonia in aspergillosis	EXCLUDED FILE	208
484.7	Pneumonia in other systemic mycoses	EXCLUDED FILE	208
484.8	Pneumonia in other infectious diseases classified elsewhere	EXCLUDED FILE	208

ICD-9	Diagnosis Description	Current file	Recommended Placement
521.40	Pathological resorption, unspecified	ANCILLARY CODES	654 DENTAL CONDITIONS WHERE TREATMENT IS CHOSEN PRIMARILY FOR AESTHETIC CONSIDERATIONS
521.41	Pathological resorption, internal	EXCLUDED FILE	654
521.42	Pathological resorption, external	EXCLUDED FILE	654
521.49	Other pathological resorption	EXCLUDED FILE	654
521.5	Hypercementosis	ANCILLARY CODES	654
523.00	Acute gingivitis, plaque induced	ANCILLARY CODES	222 DENTAL CONDITIONS (EG. PERIODONTAL DISEASE)
523.01	Acute gingivitis, non-plaque induced	ANCILLARY CODES	222
523.10	Chronic gingivitis, plaque induced	ANCILLARY CODES	222
523.11	Chronic gingivitis, non-plaque induced	ANCILLARY CODES	222
523.20	Gingival recession, unspecified	ANCILLARY CODES	222
523.21	Gingival recession, minimal	ANCILLARY CODES	222
523.22	Gingival recession, moderate	ANCILLARY CODES	222
523.23	Gingival recession, severe	ANCILLARY CODES	222
523.24	Gingival recession, localized	ANCILLARY CODES	222
523.25	Gingival recession, generalized	ANCILLARY CODES	222

ICD-9	Diagnosis Description	Current file	Recommended Placement
523.30	Aggressive periodontitis, unspecified	ANCILLARY CODES	222
523.31	Aggressive periodontitis, localized	ANCILLARY CODES	222
523.32	Aggressive periodontitis, generalized	ANCILLARY CODES	222
523.33	Acute periodontitis	ANCILLARY CODES	222
523.40	Chronic periodontitis, unspecified	ANCILLARY CODES	222
523.41	Chronic periodontitis, localized	ANCILLARY CODES	222
523.42	Chronic periodontitis, generalized	ANCILLARY CODES	222
523.5	Periodontosis	ANCILLARY CODES	222
523.6	Accretions on teeth	ANCILLARY CODES	654
523.8	Other specified periodontal diseases	ANCILLARY CODES	222
523.9	Unspecified gingival and periodontal disease	ANCILLARY CODES	222
524.32	Excessive spacing of teeth	ANCILLARY CODES	655 DENTAL CONDITIONS WHERE TREATMENT RESULTS IN MARGINAL IMPROVEMENT
525.0	Exfoliation of teeth due to systemic causes	ANCILLARY CODES	655
525.10	Acquired absence of teeth, unspecified	EXCLUDED FILE	457 DENTAL CONDITIONS (EG. MISSING TEETH, PROSTHESIS FAILURE)
525.11	Loss of teeth due to trauma	EXCLUDED FILE	457

ICD-9	Diagnosis Description	Current file	Recommended Placement
525.12	Loss of teeth due to periodontal disease	EXCLUDED FILE	457
525.13	Loss of teeth due to caries	EXCLUDED FILE	457
525.19	Other loss of teeth	EXCLUDED FILE	457
525.61	Open restoration margins	EXCLUDED FILE	655
525.62	Unrepairable overhanging of dental restorative materials	EXCLUDED FILE	655
525.63	Fractured dental restorative material without loss of material	EXCLUDED FILE	347 DENTAL CONDITIONS (EG. CARIES, FRACTURED TOOTH)
525.64	Fractured dental restorative material with loss of material	EXCLUDED FILE	347
525.65	Contour of existing restoration of tooth biologically incompatible with oral health	EXCLUDED FILE	655
525.66	Allergy to existing dental restorative material	EXCLUDED FILE	57 PREVENTIVE DENTAL SERVICES
525.8	Other specified disorders of the teeth and supporting structures	ANCILLARY CODES	655
526.61	Perforation of root canal space	EXCLUDED FILE	625 ANOMALIES OF RELATIONSHIP OF JAW TO CRANIAL BASE, MAJOR ANOMALIES OF JAW SIZE, OTHER SPECIFIED AND UNSPECIFIED DENTOFACIAL ANOMALIES
526.62	Endodontic overfill	EXCLUDED FILE	625
526.63	Endodontic underfill	EXCLUDED FILE	625
526.69	Other periradicular pathology associated with previous endodontic treatment	EXCLUDED FILE	625

ICD-9	Diagnosis Description	Current file	Recommended Placement
573.1	Hepatitis in viral diseases classified elsewhere	EXCLUDED FILE	202 CHRONIC HEPATITIS; VIRAL HEPATITIS
573.2	Hepatitis in other infectious diseases classified elsewhere	EXCLUDED FILE	202
595.4	Cystitis in diseases classified elsewhere	EXCLUDED FILE	278 UROLOGIC INFECTIONS
601.4	Prostatitis in diseases classified elsewhere	EXCLUDED FILE	521 CHRONIC PROSTATITIS, OTHER DISORDERS OF PROSTATE
604.91	Orchitis and epididymitis in diseases classified elsewhere	EXCLUDED FILE	278
608.81	Disorders of male genital organs in diseases classified elsewhere	EXCLUDED FILE	667 GENITOURINARY CONDITIONS WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY
608.85	Stricture of male genital organs	ANCILLARY CODES	667
608.86	Edema of male genital organs	EXCLUDED FILE	667
608.89	Other specified disorders of male genital organs	EXCLUDED FILE	667
629.0	Hematocele, female, not elsewhere classified	EXCLUDED FILE	536 CHRONIC PELVIC INFLAMMATORY DISEASE, PELVIC PAIN SYNDROME, DYSPAREUNIA
629.9	Unspecified disorder of female genital organs	EXCLUDED FILE	667
711.10	Arthropathy associated with Reiter's disease and nonspecific urethritis, site unspecified	EXCLUDED FILE	668 MUSCULOSKELETAL CONDITIONS WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY
711.11	Arthropathy associated with Reiter's disease and nonspecific urethritis, shoulder region	EXCLUDED FILE	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
711.12	Arthropathy associated with Reiter's disease and nonspecific urethritis, upper arm	EXCLUDED FILE	668
711.13	Arthropathy associated with Reiter's disease and nonspecific urethritis, forearm	EXCLUDED FILE	668
711.14	Arthropathy associated with Reiter's disease and nonspecific urethritis, hand	EXCLUDED FILE	668
711.15	Arthropathy associated with Reiter's disease and nonspecific urethritis, pelvic region and thigh	EXCLUDED FILE	668
711.16	Arthropathy associated with Reiter's disease and nonspecific urethritis, lower leg	EXCLUDED FILE	668
711.17	Arthropathy associated with Reiter's disease and nonspecific urethritis, ankle and foot	EXCLUDED FILE	668
711.18	Arthropathy associated with Reiter's disease and nonspecific urethritis, other specified sites	EXCLUDED FILE	668
711.19	Arthropathy associated with Reiter's disease and nonspecific urethritis, multiple sites	EXCLUDED FILE	668
711.20	Arthropathy in Behcet's syndrome, site unspecified	EXCLUDED FILE	668
711.21	Arthropathy in Behcet's syndrome, shoulder region	EXCLUDED FILE	668
711.22	Arthropathy in Behcet's syndrome, upper arm	EXCLUDED FILE	668
711.23	Arthropathy in Behcet's syndrome, forearm	EXCLUDED FILE	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
711.24	Arthropathy in Behcet's syndrome, hand	EXCLUDED FILE	668
711.25	Arthropathy in Behcet's syndrome, pelvic region and thigh	EXCLUDED FILE	668
711.26	Arthropathy in Behcet's syndrome, lower leg	EXCLUDED FILE	668
711.27	Arthropathy in Behcet's syndrome, ankle and foot	EXCLUDED FILE	668
711.28	Arthropathy in Behcet's syndrome, other specified sites	EXCLUDED FILE	668
711.29	Arthropathy in Behcet's syndrome, multiple sites	EXCLUDED FILE	668
711.30	Postdysenteric arthropathy, site unspecified	EXCLUDED FILE	668
711.31	Postdysenteric arthropathy, shoulder region	EXCLUDED FILE	668
711.32	Postdysenteric arthropathy, upper arm	EXCLUDED FILE	668
711.33	Postdysenteric arthropathy, forearm	EXCLUDED FILE	668
711.34	Postdysenteric arthropathy, hand	EXCLUDED FILE	668
711.35	Postdysenteric arthropathy, pelvic region and thigh	EXCLUDED FILE	668
711.36	Postdysenteric arthropathy, lower leg	EXCLUDED FILE	668
711.38	Postdysenteric arthropathy, other specified sites	ANCILLARY CODES	668
711.39	Postdysenteric arthropathy, multiple sites	ANCILLARY CODES	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
711.40	Arthropathy associated with other bacterial diseases, site unspecified	ANCILLARY CODES	668
711.41	Arthropathy associated with other bacterial diseases, shoulder region	ANCILLARY CODES	668
711.42	Arthropathy associated with other bacterial diseases, upper arm	ANCILLARY CODES	668
711.43	Arthropathy associated with other bacterial diseases, forearm	ANCILLARY CODES	668
711.44	Arthropathy associated with other bacterial diseases, hand	ANCILLARY CODES	668
711.45	Arthropathy associated with other bacterial diseases, pelvic region and thigh	ANCILLARY CODES	668
711.46	Arthropathy associated with other bacterial diseases, lower leg	ANCILLARY CODES	668
711.47	Arthropathy associated with other bacterial diseases, ankle and foot	ANCILLARY CODES	668
711.48	Arthropathy associated with other bacterial diseases, other specified sites	ANCILLARY CODES	668
711.49	Arthropathy associated with other bacterial diseases, multiple sites	ANCILLARY CODES	668
711.50	Arthropathy associated with other viral diseases, site unspecified	ANCILLARY CODES	668
711.51	Arthropathy associated with other viral diseases, shoulder region	ANCILLARY CODES	668
711.52	Arthropathy associated with other viral diseases, upper arm	ANCILLARY CODES	668
711.53	Arthropathy associated with other viral diseases, forearm	ANCILLARY CODES	668
711.54	Arthropathy associated with other viral diseases, hand	ANCILLARY CODES	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
711.55	Arthropathy associated with other viral diseases, pelvic region and thigh	ANCILLARY CODES	668
711.56	Arthropathy associated with other viral diseases, lower leg	ANCILLARY CODES	668
711.57	Arthropathy associated with other viral diseases, ankle and foot	ANCILLARY CODES	668
711.58	Arthropathy associated with other viral diseases, other specified sites	ANCILLARY CODES	668
711.59	Arthropathy associated with other viral diseases, multiple sites	ANCILLARY CODES	668
711.60	Arthropathy associated with mycoses, site unspecified	ANCILLARY CODES	668
711.61	Arthropathy associated with mycoses, shoulder region	ANCILLARY CODES	668
711.62	Arthropathy associated with mycoses, upper arm	ANCILLARY CODES	668
711.63	Arthropathy associated with mycoses, forearm	ANCILLARY CODES	668
711.64	Arthropathy associated with mycoses, hand	ANCILLARY CODES	668
711.65	Arthropathy associated with mycoses, pelvic region and thigh	ANCILLARY CODES	668
711.66	Arthropathy associated with mycoses, lower leg	ANCILLARY CODES	668
711.67	Arthropathy associated with mycoses, ankle and foot	ANCILLARY CODES	668
711.68	Arthropathy associated with mycoses, other specified sites	ANCILLARY CODES	668
711.69	Arthropathy associated with mycoses, involving multiple sites	ANCILLARY CODES	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
711.70	Arthropathy associated with helminthiasis, site unspecified	ANCILLARY CODES	668
711.71	Arthropathy associated with helminthiasis, shoulder region	ANCILLARY CODES	668
711.72	Arthropathy associated with helminthiasis, upper arm	ANCILLARY CODES	668
711.73	Arthropathy associated with helminthiasis, forearm	ANCILLARY CODES	668
711.74	Arthropathy associated with helminthiasis, hand	ANCILLARY CODES	668
711.75	Arthropathy associated with helminthiasis, pelvic region and thigh	ANCILLARY CODES	668
711.76	Arthropathy associated with helminthiasis, lower leg	ANCILLARY CODES	668
711.77	Arthropathy associated with helminthiasis, ankle and foot	ANCILLARY CODES	668
711.78	Arthropathy associated with helminthiasis, other specified sites	ANCILLARY CODES	668
711.79	Arthropathy associated with helminthiasis, multiple sites	ANCILLARY CODES	668
711.80	Arthropathy associated with other infectious and parasitic diseases, site unspecified	ANCILLARY CODES	668
711.81	Arthropathy associated with other infectious and parasitic diseases, shoulder region	ANCILLARY CODES	668
711.82	Arthropathy associated with other infectious and parasitic diseases, upper arm	ANCILLARY CODES	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
711.83	Arthropathy associated with other infectious and parasitic diseases, forearm	ANCILLARY CODES	668
711.84	Arthropathy associated with other infectious and parasitic diseases, hand	ANCILLARY CODES	668
711.85	Arthropathy associated with other infectious and parasitic diseases, pelvic region and thigh	ANCILLARY CODES	668
711.86	Arthropathy associated with other infectious and parasitic diseases, lower leg	ANCILLARY CODES	668
711.87	Arthropathy associated with other infectious and parasitic diseases, ankle and foot	ANCILLARY CODES	668
711.88	Arthropathy associated with other infectious and parasitic diseases, other specified sites	ANCILLARY CODES	668
711.89	Arthropathy associated with other infectious and parasitic diseases, multiple sites	ANCILLARY CODES	668
713.0	Arthropathy associated with other endocrine and metabolic disorders	ANCILLARY CODES	668
713.1	Arthropathy associated with gastrointestinal conditions other than infections	ANCILLARY CODES	668
713.2	Arthropathy associated with hematological disorders	ANCILLARY CODES	668
713.3	Arthropathy associated with dermatological disorders	ANCILLARY CODES	668
713.4	Arthropathy associated with respiratory disorders	ANCILLARY CODES	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
713.6	Arthropathy associated with hypersensitivity reaction	ANCILLARY CODES	668
713.7	Other general diseases with articular involvement	ANCILLARY CODES	668
713.8	Arthropathy associated with other conditions classifiable elsewhere	ANCILLARY CODES	668
718.70	Developmental dislocation of joint, site unspecified	ANCILLARY CODES	668
718.70	Developmental dislocation of joint, site unspecified	ANCILLARY CODES	668
718.88	Other joint derangement, not elsewhere classified, other specified sites	ANCILLARY CODES	668
718.88	Other joint derangement, not elsewhere classified, other specified sites	ANCILLARY CODES	668
720.81	Inflammatory spondylopathies in diseases classified elsewhere	EXCLUDED FILE	545 ACUTE AND CHRONIC DISORDERS OF SPINE WITHOUT NEUROLOGIC IMPAIRMENT
727.01	Synovitis and tenosynovitis in diseases classified elsewhere	EXCLUDED FILE	612 DISORDERS OF SOFT TISSUE
730.70	Osteopathy resulting from poliomyelitis, site unspecified	ANCILLARY CODES	297 NEUROLOGICAL DYSFUNCTION IN POSTURE AND MOVEMENT CAUSED BY CHRONIC CONDITIONS 381 DYSFUNCTION RESULTING IN LOSS OF ABILITY TO MAXIMIZE LEVEL OF INDEPENDENCE IN SELF-DIRECTED CARE CAUSED BY CHRONIC CONDITIONS THAT CAUSE NEUROLOGICAL DYSFUNCTION
730.71	Osteopathy resulting from poliomyelitis, shoulder region	ANCILLARY CODES	297,381
730.72	Osteopathy resulting from poliomyelitis, upper arm	ANCILLARY CODES	297,381

ICD-9	Diagnosis Description	Current file	Recommended Placement
730.73	Osteopathy resulting from poliomyelitis, forearm	ANCILLARY CODES	297,381
730.74	Osteopathy resulting from poliomyelitis, hand	ANCILLARY CODES	297,381
730.75	Osteopathy resulting from poliomyelitis, pelvic region and thigh	ANCILLARY CODES	297,381
730.76	Osteopathy resulting from poliomyelitis, lower leg	ANCILLARY CODES	297,381
730.77	Osteopathy resulting from poliomyelitis, ankle and foot	ANCILLARY CODES	297,381
730.78	Osteopathy resulting from poliomyelitis, other specified sites	ANCILLARY CODES	297,381
730.79	Osteopathy resulting from poliomyelitis, multiple sites	ANCILLARY CODES	297,381
730.80	Other infections involving bone in diseases classified elsewhere, site unspecified	ANCILLARY CODES	258 CHRONIC OSTEOMYELITIS
730.81	Other infections involving bone in diseases classified elsewhere, shoulder region	ANCILLARY CODES	258
730.82	Other infections involving bone in diseases classified elsewhere, upper arm	ANCILLARY CODES	258
730.83	Other infections involving bone in diseases classified elsewhere, forearm	ANCILLARY CODES	258
730.84	Other infections involving bone in diseases classified elsewhere, hand	ANCILLARY CODES	258
730.85	Other infections involving bone in diseases classified elsewhere, pelvic region and thigh	ANCILLARY CODES	258
730.86	Other infections involving bone in diseases classified elsewhere, lower leg	ANCILLARY CODES	258

ICD-9	Diagnosis Description	Current file	Recommended Placement
730.87	Other infections involving bone in diseases classified elsewhere, ankle and foot	ANCILLARY CODES	258
730.88	Other infections involving bone in diseases classified elsewhere, other specified sites	ANCILLARY CODES	258
730.89	Other infections involving bone in diseases classified elsewhere, multiple sites	DIAGNOSTIC WORKUP FILE	258
731.3	Major osseous defects	DIAGNOSTIC WORKUP FILE	534 DEFORMITIES OF UPPER BODY AND ALL LIMBS
731.8	Other bone involvement in diseases classified elsewhere	DIAGNOSTIC WORKUP FILE	668
733.99	Other disorders of bone and cartilage	EXCLUDED FILE	668
737.40	Curvature of spine, unspecified, associated with other conditions	DIAGNOSTIC WORKUP FILE	412 SPINAL DEFORMITY, CLINICALLY SIGNIFICANT 588 SPINAL DEFORMITY, NOT CLINICALLY SIGNIFICANT
737.41	Kyphosis associated with other conditions	DIAGNOSTIC WORKUP FILE	412,588
737.42	Lordosis associated with other conditions	DIAGNOSTIC WORKUP FILE	412,588
737.43	Scoliosis associated with other conditions	DIAGNOSTIC WORKUP FILE	412,588
756.81	Absence of muscle and tendon	EXCLUDED FILE	668

ICD-9	Diagnosis Description	Current file	Recommended Placement
759.3	Situs inversus	EXCLUDED FILE	662 CARDIOVASCULAR CONDITIONS WITH NO OR MINIMALLY EFFECTIVE TREATMENTS OR NO TREATMENT NECESSARY
774.5	Perinatal jaundice from other causes	DIAGNOSTIC WORKUP FILE	106 HEMOLYTIC DISEASE DUE TO ISOIMMUNIZATION, ANEMIA DUE TO TRANSPLACENTAL HEMORRHAGE, AND FETAL AND NEONATAL JAUNDICE
779.31	Feeding problems in newborn	EXEMPT FILE	19 FEEDING PROBLEMS IN NEWBORNS
779.32	Bilious vomiting in newborn	DIAGNOSTIC WORKUP FILE	2 BIRTH OF INFANT
779.34	Failure to thrive in newborn	EXEMPT FILE	19
848.0	Sprain of septal cartilage of nose	EXCLUDED FILE	616 SPRAINS AND STRAINS OF ADJACENT MUSCLES AND JOINTS, MINOR
848.2	Sprain of thyroid region	EXCLUDED FILE	616

Section 3.0

New Codes

Code	Code Descriptions	Proposed Placement	Notes
20604	Arthrocentesis, aspiration and/or injection, small joint or bursa (eg, fingers, toes); with ultrasound guidance, with permanent recording and reporting	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **157** PYOGENIC ARTHRITIS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **468** OSTEOARTHRITIS AND ALLIED DISORDERS **511** PERIPHERAL ENTHESOPATHIES **533** BENIGN NEOPLASM OF BONE AND ARTICULAR CARTILAGE INCLUDING OSTEOID OSTEOMAS; BENIGN NEOPLASM OF CONNECTIVE AND OTHER SOFT TISSUE **597** SYNOVITIS AND TENOSYNOVITIS	See arthrocentesis document
20606	Arthrocentesis, aspiration and/or injection, intermediate joint or bursa (eg, temporomandibular, acromioclavicular, wrist, elbow or ankle, olecranon bursa); with ultrasound guidance, with permanent recording and reporting	**50, 157, 290, 306, 468, 511, 533, 597**	See arthrocentesis document
20611	Arthrocentesis, aspiration and/or injection, major joint or bursa (eg, shoulder, hip, knee, subacromial bursa); with ultrasound guidance, with permanent recording and reporting	**50, 157, 290, 306, 422, 468, 511, 533, 597** **435** INTERNAL DERANGEMENT OF KNEE AND LIGAMENTOUS DISRUPTIONS OF THE KNEE, RESULTING IN SIGNIFICANT INJURY/IMPAIRMENT	See arthrocentesis document
20983	Ablation therapy for reduction or eradication of 1 or more bone tumors (eg, metastasis) including adjacent soft tissue when involved by tumor extension, percutaneous, including imaging guidance when performed; cryoablation	**Non-Covered List**	See Issues document
21811	Open treatment of rib fracture(s) with internal fixation, includes thoracoscopic visualization when performed, unilateral; 1-3 ribs	**112** FRACTURE OF RIBS AND STERNUM, OPEN	
21812	Open treatment of rib fracture(s) with internal fixation, includes thoracoscopic visualization when performed, unilateral; 4-6 ribs	**112** FRACTURE OF RIBS AND STERNUM, OPEN	
21813	Open treatment of rib fracture(s) with internal fixation, includes thoracoscopic visualization when performed, unilateral; 7 or more ribs	**112** FRACTURE OF RIBS AND STERNUM, OPEN	
22510	Percutaneous vertebroplasty (bone biopsy included when performed), 1 vertebral body, unilateral or bilateral injection, inclusive of all imaging guidance; cervicothoracic	**484** CLOSED DISLOCATIONS/FRACTURES OF NON-CERVICAL VERTEBRAL COLUMN WITHOUT SPINAL CORD INJURY	22520-22522 (Percutaneous vertebroplasty (bone biopsy included when performed), 1 vertebral body,unilateral or bilateral injection;...) were on line 507 (became line 484). These codes were deleted with 2015 CPT codes. The new codes include the radiologic supervision which were previously separate codes
22511	Percutaneous vertebroplasty (bone biopsy included when performed), 1 vertebral body, unilateral or bilateral injection, inclusive of all imaging guidance; lumbosacral	**484** CLOSED DISLOCATIONS/FRACTURES OF NON-CERVICAL VERTEBRAL COLUMN WITHOUT SPINAL CORD INJURY	See 22510
22512	Percutaneous vertebroplasty (bone biopsy included when performed), 1 vertebral body, unilateral or bilateral injection, inclusive of all imaging guidance; each additional cervicothoracic or lumbosacral vertebral body (List separately in addition to code f	**484** CLOSED DISLOCATIONS/FRACTURES OF NON-CERVICAL VERTEBRAL COLUMN WITHOUT SPINAL CORD INJURY	See 22510
22513	Percutaneous vertebral augmentation, including cavity creation (fracture reduction and bone biopsy included when performed) using mechanical device (eg, kyphoplasty), 1 vertebral body, unilateral or bilateral cannulation, inclusive of all imaging guidance; thoracic	**484** CLOSED DISLOCATIONS/FRACTURES OF NON-CERVICAL VERTEBRAL COLUMN WITHOUT SPINAL CORD INJURY	22523-22525 (Percutaneous vertebral augmentation, including cavity creation (fracture reduction and bone biopsy included when performed) using mechanical device, 1 vertebral body, unilateral or bilateral cannulation (eg, kyphoplasty)...) were on line 507 (became line 484). Previous separate radiologic interpretation CPT codes are now included in these codes.
22514	Percutaneous vertebral augmentation, including cavity creation (fracture reduction and bone biopsy included when performed) using mechanical device (eg, kyphoplasty), 1 vertebral body, unilateral or bilateral cannulation, inclusive of all imaging guidance; lumbar	**484** CLOSED DISLOCATIONS/FRACTURES OF NON-CERVICAL VERTEBRAL COLUMN WITHOUT SPINAL CORD INJURY	See 22513 above

Code	Code Descriptions	Proposed Placement	Notes
22515	Percutaneous vertebral augmentation, including cavity creation (fracture reduction and bone biopsy included when performed) using mechanical device (eg, kyphoplasty), 1 vertebral body, unilateral or bilateral cannulation, inclusive of all imaging guidance; each additional vertebral body	**484** CLOSED DISLOCATIONS/FRACTURES OF NON-CERVICAL VERTEBRAL COLUMN WITHOUT SPINAL CORD INJURY	See 22513 above
22858	Total disc arthroplasty (artificial disc), anterior approach, including discectomy with end plate preparation (includes osteophytectomy for nerve root or spinal cord decompression and microdissection); second level, cervical	**374** DISORDERS OF SPINE WITH NEUROLOGIC IMPAIRMENT **545** ACUTE AND CHRONIC DISORDERS OF SPINE WITHOUT NEUROLOGIC IMPAIRMENT	22856 (Total disc arthroplasty (artificial disc), anterior approach, including discectomy with end plate preparation (includes osteophytectomy for nerve root or spinal cord decompression and microdissection); single interspace, cervical) is on lines 374,545
27279	Arthrodesis, sacroiliac joint, percutaneous or minimally invasive (indirect visualization), with image guidance, includes obtaining bone graft when performed, and placement of transfixing device	**187** FRACTURE OF PELVIS, OPEN AND CLOSED	27280 (Arthrodesis, sacroiliac joint (including obtaining graft)) was on line 189 (became 187), deleted with 2015 CPT codes
33270	Insertion or replacement of permanent subcutaneous implantable defibrillator system, with subcutaneous electrode, including defibrillation threshold evaluation, induction of arrhythmia, evaluation of sensing for arrhythmia termination, and programming or	**103** CARDIOMYOPATHY **115** CONGENITAL HEART BLOCK; OTHER OBSTRUCTIVE ANOMALIES OF HEART **286** LIFE-THREATENING CARDIAC ARRHYTHMIAS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT	Other implantable defibrillator systems on 103, 115, 286, 290
33271	Insertion of subcutaneous implantable defibrillator electrode	**103, 115, 286, 290**	See 33270
33272	Removal of subcutaneous implantable defibrillator electrode	**103, 115, 286, 290**	See 33270
33273	Repositioning of previously implanted subcutaneous implantable defibrillator electrode	**103, 115, 286, 290**	See 33270
33418	Transcatheter mitral valve repair, percutaneous approach, including transseptal puncture when performed; initial prosthesis	**73** ACUTE AND SUBACUTE ISCHEMIC HEART DISEASE, MYOCARDIAL INFARCTION **86** MYOCARDITIS, PERICARDITIS, AND ENDOCARDITIS 93 DISCORDANT CARDIOVASCULAR CONNECTIONS **94** CONGENITAL MITRAL VALVE STENOSIS/INSUFFICIENCY **115** CONGENITAL HEART BLOCK; OTHER OBSTRUCTIVE ANOMALIES OF HEART **190** RHEUMATIC MULTIPLE VALVULAR DISEASE **193** CHRONIC ISCHEMIC HEART DISEASE **261** DISEASES OF MITRAL, TRICUSPID, AND PULMONARY VALVES **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **368** ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS	mitral valve repair open procedures are on lines 73, 86, 94, 115, 190, 193, 261, 290, 368
33419	Transcatheter mitral valve repair, percutaneous approach, including transseptal puncture when performed; additional prosthesis(es) during same session (List separately in addition to code for primary procedure)	**73, 86, 94, 115, 190, 193, 261, 290, 368**	See 33418 above
33946	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; initiation, veno-venous	**11** RESPIRATORY CONDITIONS OF FETUS AND NEWBORN **48** COARCTATION OF THE AORTA **71** VENTRICULAR SEPTAL DEFECT **73** ACUTE AND SUBACUTE ISCHEMIC HEART DISEASE, MYOCARDIAL INFARCTION **74** CONGENITAL PULMONARY VALVE ANOMALIES **81** PATENT DUCTUS ARTERIOSUS; AORTIC PULMONARY FISTULA/WINDOW **86** MYOCARDITIS, PERICARDITIS, AND ENDOCARDITIS **89** ENDOCARDIAL CUSHION DEFECTS **90** CONGENITAL PULMONARY VALVE ATRESIA **93** DISCORDANT CARDIOVASCULAR CONNECTIONS **94** CONGENITAL MITRAL VALVE STENOSIS/INSUFFICIENCY **102** HEART FAILURE **109** TETRALOGY OF FALLOT (TOF); CONGENITAL VENOUS ABNORMALITIES **110** CONGENITAL STENOSIS AND INSUFFICIENCY OF AORTIC VALVE **115** CONGENITAL HEART BLOCK; OTHER OBSTRUCTIVE ANOMALIES OF HEART **123** ATRIAL SEPTAL DEFECT, SECUNDUM **132** COMMON TRUNCUS **134** TOTAL ANOMALOUS PULMONARY VENOUS CONNECTION **138** INTERRUPTED AORTIC ARCH **142** EBSTEIN'S ANOMALY **180** COMMON VENTRICLE **186** SEPTICEMIA **192** CONGENITAL TRICUSPID ATRESIA AND STENOSIS **236** HYPOPLASTIC LEFT HEART SYNDROME	Lines with diagnoses of respiratory failure (11, 237), cardiac failure lines with treatments including VAD procedures (86, 102), cardiogenic shock (73), septic shock (186), persistant fetal circulation (81), congenital heart disease (48, 71, 74, 89, 90, 93, 94, 109, 110, 115, 123, 132, 134, 138, 142, 180, 192, 236), cardiac transplant and complications thereof (244, 267) Current CPT codes 33960, 33961 (Prolonged extracorporeal circulation for cardiopulmonary insufficiency) are Exempt (always covered). These codes are deleted with 2015 CPT code set. Exempt (always covered) would be another option; however, virtually all diagnoses for which ECMO is used should be on these lines.

Code	Code Descriptions	Proposed Placement	Notes
33947	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; initiation, veno-arterial	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33948	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; daily management, each day, veno-venous	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33949	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; daily management, each day, veno-arterial	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33951	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; insertion of peripheral (arterial and/or venous) cannula(e), percutaneous, birth through 5 years of age (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33952	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; insertion of peripheral (arterial and/or venous) cannula(e), percutaneous, 6 years and older (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33953	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; insertion of peripheral (arterial and/or venous) cannula(e), open, birth through 5 years of age	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33954	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; insertion of peripheral (arterial and/or venous) cannula(e), open, 6 years and older	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33955	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; insertion of central cannula(e) by sternotomy or thoracotomy, birth through 5 years of age	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33956	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; insertion of central cannula(e) by sternotomy or thoracotomy, 6 years and older	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33957	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; reposition peripheral (arterial and/or venous) cannula(e), percutaneous, birth through 5 years of age (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33958	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; reposition peripheral (arterial and/or venous) cannula(e), percutaneous, 6 years and older (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33959	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; reposition peripheral (arterial and/or venous) cannula(e), open, birth through 5 years of age (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33962	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; reposition peripheral (arterial and/or venous) cannula(e), open, 6 years and older (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33963	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; reposition of central cannula(e) by sternotomy or thoracotomy, birth through 5 years of age (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33964	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; reposition central cannula(e) by sternotomy or thoracotomy, 6 years and older (includes fluoroscopic guidance, when performed)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33965	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; removal of peripheral (arterial and/or venous) cannula(e), percutaneous, birth through 5 years of age	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33966	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; removal of peripheral (arterial and/or venous) cannula(e), percutaneous, 6 years and older	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above

Code	Code Descriptions	Proposed Placement	Notes
33969	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; removal of peripheral (arterial and/or venous) cannula(e), open, birth through 5 years of age	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33984	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; removal of peripheral (arterial and/or venous) cannula(e), open, 6 years and older	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33985	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; removal of central cannula(e) by sternotomy or thoracotomy, birth through 5 years of age	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33986	Extracorporeal membrane oxygenation (ECMO)/extracorporeal life support (ECLS) provided by physician; removal of central cannula(e) by sternotomy or thoracotomy, 6 years and older	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33987	Arterial exposure with creation of graft conduit (eg, chimney graft) to facilitate arterial perfusion for ECMO/ECLS (List separately in addition to code for primary procedure)	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33988	Insertion of left heart vent by thoracic incision (eg, sternotomy, thoracotomy) for ECMO/ECLS	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
33989	Removal of left heart vent by thoracic incision (eg, sternotomy, thoracotomy) for ECMO/ECLS	**11, 48, 71, 73, 74, 81, 86, 89, 90, 93, 94, 102, 109, 110, 115, 123, 132, 134, 138, 142, 180, 186, 192, 236, 237, 244, 267**	See 33946 above
34839	Physician planning of a patient-specific fenestrated visceral aortic endograft requiring a minimum of 90 minutes of physician time	**84** INJURY TO INTERNAL ORGANS **257** ARTERIAL EMBOLISM/THROMBOSIS: ABDOMINAL AORTA, THORACIC AORTA **280** INJURY TO BLOOD VESSELS OF THE THORACIC CAVITY **289** DISSECTING OR RUPTURED AORTIC ANEURYSM **330** NON-DISSECTING ANEURYSM WITHOUT RUPTURE	Endovascular aortic graft procedures (CPT 34841-34848) are on line 84,257,280,289,330
37218	Transcatheter placement of intravascular stent(s), intrathoracic common carotid artery or innominate artery, open or percutaneous antegrade approach, including angioplasty, when performed, and radiological supervision and interpretation	**322** STROKE **419** TRANSIENT CEREBRAL ISCHEMIA; OCCLUSION/STENOSIS OF PRECEREBRAL ARTERIES WITHOUT OCCLUSION	Similar code 37217 (Transcatheter placement of intravascular stent(s), intrathoracic common carotid artery or innominate artery by retrograde treatment, open ipsilateral cervical carotid artery exposure) is on lines 322, 419
43180	Esophagoscopy, rigid, transoral with diverticulectomy of hypopharynx or cervical esophagus (eg, Zenker's diverticulum), with cricopharyngeal myotomy, includes use of telescope or operating microscope and repair, when performed	**384** ESOPHAGITIS; ESOPHAGEAL AND INTRAESOPHAGEAL HERNIAS **519** ESOPHAGITIS AND GERD; ESOPHAGEAL SPASM; ASYMPTOMATIC DIAPHRAGMATIC HERNIA	530.6 (Diverticulum of esophagus, acquired) is on lines 384,519
44381	Ileoscopy, through stoma; with transendoscopic balloon dilation	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	Similar code 44379 (Small intestinal endoscopy, enteroscopy beyond second portion of duodenum, including ileum; with transendoscopic stent) is on lines 46,105 Add 44379 to lines 32, 161, and 647
44384	Ileoscopy, through stoma; with placement of endoscopic stent (includes pre- and post-dilation and guide wire passage, when performed)	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	See 44381 above
44401	Colonoscopy through stoma; with ablation of tumor(s), polyp(s), or other lesion(s) (includes pre-and post-dilation and guide wire passage, when performed)	**46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **60** ULCERS, GASTRITIS, DUODENITIS, AND GI HEMORRHAGE **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **170** ANAL, RECTAL AND COLONIC POLYPS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	Simlar code 44394 (Colonoscopy through stoma; with removal of tumor(s), polyp(s), or other lesion(s) by snare technique) is on lines 46,105,161,170,647 Similar code 45384 (Colonoscopy, flexible; with removal of tumor(s), polyp(s), or other lesion(s) by hot biopsy forceps) is on lines 46,60,105,161,170,647

Code	Code Descriptions	Proposed Placement	Notes
44402	Colonoscopy through stoma; with endoscopic stent placement (including pre- and post-dilation and guide wire passage, when performed)	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	45327 (Proctosigmoidoscopy, rigid; with transendoscopic stent placement (includes predilation)) is on lines 46,105,647 Lines 32, 161 are also appropriate Add 45327 to lines 32 and 161
44403	Colonoscopy through stoma; with endoscopic mucosal resection	**Diagnostic Procedures List**	Similar code 43254 is Diagnostic
44404	Colonoscopy through stoma; with directed submucosal injection(s), any substance	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **60** ULCERS, GASTRITIS, DUODENITIS, AND GI HEMORRHAGE **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **170** ANAL, RECTAL AND COLONIC POLYPS **189** DIVERTICULITIS OF COLON **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	45381 (Colonoscopy, flexible; with directed submucosal injection(s), any substance) is on lines 32,46,60,105,161,170,189,480,647,629 480 and 629 are not appropriate for colonoscopy through stoma
44405	Colonoscopy through stoma; with transendoscopic balloon dilation	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	Similar code 45386 (Colonoscopy, flexible; with transendoscopic balloon dilation) is on lines 32,46,105,161 Add 45386 to line 647
44406	Colonoscopy through stoma; with endoscopic ultrasound examination, limited to the sigmoid, descending, transverse, or ascending colon and cecum and adjacent structures	**Diagnostic Procedures List**	
44407	Colonoscopy through stoma; with transendoscopic ultrasound guided intramural or transmural fine needle aspiration/biopsy(s), includes endoscopic ultrasound examination limited to the sigmoid, descending, transverse, or ascending colon and cecum and adjace	**Diagnostic Procedures List**	
44408	Colonoscopy through stoma; with decompression (for pathologic distention) (eg, volvulus, megacolon), including placement of decompression tube, when performed	**46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION	Megacolon and volvulus diagnoses are on line 46 Hirschsprung's disease is on line 105
45346	Sigmoidoscopy, flexible; with ablation of tumor(s), polyp(s), or other lesion(s) (includes pre- and post-dilation and guide wire passage, when performed)	**46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **60** ULCERS, GASTRITIS, DUODENITIS, AND GI HEMORRHAGE **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **170** ANAL, RECTAL AND COLONIC POLYPS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	Simlar code 44394 (Colonoscopy through stoma; with removal of tumor(s), polyp(s), or other lesion(s) by snare technique) is on lines 46,105,161,170,647 Similar code 45384 (Colonoscopy, flexible; with removal of tumor(s), polyp(s), or other lesion(s) by hot biopsy forceps) is on lines 46,60,105,161,170,647
45347	Sigmoidoscopy, flexible; with placement of endoscopic stent (includes pre- and post-dilation and guide wire passage, when performed)	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	45327 (Proctosigmoidoscopy, rigid; with transendoscopic stent placement (includes predilation)) is on lines 46,105,647 Lines 32, 161 are also appropriate
45349	Sigmoidoscopy, flexible; with endoscopic mucosal resection	**Diagnostic Procedures List**	
45350	Sigmoidoscopy, flexible; with band ligation(s) (eg, hemorrhoids)	**480** THROMBOSED AND COMPLICATED HEMORRHOIDS **629** UNCOMPLICATED HEMORRHOIDS	Similar code 46947 (Hemorrhoidopexy) is on lines 480, 629

Code	Code Descriptions	Proposed Placement	Notes
45388	Colonoscopy, flexible; with ablation of tumor(s), polyp(s), or other lesion(s) (includes pre- and post-dilation and guide wire passage, when performed)	**46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **60** ULCERS, GASTRITIS, DUODENITIS, AND GI HEMORRHAGE **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **170** ANAL, RECTAL AND COLONIC POLYPS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	Similar code 45384 (Colonoscopy, flexible; with removal of tumor(s), polyp(s), or other lesion(s) by hot biopsy forceps) is on lines 46,60,105,161,170,647
45389	Colonoscopy, flexible; with endoscopic stent placement (includes pre- and post-dilation and guide wire passage, when performed)	**32** REGIONAL ENTERITIS, IDIOPATHIC PROCTOCOLITIS, ULCERATION OF INTESTINE **46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION **161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS **647** BENIGN NEOPLASMS OF DIGESTIVE SYSTEM	45327 (Proctosigmoidoscopy, rigid; with transendoscopic stent placement (includes predilation)) is on lines 46,105,647 Lines 32, 161 are also appropriate
45390	Colonoscopy, flexible; with endoscopic mucosal resection	**Diagnostic Procedures List**	
45393	Colonoscopy, flexible; with decompression (for pathologic distention) (eg, volvulus, megacolon), including placement of decompression tube, when performed	**46** INTUSSCEPTION, VOLVULUS, INTESTINAL OBSTRUCTION, HAZARDOUS FOREIGN BODY IN GI TRACT WITH RISK OF PERFORATION OR OBSTRUCTION **105** CONGENITAL ANOMALIES OF DIGESTIVE SYSTEM AND ABDOMINAL WALL EXCLUDING NECROSIS; CHRONIC INTESTINAL PSEUDO-OBSTRUCTION	Megacolon and volvulus are on line 46 Hirschsprung's disease is on line 105
45398	Colonoscopy, flexible; with band ligation(s) (eg, hemorrhoids)	**480** THROMBOSED AND COMPLICATED HEMORRHOIDS **629** UNCOMPLICATED HEMORRHOIDS	Similar code 46947 (Hemorrhoidopexy) is on lines 480, 629
45399	Unlisted procedure, colon	**Ancillary Codes File**	Will require manual review
46601	Anoscopy; diagnostic, with high-resolution magnification (HRA) (eg, colposcope, operating microscope) and chemical agent enhancement, including collection of specimen(s) by brushing or washing, when performed	**Diagnostic Procedures List**	
46607	Anoscopy; with high-resolution magnification (HRA) (eg, colposcope, operating microscope) and chemical agent enhancement, with biopsy, single or multiple	**Diagnostic Procedures List**	
47383	Ablation, 1 or more liver tumor(s), percutaneous, cryoablation	**Non-Covered List**	See Issues document
52441	Cystourethroscopy, with insertion of permanent adjustable transprostatic implant; single implant	**331** FUNCTIONAL AND MECHANICAL DISORDERS OF THE GENITOURINARY SYSTEM INCLUDING BLADDER OUTLET OBSTRUCTION	Similar HCPCS codes C9739-C9740 (Cystourethroscopy with transprostatic implant; 1 to 3 implants or 4 or more implants) care on line 331 **NICE 2014**: Current evidence on the efficacy and safety of insertion of prostatic urethral lift implants to treat lower urinary tract symptoms secondary to benign prostatic hyperplasia is adequate to support the use of this procedure
52442	Cystourethroscopy, with insertion of permanent adjustable transprostatic implant; each additional permanent adjustable transprostatic implant (List separately in addition to code for primary procedure)	**331** FUNCTIONAL AND MECHANICAL DISORDERS OF THE GENITOURINARY SYSTEM INCLUDING BLADDER OUTLET OBSTRUCTION	See 52441
62302	Myelography via lumbar injection, including radiological supervision and interpretation; cervical	**Diagnostic Procedures List**	Other myelography codes (62284, 72240, 72255, etc.) are Diagnostic
62303	Myelography via lumbar injection, including radiological supervision and interpretation; thoracic	**Diagnostic Procedures List**	
62304	Myelography via lumbar injection, including radiological supervision and interpretation; lumbosacral	**Diagnostic Procedures List**	
62305	Myelography via lumbar injection, including radiological supervision and interpretation; 2 or more regions (eg, lumbar/thoracic, cervical/thoracic, lumbar/cervical, lumbar/thoracic/cervical)	**Diagnostic Procedures List**	
64486	Transversus abdominis plane (TAP) block (abdominal plane block, rectus sheath block) unilateral; by injection(s) (includes imaging guidance, when performed)	**Ancillary Codes File**	See Issues document
64487	Transversus abdominis plane (TAP) block (abdominal plane block, rectus sheath block) unilateral; by continuous infusion(s) (includes imaging guidance, when performed)	**Ancillary Codes File**	See Issues document
64488	Transversus abdominis plane (TAP) block (abdominal plane block, rectus sheath block) bilateral; by injections (includes imaging guidance, when performed)	**Ancillary Codes File**	See Issues document

Code	Code Descriptions	Proposed Placement	Notes
64489	Transversus abdominis plane (TAP) block (abdominal plane block, rectus sheath block) bilateral; by continuous infusions (includes imaging guidance, when performed)	**Ancillary Codes File**	See Issues document
66179	Aqueous shunt to extraocular equatorial plate reservoir, external approach; without graft	**143** GLAUCOMA, OTHER THAN PRIMARY ANGLE-CLOSURE **247** PRIMARY ANGLE-CLOSURE GLAUCOMA	66180 (with graft) is on lines 143,247
66184	Revision of aqueous shunt to extraocular equatorial plate reservoir; without graft	**143** GLAUCOMA, OTHER THAN PRIMARY ANGLE-CLOSURE **247** PRIMARY ANGLE-CLOSURE GLAUCOMA	66185 (with graft) is on line 143 Add 66185 to line 247
76641	Ultrasound, breast, unilateral, real time with image documentation, including axilla when performed; complete	**Diagnostic Procedures List**	
76642	Ultrasound, breast, unilateral, real time with image documentation, including axilla when performed; limited	**Diagnostic Procedures List**	
77061	Digital breast tomosynthesis; unilateral	**Non-Covered List**	See Issues document
77062	Digital breast tomosynthesis; bilateral	**Non-Covered List**	See Issues document
77063	Screening digital breast tomosynthesis, bilateral (List separately in addition to code for primary procedure)	**Non-Covered List**	See Issues document
77085	Dual-energy X-ray absorptiometry (DXA), bone density study, 1 or more sites; axial skeleton (eg, hips, pelvis, spine), including vertebral fracture assessment	**Diagnostic Procedures List**	Similar code 77080 and 77081 are diagnostic
77086	Vertebral fracture assessment via dual-energy X-ray absorptiometry (DXA)	**Diagnostic Procedures List**	Similar code 77080 and 77081 are diagnostic
77306	Teletherapy isodose plan; simple (1 or 2 unmodified ports directed to a single area of interest), includes basic dosimetry calculation(s)	**97,116,117,119,129,130,133,137,139,161,195,199,203,204,212,214,162, 218,219,233,238,241,242,262,263,265,266,274,279,291,292,299,319,320 ,321,333,359,376, 401, 402, 424, 439,533,600,611**	Current teletherapy CPT codes (77305, 77310, 77315) have been deleted and replaced with 77306 and 77307. The old CPT codes for teletherapy planning where on the radiation therapy lines.
77307	Teletherapy isodose plan; complex (multiple treatment areas, tangential ports, the use of wedges, blocking, rotational beam, or special beam considerations), includes basic dosimetry calculation(s)	**97,116,117,119,129,130,133,137,139,161,195,199,203,204,212,214,162, 218,219,233,238,241,242,262,263,265,266,274,279,291,292,299,319,320 ,321,333,359,376, 401, 402, 424, 439,533,600,611**	See 77306
77316	Brachytherapy isodose plan; simple (calculation[s] made from 1 to 4 sources, or remote afterloading brachytherapy, 1 channel), includes basic dosimetry calculation(s)	**117, 137, 161, 195, 203, 212, 262, 265, 266, 274, 291, 292, 299, 320, 333, 376, 439, 465, 600**	Current brachytherapy CPT codes (77326, 77327, 77328) have been deleted and replaced with CPT 77316-77318. The old CPT codes for brachytherapy were on these 19 radiation therapy lines
77317	Brachytherapy isodose plan; intermediate (calculation[s] made from 5 to 10 sources, or remote afterloading brachytherapy, 2-12 channels), includes basic dosimetry calculation(s)	**117, 137, 161, 195, 203, 212, 262, 265, 266, 274, 291, 292, 299, 320, 333, 376, 439, 465, 600**	See 77316
77318	Brachytherapy isodose plan; complex (calculation[s] made from over 10 sources, or remote afterloading brachytherapy, over 12 channels), includes basic dosimetry calculation(s)	**117, 137, 161, 195, 203, 212, 262, 265, 266, 274, 291, 292, 299, 320, 333, 376, 439, 465, 600**	See 77316
77385	Intensity modulated radiation treatment delivery (IMRT), includes guidance and tracking, when performed; simple	**97,116,117,119,129,130,133,137,139,161,195,199,203,204,212,214,162, 218,219,233,238,241,242,262,263,265,266,274,279,291,292,299,319,320 ,321,333,359,376, 401, 402, 424, 439,533,600,611**	These codes replace CPT 77418 (Intensity modulated treatment delivery, single or multiple fields/arcs, via narrow spatially and temporally modulated beams, binary, dynamic MLC, per treatment session) which was on the radiation therapy lines
77386	Intensity modulated radiation treatment delivery (IMRT), includes guidance and tracking, when performed; complex	**97,116,117,119,129,130,133,137,139,161,195,199,203,204,212,214,162, 218,219,233,238,241,242,262,263,265,266,274,279,291,292,299,319,320 ,321,333,359,376, 401, 402, 424, 439,533,600,611**	See 77385
77387	Guidance for localization of target volume for delivery of radiation treatment delivery, includes intrafraction tracking, when performed	**41,75,85,97,116,117,119,129,130,133,137,139,161,195,199,203,204,212, 214,162,218,219,233,238,241,242,262,263,265,266,274,279,291,292,299 ,319,320,321,333,359,376, 401, 402, 424, 439,533,600,611**	Radiation therapy lines
80163	Digoxin; free	**Diagnostic Procedures List**	
80165	Valproic acid (dipropylacetic acid); free	**Diagnostic Procedures List**	
80300	Drug screen, any number of drug classes from Drug Class List A; any number of non-TLC devices or procedures, (eg, immunoassay) capable of being read by direct optical observation, including instrumented-assisted when performed (eg, dipsticks, cups, cards,	**Diagnostic Procedures List**	
80301	Drug screen, any number of drug classes from Drug Class List A; single drug class method, by instrumented test systems (eg, discrete multichannel chemistry analyzers utilizing immunoassay or enzyme assay), per date of service	**Diagnostic Procedures List**	
80302	Drug screen, presumptive, single drug class from Drug Class List B, by immunoassay (eg, ELISA) or non-TLC chromatography without mass spectrometry (eg, GC, HPLC), each procedure	**Diagnostic Procedures List**	

Code	Code Descriptions	Proposed Placement	Notes
80303	Drug screen, any number of drug classes, presumptive, single or multiple drug class method; thin layer chromatography procedure(s) (TLC) (eg, acid, neutral, alkaloid plate), per date of service	**Diagnostic Procedures List**	
80304	Drug screen, any number of drug classes, presumptive, single or multiple drug class method; not otherwise specified presumptive procedure (eg, TOF, MALDI, LDTD, DESI, DART), each procedure	**Diagnostic Procedures List**	
80320	Alcohols	**Diagnostic Procedures List**	
80321	Alcohol biomarkers; 1 or 2	**Diagnostic Procedures List**	
80322	Alcohol biomarkers; 3 or more	**Diagnostic Procedures List**	
80323	Alkaloids, not otherwise specified	**Diagnostic Procedures List**	
80324	Amphetamines; 1 or 2	**Diagnostic Procedures List**	
80325	Amphetamines; 3 or 4	**Diagnostic Procedures List**	
80326	Amphetamines; 5 or more	**Diagnostic Procedures List**	
80327	Anabolic steroids; 1 or 2	**Diagnostic Procedures List**	
80328	Anabolic steroids; 3 or more	**Diagnostic Procedures List**	
80329	Analgesics, non-opioid; 1 or 2	**Diagnostic Procedures List**	
80330	Analgesics, non-opioid; 3-5	**Diagnostic Procedures List**	
80331	Analgesics, non-opioid; 6 or more	**Diagnostic Procedures List**	
80332	Antidepressants, serotonergic class; 1 or 2	**Diagnostic Procedures List**	
80333	Antidepressants, serotonergic class; 3-5	**Diagnostic Procedures List**	
80334	Antidepressants, serotonergic class; 6 or more	**Diagnostic Procedures List**	
80335	Antidepressants, tricyclic and other cyclicals; 1 or 2	**Diagnostic Procedures List**	
80336	Antidepressants, tricyclic and other cyclicals; 3-5	**Diagnostic Procedures List**	
80337	Antidepressants, tricyclic and other cyclicals; 6 or more	**Diagnostic Procedures List**	
80338	Antidepressants, not otherwise specified	**Diagnostic Procedures List**	
80339	Antiepileptics, not otherwise specified; 1-3	**Diagnostic Procedures List**	
80340	Antiepileptics, not otherwise specified; 4-6	**Diagnostic Procedures List**	
80341	Antiepileptics, not otherwise specified; 7 or more	**Diagnostic Procedures List**	
80342	Antipsychotics, not otherwise specified; 1-3	**Diagnostic Procedures List**	
80343	Antipsychotics, not otherwise specified; 4-6	**Diagnostic Procedures List**	
80344	Antipsychotics, not otherwise specified; 7 or more	**Diagnostic Procedures List**	
80345	Barbiturates	**Diagnostic Procedures List**	
80346	Benzodiazepines; 1-12	**Diagnostic Procedures List**	
80347	Benzodiazepines; 13 or more	**Diagnostic Procedures List**	
80348	Buprenorphine	**Diagnostic Procedures List**	
80349	Cannabinoids, natural	**Diagnostic Procedures List**	
80350	Cannabinoids, synthetic; 1-3	**Diagnostic Procedures List**	
80351	Cannabinoids, synthetic; 4-6	**Diagnostic Procedures List**	
80352	Cannabinoids, synthetic; 7 or more	**Diagnostic Procedures List**	
80353	Cocaine	**Diagnostic Procedures List**	
80354	Fentanyl	**Diagnostic Procedures List**	
80355	Gabapentin, non-blood	**Diagnostic Procedures List**	
80356	Heroin metabolite	**Diagnostic Procedures List**	
80357	Ketamine and norketamine	**Diagnostic Procedures List**	
80358	Methadone	**Diagnostic Procedures List**	
80359	Methylenedioxyamphetamines (MDA, MDEA, MDMA)	**Diagnostic Procedures List**	
80360	Methylphenidate	**Diagnostic Procedures List**	
80361	Opiates, 1 or more	**Diagnostic Procedures List**	
80362	Opioids and opiate analogs; 1 or 2	**Diagnostic Procedures List**	
80363	Opioids and Opiate analogs; 3 or 4	**Diagnostic Procedures List**	
80364	Opioids and Opiate analogs; 5 or more	**Diagnostic Procedures List**	
80365	Oxycodone	**Diagnostic Procedures List**	
80366	Pregabalin	**Diagnostic Procedures List**	
80367	Propoxyphene	**Diagnostic Procedures List**	
80368	Sedative hypnotics (non-benzodiazepines)	**Diagnostic Procedures List**	
80369	Skeletal muscle relaxants; 1 or 2	**Diagnostic Procedures List**	
80370	Skeletal muscle relaxants; 3 or more	**Diagnostic Procedures List**	
80371	Stimulants, synthetic	**Diagnostic Procedures List**	
80372	Tapentadol	**Diagnostic Procedures List**	
80373	Tramadol	**Diagnostic Procedures List**	
80374	Stereoisomer (enantiomer) analysis, single drug class	**Diagnostic Procedures List**	
80375	Drug(s) or substance(s), definitive, qualitative or quantitative, not otherwise specified; 1-3	**Diagnostic Procedures List**	
80376	Drug(s) or substance(s), definitive, qualitative or quantitative, not otherwise specified; 4-6	**Diagnostic Procedures List**	
80377	Drug(s) or substance(s), definitive, qualitative or quantitative, not otherwise specified; 7 or more	**Diagnostic Procedures List**	
81246	FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (eg, D835, I836)	**97** CHILDHOOD LEUKEMIAS **401** ACUTE PROMYELOCYTIC LEUKEMIA **402** ACUTE MYELOID LEUKEMIA Treatment: BONE MARROW TRANSPLANT **241** MYELOID DISORDERS	See Issues document

Code	Code Descriptions	Proposed Placement	Notes
81288	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter methylation analysis	**161** CANCER OF COLON, RECTUM, SMALL INTESTINE AND ANUS	Test done on a tumor , may affect treatment or surveillance
81313	PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)	**Non-Covered List**	See Issues document
81410	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis panel, must include sequencing of at least 9 genes, including FBN1, TGFBR1, TGFBR2, COL3A1,	**Diagnostic List**	Genetics review
81411	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); duplication/deletion analysis panel, must include analyses for TGFBR1, TGFBR2, MYH11, and COL3A1	**Diagnostic List**	Genetics review
81415	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis	**Diagnostic List**	Genetics review
81416	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)	**Diagnostic List**	Genetics review
81417	Exome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)	**Non-Covered List**	Genetics review
81420	Fetal chromosomal aneuploidy (eg, trisomy 21, monosomy X) genomic sequence analysis panel, circulating cell-free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18, and 21	**1** PREGNANCY	Prenatal testing guideline applies
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis	**Non-Covered List**	Genetics review
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)	**Non-Covered List**	Genetics review
81427	Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)	**Non-Covered List**	Genetics review
81430	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3	**Diagnostic List**	Genetics review
81431	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes	**Diagnostic List**	Genetics review
81435	Hereditary colon cancer syndromes (eg, Lynch syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include analysis of at least 7 genes, including APC, CHEK2, MLH1, MSH2, MSH6, MUTYH, and PMS2	**Diagnostic List**	Genetics review
81436	Hereditary colon cancer syndromes (eg, Lynch syndrome, familial adenomatosis polyposis); duplication/deletion gene analysis panel, must include analysis of at least 8 genes, including APC, MLH1, MSH2, MSH6, PMS2, EPCAM, CHEK2, and MUTYH	**Diagnostic List**	Genetics review
81440	Nuclear encoded mitochondrial genes (eg, neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including BCS1L, C10orf2, COQ2, COX10, DGUOK, MPV17, OPA1, PDSS2, POLG, POLG2, RRM2B, SCO1, SCO2, SLC25A4, S	**Diagnostic List**	Genetics review
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or re	**Diagnostic List**	ALK, BRAF, KRAS, EGFR, PTEN appear to be standard testing
81450	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, DNA and RNA analysis when performed, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, KIT, MLL, NRAS, NPM1, NOTCH1), interrogation for sequence variants	**Diagnostic List**	NPMI, BRAF, IDH, KRAS, FTL3 appear to be standard testing

Code	Code Descriptions	Proposed Placement	Notes
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA and RNA analysis when performed, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, N	**Diagnostic List**	ALK, BRAF, KRAS, EGFR, NPM1, FTL3 appear to be standard testing
81460	Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary op	**Diagnostic List**	Genetics review
81465	Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed	**Diagnostic List**	Genetics review
81470	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL,	**Non-Covered List**	Genetics review
81471	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL,	**Non-Covered List**	Genetics review
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score	**195** CANCER OF BREAST; AT HIGH RISK OF BREAST CANCER	See issues document
83006	Growth stimulation expressed gene 2 (ST2, Interleukin 1 receptor like-1)	**Non-Covered List**	See issues document
87505	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe tech, 3-5 targets	**Diagnostic Procedures List**	
87506	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe tech, 6-11 targets	**Diagnostic Procedures List**	
87507	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe tech, 12-25 targets	**Diagnostic Procedures List**	
87623	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), low-risk types (eg, 6, 11, 42, 43, 44)	**Diagnostic Procedures List**	Non-oncogenic, or low-risk HPV types, such as HPV 6 or 11, can cause (1) benign or low-grade abnormalities of cervical cells, (2) anogenital warts, and (3) a disease of the respiratory tract called recurrent respiratory papillomatosis (RRP).
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)	**Diagnostic Procedures List**	G0123 (pap smear) is diagnostic
87625	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed	**Diagnostic Procedures List**	G0123 (pap smear) is diagnostic
87806	Infectious agent antigen detection by immunoassay with direct optical observation; HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies	**Diagnostic Procedures List**	
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)	**Diagnostic Procedures List**	
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure	**Diagnostic Procedures List**	
88364	In situ hybridization (eg, FISH), per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure)	**Diagnostic Procedures List**	FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification.[2] FISH can also be used to detect and localize specific RNA targets (mRNA, lncRNA and miRNA) in cells, circulating tumor cells, and tissue samples.
88366	In situ hybridization (eg, FISH), per specimen; each multiplex probe stain procedure	**Diagnostic Procedures List**	

Code	Code Descriptions	Proposed Placement	Notes
88369	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure)	**Diagnostic Procedures List**	In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e., probe) to localize a specific DNA or RNA sequence in a portion or section of tissue
88373	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure)	**Diagnostic Procedures List**	
88374	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each multiplex probe stain procedure	**Diagnostic Procedures List**	
88377	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each multiplex probe stain procedure	**Diagnostic Procedures List**	
89337	Cryopreservation, mature oocyte(s)	**Non-Covered List**	In vitro fertilization is an excluded procedures
90630	Influenza virus vaccine, quadrivalent (IIV4), split virus, preservative free, for intradermal use	**3** PREVENTION SERVICES WITH EVIDENCE OF EFFECTIVENESS	
90651	Human Papillomavirus vaccine types 6, 11, 16, 18, 31, 33, 45, 52, 58, nonavalent (HPV), 3 dose schedule, for intramuscular use	**3** PREVENTION SERVICES WITH EVIDENCE OF EFFECTIVENESS	
91200	Liver elastography, mechanically induced shear wave (eg, vibration), without imaging, with interpretation and report	**Non-Covered List**	See Issues document
92145	Corneal hysteresis determination, by air impulse stimulation, unilateral or bilateral, with interpretation and report	**Non-Covered List**	See Issues document
93260	Programming device evaluation (in person) with iterative adjustment of the implantable device to test the function of the device and select optimal permanent programmed values with analysis, review and report by a physician or other qualified health care	**Ancillary Codes File**	DMAP will need to manually review
93261	Interrogation device evaluation (in person) with analysis, review and report by a physician or other qualified health care professional, includes connection, recording and disconnection per patient encounter; implantable subcutaneous lead defibrillator sy	**Ancillary Codes File**	DMAP will need to manually review
93355	Echocardiography, transesophageal (TEE) for guidance of a transcatheter intracardiac or great vessel(s) structural intervention(s) (eg,TAVR, transcathether pulmonary valve replacement, mitral valve repair, paravalvular regurgitation repair, left atrial ap	**48** COARCTATION OF THE AORTA **71** VENTRICULAR SEPTAL DEFECT **73** ACUTE AND SUBACUTE ISCHEMIC HEART DISEASE, MYOCARDIAL INFARCTION **74** CONGENITAL PULMONARY VALVE ANOMALIES **81** PATENT DUCTUS ARTERIOSUS; AORTIC PULMONARY FISTULA/WINDOW **86** MYOCARDITIS, PERICARDITIS, AND ENDOCARDITIS **89** ENDOCARDIAL CUSHION DEFECTS **90** CONGENITAL PULMONARY VALVE ATRESIA **93** DISCORDANT CARDIOVASCULAR CONNECTIONS **94** CONGENITAL MITRAL VALVE STENOSIS/INSUFFICIENCY **102** HEART FAILURE **109** TETRALOGY OF FALLOT (TOF); CONGENITAL VENOUS ABNORMALITIES **110** CONGENITAL STENOSIS AND INSUFFICIENCY OF AORTIC VALVE **115** CONGENITAL HEART BLOCK; OTHER OBSTRUCTIVE ANOMALIES OF HEART **123** ATRIAL SEPTAL DEFECT, SECUNDUM **132** COMMON TRUNCUS **134** TOTAL ANOMALOUS PULMONARY VENOUS CONNECTION **138** INTERRUPTED AORTIC ARCH **142** EBSTEIN'S ANOMALY **180** COMMON VENTRICLE **190** RHEUMATIC MULTIPLE VALVULAR DISEASE **192** CONGENITAL TRICUSPID ATRESIA AND STENOSIS **193** CHRONIC ISCHEMIC HEART DISEASE **227** DISEASES AND DISORDERS OF AORTIC VALVE	All congenital heart defect lines, valvular disorder lines and major heart structural defect lines Alternative: Diagnostic Procedures File
93644	Electrophysiologic evaluation of subcutaneous implantable defibrillator (includes defibrillation threshold evaluation, induction of arrhythmia, evaluation of sensing for arrhythmia termination, and programming or reprogramming of sensing or therapeutic pa	**103** CARDIOMYOPATHY **115** CONGENITAL HEART BLOCK; OTHER OBSTRUCTIVE ANOMALIES OF HEART **286** LIFE-THREATENING CARDIAC ARRHYTHMIAS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT	Implantable defibrillator systems on 103, 115, 286, 290
93702	Bioimpedance spectroscopy (BIS), extracellular fluid analysis for lymphedema assessment(s)	**Non-Covered List**	See Issues document

Code	Code Descriptions	Proposed Placement	Notes
93895	Quantitative carotid intima media thickness and carotid atheroma evaluation, bilateral	**Non-Covered List**	See Issues document
96127	Brief emotional/behavioral assessment (eg, depression inventory, attention-deficit/hyperactivity disorder [ADHD] scale), with scoring and documentation, per standardized instrument	**Inpatient and outpatient medical lines**	Alcohol/drug use assessment tools (SBIRT; CPT 99408, 99409) are on all inpatient and outpatient medical lines
97607	Negative pressure wound therapy, (eg, vacuum assisted drainage collection), utilizing disposable, non-durable medical equipment including provision of exudate management collection system, topical application(s), wound assessment, and instructions for ong	**8,30,51,84,209,211,239, 290, 383, 427, 503, 612**	See Negative Pressure Wound Therapy document
97608	Negative pressure wound therapy, (eg, vacuum assisted drainage collection), utilizing disposable, non-durable medical equipment including provision of exudate management collection system, topical application(s), wound assessment, and instructions for ong	**8,30,51,84,209,211,239, 290, 383, 427, 503, 612**	See Negative Pressure Wound Therapy document
99184	Initiation of selective head or total body hypothermia in the critically ill neonate, includes appropriate patient selection by review of clinical, imaging and laboratory data, confirmation of esophageal temperature probe location, evaluation of amplitude	**Inpatient medical lines**	
99188	Application of topical fluoride varnish by a physician or other qualified health care professional	**3** PREVENTION SERVICES WITH EVIDENCE OF EFFECTIVENESS **57** PREVENTIVE DENTAL SERVICES	D1206 (Topical application of fluoride varnish) is on lines 3 and 57
99490	Chronic care management services, at least 20 minutes of clinical staff time directed by a physician or other qualified health care professional, per calendar month, with the following required elements: multiple (two or more) chronic conditions expected	**Outpatient medical visit lines**	
99497	Advance care planning including the explanation and discussion of advance directives such as standard forms (with completion of such forms, when performed), by the physician or other qualified health care professional; first 30 minutes, face-to-face with	**Outpatient medical visit lines**	
99498	Advance care planning including the explanation and discussion of advance directives such as standard forms (with completion of such forms, when performed), by the physician or other qualified health care professional; each additional 30 minutes (List sep	**Outpatient medical visit lines**	

code	Code	Proposed	Notes
20983	Ablation therapy for reduction or		20982 (Ablation therapy... radiofrequency) is

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Clinical Policy Bulletin:
Tumor Markers
Number: 0352

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Review History
Definitions
Additional Information
Clinical Policy Bulletin Notes

Policy

1. Aetna considers any of the following serum tumor markers for the stated indication medically necessary:
    1. Prostate-specific antigen (PSA) for prostate cancer screening (see CPB 0521 - Prostate Cancer Screening), staging, monitoring response to therapy, and detecting disease recurrence.
    2. Carcinoembryonic antigen (CEA) for any of the following:
        1. As a preoperative prognostic indicator in members with known colorectal carcinoma or mucinous appendiceal carcinoma when it will assist in staging and surgical treatment planning; *or*
        2. To detect asymptomatic recurrence of colorectal cancer after surgical and/or medical treatment for the diagnosis of colorectal cancer (not as a screening test for colorectal cancer); *or*
        3. To monitor response to treatment for metastatic cancer.
    3. 1p19q codeletion molecular cytogenetic analysis for astrocytoma and gliomas
    4. ALK gene fusion as a molecular biomarker in non-small cell lung cancer
    5. ALK gene rearrangement for diffuse large B cell lymphoma, peripheral T-cell lymphoma, and post-transplant lymphoproliferative disorder
    6. ALK translocations for selecting candidates for crizotinib (Xalkori) in inflammatory myofibroblastic tumor
    7. APC for familial adenomatous polyposis when criteria are met in CPB 140 - Genetic Testing; and for desmoid fibromatosis; experimental for other indications.
    8. Afirma Thyroid FNA analysis for assessing fine needle aspiration samples from thyroid nodules that are indeterminate; experimental for other indications.
    9. BCR/ABL fluorescent in situ hybridization (FISH) for lymphoblastic lymphoma, acute myeloid leukemia, acute lymphocytic leukemia and chronic myelogenous leukemia; experimental for other indications.
    10. BRAF V600 mutation for hairy cell leukemia; gastrointestinal stromal tumors; Lynch syndrome testing for persons meeting criteria in CPB 140 - Genetic Testing; melamoma for vemurafenib, dabrafenib, and trametinib (see CPB 743 - Pharmacogenomic and Pharmacodynamic Testing; and colorectal cancer if KRAS nonmutated; experimental for other indications.
    11. Cancer antigen 125 (CA 125) levels for any of the following:

1. As a preoperative diagnostic aid in women with ovarian masses that are suspected to be malignant, such that arrangements can be made for intraoperative availability of a gynecological oncologist if the CA 125 is increased; *or*
2. As a screening test for ovarian cancer when there is a history of hereditary cancer syndrome (a pattern of clusters of ovarian cancer within two or more generations); *or*
3. Diagnosis of ovarian cancer in women with new symptoms (bloating, pelvic or abdominal pain, difficulty eating or feeling full quickly, or urinary frequency and urgency) that have persisted for three or more weeks, where the clinician has performed a pelvic and rectal examination and suspects ovarian cancer; *or*
4. In members with adenocarcinoma of unknown primary, to rule out ovarian cancer; *or*
5. In members with known ovarian cancer, as an aid in the monitoring of disease, response to treatment, detection of recurrent disease, or assessing value of performing second-look surgery.

12. CA 19-9 to monitor the clinical response to therapy or detect early recurrence of disease in members with known gastric cancer, pancreatic cancer, gallbladder cancer, cholangiocarcinoma or adenocarcinoma of the ampulla of Vater.
13. CA 19-9 to rule out cholangiocarcinoma in persons with primary sclerosing cholangitis undergoing liver transplantation.
14. CA 19-9 as a tumor marker for mucinous appendiceal carcinoma.
15. CD 20, for determining eligibility for anti-CD20 treatment (rituximab) -- see CPB 0314 - Rituximab (Rituxan).
16. CD 25, for determining eligibility for denileukin diftitox (Ontak) treatment.
17. CD 31 immunostaining, for diagnosis of angiosarcoma.
18. CD 33, for determining eligibility for anti-CD33 (gemtuzumab, Mylotarg) treatment.
19. CD 52, for determining eligibility for anti-CD52 (alemtuzumab, Campath) treatment.
20. CD117 (c-kit), for determining eligibility for treatment with imatinib mesylate (Gleevec).
21. Cyclin D1, for diagnosis and predicting disease recurrence of mantle cell lymphoma.
22. Epidermal growth factor receptor (EGFR) testingfor tyrosine kinase inhibitors (erlotinib (Tarceva), gefitinib (Iressa), afatinib (Gilotrif)) in non-small cell lung cancer.
23. Human epidermal growth factor receptor 2 (HER2) evaluation in breast, gastric and esophageal cancer - see CPB 0313 - Trastuzumab (Herceptin), Ado-Trastuzumab (Kadcyla) and Pertuzumab (Perjeta).
24. IGH@ (Immunoglobulin heavy chain locus), gene rearrangement analysis to detect abnormal clonal population(s) in non-Hodgkin's lymphomas,hairy cell leukemia, and post-transplant lymphoproliferative disorder.
25. IGK@ (Immunoglobulin kappa light chain locus), gene rearrangement analysis, evaluation to detect abnormal clonal population(s) for non-Hodgkin's lymphoma, systemic light chain amyloidosis
26. IDH mutation for glioma
27. K-ras (KRAS) mutation analysis, with BRAF reflex testing, to predict non-response to cetuximab (Erbitux) and panitumumab (Vectibix) in the treatment of anal adenocarcinoma, metastatic colorectal cancer and small bowel adenocarcinoma; K-ras (KRAS) mutation analysis to predict non-response to erlotinib (Tarceva) in the treatment of non-small cell lung cancer; experimental for all other indications.
28. Measurement of estrogen and progesterone receptors on primary breast cancers, and on metastatic lesions if the results would influence treatment planning.
29. MLH1, MSH2, MSH6 for persons meeting HNPCC/Lynch Syndrome testing criteria in CPB 140 - Genetic Testing; colorectal cancer in persons under age 50; and all

persons with Stage II colon cancer; experimental for all other indications,

30. Mycosis fungoides, diagnosis: polymerase chain reaction (PCR) for T-cell receptor gamma chain gene rearrangement as an adjunct to the histopathologic diagnosis of mycosis fungoides.
31. Myeloperoxidase (MPO) immunostaining, FLT3-ITD, CEBPA mutation, NPM1 mutation, and KIT mutation for diagnosis of acute myeloid leukemia.
32. NPM1 in acute myeloid leukemia; experimental for other indications.
33. PML/RARA for acute promyelocytic leukemia; experimental for all other indications.
34. PTEN for persons meeting Cowden syndrome testing criteria in CPB 140 - Genetic Testing; experimental for all other indications.
35. Placental alkaline phosphatase (PLAP), to diagnose germ cell seminoma and non-seminoma germ cell tumors in unknown primary cancers.
36. ROS-1 to predict response to crizotinib (Xalkori) for the treatment of non-small cell lung cancer (NSCLC).
37. Serial measurements of alpha fetoprotein (AFP) to diagnose germ cell tumors in members with adenocarcinoma, or carcinoma not otherwise specified, involving mediastinal nodes; or the diagnosis and monitoring of hepatocellular carcinoma (e.g., before considering liver transplantation).
38. Serial measurements of CA 15-3 (also known as CA 27-29 or Truquant RIA) in following the course of treatment in women diagnosed with breast cancer, especially advanced metastatic breast cancer (an increasing CA 15-3 level may suggest treatment failure).
39. Serial measurements of human chorionic gonadotropin (HCG) to diagnose germ cell tumors in members with adenocarcinoma, or carcinoma not otherwise specified, involving mediastinal nodes, or to monitor treatment in members with known trophoblastic tumors (invasive hydatidiform moles and choriocarcinomas) and germinal cell tumors (teratocarcinoma and embryonal cell carcinoma) of the ovaries or testes, or to monitor for relapse after remission is achieved.
40. Serial measurements of AFP and HCG together to diagnose and monitor testicular cancer.
41. Steroid hormone receptor status in both pre-menopausal and post-menopausal members to identify individuals most likely to benefit from endocrine forms of adjuvant therapy and therapy for recurrent or metastatic breast cancer.
42. TCB@ (T cell antigen receptor, beta), gene rearrangement analysis to detect abnormal clonal population(s); for T-cell prolymphocytic leukemia
43. TCG@ (T cell receptor, gamma), gene rearrangement analysis for T-cell prolymphocytic leukemia
44. Oncotype Dx (also known as 21 gene RT-PCR test) to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

    1. Breast cancer is nonmetastatic (node negative[1]); *and*
    2. Breast tumor is estrogen receptor positive; *and*
    3. Breast tumor is HER2 receptor negative or breast tumor is HER2 receptor positive and less than 1 cm in diameter. (Rationale: adjuvant chemotherapy with trastuzumab (Herceptin) is considered to be medically necessary regardless of an Oncotype Dx score for HER2 receptor positive lesions 1 cm or more in diameter); *and*
    4. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); *and*
    5. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy (i.e., member will forgo adjuvant chemotherapy if Oncotype Dx score is low).

45. Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-

1) for the determination of prognosis in patients with newly diagnosed, node negative breast cancer.

46. ZAP-70, for assessing prognosis and need for aggressive therapy in persons with chronic lymphocytic leukemia.

[1] Either standard node dissection negative by hematoxylin and eosin (H&E) staining or sentinel node negative by H&E staining (if sentinel node is negative by H&E, but immunoassay is positive, then still considered node negative for this purpose). In addition, women with isolated tumor cells in lymph nodes (micrometastases) are considered node negative.

More than one Oncotype Dx test may be medically necessary for persons with breast cancer who have two or more histologically distinct tumors that meet medical necessity criteria. Repeat Oncotype Dx testing or testing of multiple tumor sites in the same person has no proven value for other indications. Oncotype Dx is considered experimental and investigational for node positive breast cancer, ductal carcinoma in situ (OncotypeDx DCIS), colon cancer (OncotypeDx Colon), prostate cancer (OncotypeDx Prostate) and all other indications.

2. Aetna considers the bladder tumor antigen (BTA) Stat test, the nuclear matrix protein (NMP22) test, the fibrin/fibrinogen degradation products (Aura-Tek FDfP) test, or the UroVysion fluorescent in situ hybridization (FISH) test medically necessary in any of the following conditions:

    1. Follow-up of treatment for bladder cancer; *or*
    2. Monitoring for eradication of bladder cancer; *or*
    3. Recurrences after eradication.

    Aetna considers the BTA Stat test, the NMP22 test, the Aura-Tek FDP test, or the UroVysion fluorescent in situ hybridization (FISH) test experimental and investigational for screening of bladder cancer, evaluation of hematuria, and all other indications.

3. Aetna considers the use of the ImmunoCyte immunohistochemistry test medically necessary as an adjunct to cystoscopy or cytology in the monitoring of persons with bladder cancer.

    Aetna considers the ImmunoCyte immunohistochemistry test experimental and investigational in the diagnosis of bladder cancer or for screening for bladder cancer in asymptomatic persons.

4. Aetna considers genetic testing for Janus Kinase 2 (JAK2) mutations in persons with chronic myeloproliferative disorders (CMPDs) medically necessary for the following indications: 1) qualitative assessment of JAK2-V617F sequence variant using methods with detection thresholds of up to 5% for initial diagnostic assessment of adult patients presenting with symptoms of CMPD; 2) diagnostic assessment of polycythemia vera in adults; and 3) differential diagnosis of essential thrombocytosis and primary myelofibrosis from reactive conditions in adults.

    Aetna considers genetic testing for Janus Kinase 2 (JAK2) mutations in persons with chronic myeloproliferative disorders (CMPDs) experimental and investigational for any other indication including: 1) diagnostic assessment of myeloproliferative disorders in children; 2) quantitative assessment of JAK2-V617F allele burden subsequent to qualitative detection of JAK2-V617F.

5. Aetna considers each of the following experimental and investigational. The peer reviewed medical literature does not support these tests as having sufficient sensitivity or specificity necessary to define their clinical role:

1. CEA used for all other indications including any of the following:

    1. As a screening test for colorectal cancer; or
    2. As a sole determinant to treat a colorectal cancer member with adjuvant therapy or systemic therapy for presumed metastatic disease; or
    3. For diagnosis, prognosis, or monitoring of treatment in members with lung cancer; or
    4. For routine use of CEA alone for monitoring response to treatment of colorectal when there are other simple tests available to indicate a response; or
    5. For screening, diagnosis, staging or routine surveillance of breast cancer.

2. AFP for the diagnosis of trophoblastic tumors and other oncologic indications.
3. Assaying for loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) or deleted in colon cancer (DCC) protein (18q-LOH/DCC) for colorectal cancer.
4. Biomarker Translation (BT) test for breast cancer and other indications.
5. BluePrint molecular subtyping profile for breast cancer
6. BRAF mutation analysis in lung cancer, thyroid cancer.
7. Breast Cancer Gene Expression Ratio (HOXB13:IL17BR).
8. BreastNext Next-Gen Cancer Panel
9. CA 125 for all other indications including use as a screening test for colorectal cancer or ovarian cancer (other than as indicated above) or for differential diagnosis of members with symptoms of colonic disease.
10. CA 19-9 for all other indications including breast, colorectal, esophageal, gastro-esophageal, liver, or uterine cancer; pancreatic pseudocyst; screening persons with primary sclerosing cholangiitis without signs or symptoms of cholangiocarcinoma; or screening persons with primary sclerosing cholangitis for development of cholangiocarcinoma.
11. CancerNext Next-Gen Cancer Panel
12. Carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6) for predicting the risk of breast cancer.
13. Carcinoembryonic antigen cellular adhesion molecule-7 (CEACAM-7) expression as a predictive marker for rectal cancer recurrence.
14. Caris Target Now Molecular Profiling Test.
15. CellSearch assay (for all cancers).
16. CK5, CK14, p63, and Racemase P504S testing for prostate cancer.
17. c-Met expression for predicting prognosis in persons with advanced NSCLC and colorectal cancer, and other indications.
18. Cofilin (CFL1) as a prognostic and drug resistance marker in non-small cell lung cancer.
19. ColonSentry test for screening of colorectal cancer.
20. Colonext Next-Gen Cancer Panel
21. ColoPrint, CIMP, LINE-1 hypomethylation, and Immune cells for colon cancer.
22. Colorectal Cancer DSA (Almac Diagnostics, Craigavon, UK)
23. CxBladder Test
24. Cyclin D1 and FADD (Fas-associated protein with death domain) for head and neck squamous cell carcinoma
25. DecisionDx-UM (uveal melanoma) (Castle Biosciences, Phoenix, AZ)
26. DCIS Recurrence Score.
27. Des-gamma-carboxy prothrombin (DCP) (also known as "prothrombin produced by vitamin K absence or antagonism II" [PIVKA II]) for diagnosing and monitoring hepatocellular carcinoma and other indications.
28. *Early*CDT-Lung test.
29. EGFR gene expression analysis for transitional (urothelial) cell cancer
30. EGFRVIII for glioblastoma multiforme
31. EML4-ALK as a diagnostic tool for stage IV non-small-cell lung cancer.
32. Estrogen and progesterone receptors when used alone to assign a member with breast

cancer to prognostic groupings since they are relatively weak predictors of long-term relapse and breast cancer related mortality rates.

33. Excision repair cross-complementation group 1 protein (ERCC1) for persons with NSCLC, colon or with gastric cancer who are being considered for treatment with platinum-based chemotherapy, and other indications.
34. FoundationOne and FoundationOne Heme
35. Gene hypermethylation for prostate cancer
36. GeneKey (GeneKey Corp., Boston, MA)
37. GeneSearch Breast Lymph Node (BLN) assay.
38. Glutathione-S-transferase P1 (GSTP1) for screening, detection and management of prostate cancer.
39. HE4 for ovarian cancer and other indications.
40. HERmark testing for breast cancer and other indications.
41. Insight DX Breast Cancer Profile
42. Mammaprint.
43. Mammostrat
44. Microarray-based gene expression profile testing using the MyPRS test for multiple myeloma.
45. Micro-RNAs (miRNAs) miRview mets and miRview mets2 (Rosetta Genomics Laboratories, Philadelphia, PA; Rosetta Genomics Ltd., Rehovot, Israel)
46. Mirinform Thyroid Cancer Test
47. Mucin 4 expression as a predictor of survival in colorectal cancer.
48. My Prognostic Risk Signature (MyPRS) (Signal Genetics LLC, New York, NY)
49. NRAS mutation for selecting persons with metastatic colorectal cancer who may benefit from (i) EGFR-targeted monoclonal antibody therapies cetuximab and panitumumab or (ii) anti-VEGF antibody bevacizumab; to predict disease prognosis and select persons with melanoma who may benefit from tyrosine kinase inhibitor therapies, and other indications.
50. OVA1 test.
51. OvaCheck test
52. Ovanext Next-Gen Cancer Panel.
53. OvaSure.
54. OncInsights (Intervention Insights, Grand Rapids, MI)
55. Panexia test
56. Pathwork Tissue of Origin test.
57. Phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide gene (PIK3CA) for predicting disease prognosis and selecting individuals with metastatic colorectal cancer who are being considered for treatment with EGFR antagonists cetuximab and panitumumab, and other indications.
58. PreOvar test for the KRAS-variant to determine ovarian cancer risk.
59. Previstage GCC for colorectal cancer
60. Prolaris for prostate cancer
61. ProOnc TumorSourceDx test (Prometheus Laboratories, San Diego, CA) to identify tissue or origin for metastatic tumor.
62. Prostate core mitotic test
63. Prostate Px and Post-Op Px for predicting recurence of prostate cancer.
64. Proveri prostate cancer assay (PPCA)
65. PTEN gene expression for non-small cell lung cancer
66. Ras oncogenes (except KRAS and BRAF).
67. ResponseDx Colon
68. Ribonucleotide reductase subunit M1 (RRM1) for persons with NSCLC who are being considered for treatment with gemcitabine-based chemotherapy, and other indications.
69. ROS1 re-arrangements for persons with lung cancer who may benefit from crizotinib therapy, and other indications.
70. Rotterdam Signature 76-gene panel.

71. Serum amyloid A as a biomarker for endometrial endometrioid carcinoma to monitor disease recurrence and rtargetesponse to therapy.
72. Single nucleotide polymorphisms for breast cancer (Oncovue, Brevagen)
73. TargetPrint gene expression test for evaluation of estrogen receptor, progesterone receptor, and HER2receptor status in breast cancer.
74. The 41-gene signature assay
75. Theros Breast Cancer Index.
76. Theros CancerType ID (bioTheranostics Inc., San Diego, CA)
77. Thymidylate synthase..
78. TMPRSS fusion genes for prostate cancer.
79. Topographic genotyping (PathFinderTG).
80. Total (whole) gene sequencing for cancer.
81. UroCor cytology panels (DD23 and P53) for bladder cancer
82. Vascular Endothelial Growth Factor (VEGF).
83. Vascular endothelial growth factor receptor 2 (VEGFR2) expression for identifying persons with colorectal cancer that is likely to respond to VEGF inhibition, and other indications.
84. Veristrat.
85. WT1 mutation, RUNX1 mutation, MLL-PTD, IDH1 mutation, IDH2 R172, IDH2 codon 140 mutation.
86. Any of the following circulating tumor markers also is considered experimental and investigational for screening asymptomatic subjects for cancer, diagnosis, staging, routine surveillance of cancer and monitoring the response to treatment:

a2-PAG	CA-SCC	MAM-6	TAG12
AMACR	Cathepsin-D, Cathepsin-L	Motility-related protein (MRP)	TAG72
Bcl-2	Cyclin E (fragments or whole length)	Multidrug resistance glycoprotein (Mdr1)	TAG72.3
BCM	DU-PAN-2	Murine double minute 2 (MDM2)	TAG72.5
CA195	Early prostate cancer antigen (EPCA)	NSE	TATI
CA242	Guanylyl cyclase C (Previstage GCC molecular test)	p53 (TP53)	Thrombospondin-1 (THBS-1)
CA50	Hepsin	PCA3 (DD3) / UpM3	Thymosin B15
CA549	Human kallikrein 2 (HK2)	PNA/ELLA	TNF-a
CA72-4	LASA	Prostate stem cell antigen (PSCA)	Topoisomerase II Alpha (TOP2A)

CAM17-1	LPA	SCC	TPA
CAM26	M 26	SLEX	Thymosin B15
CAM29	M 29	SPAN-1	Nuclear Matrix Protein 66 (NMP66)
CAR-3	MSA	SLX	Anti-malignin antibody screen (AMAS) test
CYFRA21-1	MCA	ST-439	

Background

Tumor markers are substances normally produced in low quantities by cells in the body. Detection of a higher-than-normal serum level by radioimmunoassay or immunohistochemical techniques usually indicates the presence of a certain type of cancer. Currently, the main use of tumor mfarkers is to assess a cancer's response to treatment and to check for recurrence. In some types of cancer, tumor marker levels may reflect the extent or stage of the disease and can be useful in predicting how well the disease will respond to treatment. A decrease or return to normal in the level of a tumor marker may indicate that the cancer has responded favorably to therapy. If the tumor marker level rises, it may indicate that the cancer is spreading. Finally, measurements of tumor marker levels may be used after treatment has ended as a part of follow-up care to check for recurrence.

However, in many cases the literature states that measurements of tumor marker levels alone are insufficient to diagnose cancer for the following reasons: (1) tumor marker levels can be elevated in people with benign conditions; (2) tumor marker levels are not elevated in every person with cancer, especially in the early stages of the disease; and (3) many tumor markers are not specific to a particular type of cancer; and (4) the level of a tumor marker can be elevated by more than one type of cancer.

Elevated levels of Prostate-Specific Antigen (PSA) may be found in the blood of men with benign prostate conditions, such as prostatitis and benign prostatic hyperplasia (BPH), or with a malignant growth in the prostate. While PSA does not allow distinction between benign prostate conditions and cancer, an elevated PSA level may indicate that other tests are necessary to determine whether cancer is present. PSA levels have been shown to be useful in monitoring the effectiveness of prostate cancer treatment, and in checking for recurrence after treatment has ended. Use of PSA for screening remains very controversial. Although researchers are in the process of studying the value of PSA along with digital rectal exams for routine screening of men ages 55 to 74 for prostate cancer; and the literature does not show at this time whether using PSA to screen for prostate cancer actually does reduce the number of deaths caused by this cancer. The American Cancer Society recommends annual screening with PSA and digital rectal exam for African American men and men with familial tendency age 40 or older and all men age 50 or older.

In a review on biomarkers for prostate cancer detection, Parekh, et al. (2007) stated that prostate stem cell antigen, alpha-methyl coenzyme-A racemase, PCA3, early prostate cancer antigen, hepsin and human kallikrein 2 are promising markers that are currently undergoing validation. An assessment of PCA3 prepared for the Agency for Healthcare Research and Quality (2013) concluded: "For diagnostic accuracy, there was a low strength of evidence that PCA3 had better diagnostic accuracy for positive biopsy results than tPSA elevations, but insufficient evidence that this led to improved intermediate or long-term health outcomes. For all other settings, comparators, and outcomes, there was insufficient evidence."

Hutchinson et al (2005) stated that in tissue-based assays, thymosin beta15 (B15) has been shown to correlate with prostate cancer and with recurrence of malignancy. To be clinically effective, it must be shown that thymosin B15 is released by the tumor into body fluids in detectable concentrations. These researchers developed a quantitative assay that can measure clinically relevant levels of thymosin B15 in human urine. Sixteen antibodies were raised against recombinant thymosin B15 and/or peptide conjugates. One antibody, having stable characteristics over the wide range of pH and salt concentrations found in urine and minimal cross-reactivity with other beta thymosins, was used to develop a competitive enzyme-linked immunosorbent assay (ELISA). Urinary thymosin B15 concentration was determined for control groups; normal (n = 52), prostate intraepithelial neoplasia (PIN, n = 36), and patients with prostate cancer; untreated (n = 7) with subsequent biochemical failure, radiation therapy (n = 17) at risk of biochemical recurrence. The operating range of the competition ELISA fell between 2.5 and 625 ng/ml. Recoveries exceeded 75%, and the intra- and inter-assay coefficients of variability were 3.3% and 12.9%, respectively. No cross-reactivity with other urine proteins was observed. A stable thymosin B15 signal was recovered from urine specimens stored at -20 degrees C for up to 1 year. At a threshold of 40 (ng/dl)/microg protein/mg creatinine), the assay had a sensitivity of 58% and a specificity of 94%. Relative to the control groups, thymosin B15 levels were greater than this threshold in a significant fraction of patients with prostate cancer ($p < 0.001$), including 5 of the 7 patients who later experienced PSA recurrence. The authors concluded that an ELISA that is able to detect thymosin B15 at clinically relevant concentrations in urine from patients with prostate cancer has been established. They noted that the assay will provide a tool for future clinical studies to validate urinary thymosin B15 as a predictive marker for recurrent prostate cancer.

Carcinoembryonic antigen (CEA) is a normal cell product that is over-expressed by adenocarcinomas, primarily of the colon, rectum, breast, and lung. It is normally found in small amounts in the blood of most healthy people, but may become elevated in people who have cancer or some benign conditions. According to the available literature, the primary use of CEA is in monitoring colorectal cancer, especially when the disease has metastasized. CEA is also used after treatment to check for recurrence of colorectal cancer. However, the literature indicates a wide variety of other cancers can produce elevated levels of this tumor marker, including melanoma; lymphoma; and cancers of the breast, lung, pancreas, stomach, cervix, bladder, kidney, thyroid, liver, and ovary. Elevated CEA levels can also occur in patients with non-cancerous conditions, including inflammatory bowel disease, pancreatitis, and liver disease.

The American Society of Clinical Oncology (ASCO)'s update of recommendations for the use of tumor markers in gastrointestinal cancer (Gershon, et al., 2006) stated that post-operative CEA levels should be performed every 3 months for stage II and III disease for at least 3 years if the patient is a potential candidate for surgery or chemotherapy of metastatic disease.

The Gynecologic Cancer Foundation, the Society of Gynecologic Oncologists, and the American Cancer Society have issued a consensus statement to promote early detection of ovarian cancer, which recommends that women who have symptoms, including bloating, pelvic or abdominal pain, difficulty eating or feeling full quickly, and urinary frequency and urgency, are urged to see a gynecologist if symptoms are new and persist for more than three weeks (ACS, 2007; SGO, 2007). Ovarian cancer is among the deadliest types of cancer because diagnosis usually comes very late, after the cancer has spread. If the cancer is found and surgically removed before it spreads outside the ovary, the five year survival rate is 93%. However, only 19% of cases are

detected early enough for that kind of successful intervention. It is estimated that 22,430 new cases and 15,280 deaths will be reported in 2007 (ACS, 2007). The consensus statement recommendations are based on studies that show the above symptoms appeared in women with ovarian cancer more than in other women (Goff, et al., 2004; Daly & Ozols, 2004). The recommendations acknowledge that there is not consensus on what physicians should do when patients present with these symptoms. According to a consensus statement issued by the Gynecologic Cancer Foundation, pelvic and rectal examination in women with the symptoms is one first step. If there is a suspicion of cancer, the next step may be a transvaginal ultrasound to check the ovaries for abnormal growths, enlargement, or telltale pockets of fluid that can indicate cancer. Testing for CA-125 levels should also be considered.

There is no evidence available that measurement of CA-125 can be effectively used for widespread screening to reduce mortality from ovarian cancer, nor that the use of this test would result in decreased rather than increased morbidity and mortality. According to the available literature, not all women with elevated CA 125 levels have ovarian cancer. CA 125 levels may also be elevated by cancers of the uterus, cervix, pancreas, liver, colon, breast, lung, and digestive tract. Non-cancerous conditions that can cause elevated CA 125 levels include endometriosis, pelvic inflammatory disease, peritonitis, pancreatitis, liver disease, and any condition that inflames the pleura. Menstruation and pregnancy can also cause an increase in CA 125. However, according to the available literature, changes in CA 125 levels can be effectively used in the management of treatment for ovarian cancer. In women with ovarian cancer being treated with chemotherapy, the literature suggests a falling CA 125 level generally indicates that the cancer is responding to treatment and increased survival is expected. Increasing CA 125 levels during or after treatment, on the other hand, may suggest that the cancer is not responding to therapy or that residual cancer remains. According to the available literature, failure of the CA 125 level to return to normal after three cycles of chemotherapy indicates residual tumor, early treatment failure and decreased survival. Under accepted guidelines, CA 125 levels can also be used to monitor patients for recurrence of ovarian cancer. Although an elevated CA 125 level is highly correlated with the presence of ovarian cancer, the literature suggests a normal value does not exclude residual or recurrent disease.

Aetna's preventive services guidelines are based on the recommendations of leading primary care medical professional organizations and federal public health agencies. None of these organizations recommend routine screening of average-risk, asymptomatic women with serum CA-125 levels for ovarian cancer. These organizations have concluded that serum CA-125 levels are not sufficiently sensitive or specific for use as a screening test for ovarian cancer, and that the harms of such screening outweigh the benefits.

The American College of Obstetricians and Gynecologists (2002) has stated that "[u]nfortunately, there is no screening test for ovarian cancer that has proved effective in screening low-risk asymptomatic women. Measurement of CA 125 levels and completion of pelvic ultrasonography (both abdominal and transvaginal) have been the two tests most thoroughly evaluated.... Data suggest that currently available tests do not appear to be beneficial for screening low-risk, asymptomatic women because their sensitivity, specificity, positive predictive value, and negative predictive value have all been modest at best. Because of the low incidence of disease, reported to be approximately one case per 2,500 women per year, it has been estimated that a test with even 100% sensitivity and 99% specificity would have a positive predictive value of only 4.8%, meaning 20 of 21 women undergoing surgery would not have primary ovarian cancer. Unfortunately, no test available approaches this level of sensitivity or specificity."

The National Cancer Institute (2004) has stated: "There is insufficient evidence to establish that screening for ovarian cancer with serum markers such as CA 125 levels, transvaginal ultrasound, or pelvic examinations would result in a decrease in mortality from ovarian cancer. A serious potential harm is the false-positive test result, which may lead to anxiety and invasive diagnostic procedures. There is good evidence that screening for ovarian cancer with the tests above would result in more diagnostic laparoscopies and laparotomies than new ovarian cancers found. Unnecessary oophorectomies may also result."

The U.S. Preventive Services Task Force (2004) recommends against routine screening with serum CA-125 level for ovarian cancer. The Task Force concluded that the potential harms of such screening outweigh the potential benefits.

A variety of other tumor markers have been investigated for early detection of ovarian cancer as well as different combinations of tumor markers complementary to CA 125 that could potentially offer greater sensitivity and specificity than CA 125 alone. Preliminary studies on HE4 (human epididymis protein 4), a relatively new marker for ovarian cancer, reported similar sensitivity to CA 125 when comparing ovarian cancer cases to healthy controls, and a higher sensitivity when comparing ovarian cancer cases to benign gynecologic disease (Hellstrom, et al., 2003 & 2008; Moore, et al., 2008;) However, an assessment on genomic tests for ovarian cancer prepared by Duke University for the Agency for Healthcare Research and Quality (AHRQ, 2006) stated, "Although research remains promising, adaptation of genomic tests into clinical practice must await appropriately designed and powered studies in relevant clinical settings." Further studies are needed to determine if HE4 significantly adds to the sensitivity of CA 125 while maintaining a high specificity.

CA 15-3 is a serum cancer antigen that has been used in the management of patients with breast cancer. According to the available literature, its low detection rate in early stage disease indicates that CA 15-3 cannot be used to screen or diagnose patients with breast cancer. It has been widely used to monitor the effectiveness of treatment for metastatic cancer. Cancers of the ovary, lung, and prostate may also raise CA 15-3 levels. The literature indicates elevated levels of CA 15-3 may be associated with non-cancerous conditions, such as benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis.

Similar to the CA 15-3 antigen, CA 27-29 is found in the blood of most breast cancer patients. The literature indicates CA 27-29 levels may be used in conjunction with other procedures (such as mammograms and measurements of other tumor marker levels) to check for recurrence in women previously treated for stage II and stage III breast cancer. CA 27-29 levels can also be elevated by cancers of the colon, stomach, kidney, lung, ovary, pancreas, uterus, and liver. First trimester pregnancy, endometriosis, ovarian cysts, benign breast disease, kidney disease, and liver disease are non-cancerous conditions that can also elevate CA 27-29 levels.

Initially found in colorectal cancer patients, CA 19-9 has also been identified in patients with pancreatic, stomach, hepatocellular cancer, and bile duct cancer. In those who have pancreatic cancer, the literature indicates higher levels of CA 19-9 tend to be associated with more advanced disease. Although the sensitivity of the CA 19-9 level in patients with pancreatic cancer is relatively high, the specificity is lowered by elevations that occur in patients with benign pancreatic or liver disease. Non-cancerous conditions that may elevate CA 19-9 levels include gallstones, pancreatitis, cirrhosis of the liver, and cholecystitis. Although excellent correlations have been reported between CA 19-9 measurements and relapse in patients with pancreatic cancer who are followed after surgical resection, no patient has been salvaged by the earlier diagnosis of relapse, a fact that reflects the lack of effective therapy.

Guidelines from the National Comprehensive Cancer Network (NCCN, 2010) state that measurement of CA 19-9 should be considered in evaluating patients with intrahepatic or extrahepatic cholangiocarcinoma and gallbladder cancer. The guidelines note that CA 19-9 is often elevated in persons with cholangiocarcinoma or gallbladder cancer, although this marker is not specific for these cancers. Nehls, et al. (2004) considered CA19-9 as one of the several new potential tumor markers for the diagnosis of cholangiocarcinoma. Levy, et al. (2005) aimed to characterize the test properties of CA 19-9 and of a change in CA 19-9 over time in predicting cholangiocarcinoma in patients with primary sclerosing cholangitis. Charts of 208 patients were reviewed. Fourteen patients had cholangiocarcinoma. Median CA 19-9 was higher with cholangiocarcinoma (15 versus 290 U/ml, $p < 0.0001$). A cutoff of 129 U/ml provided: sensitivity 78.6%, specificity 98.5%, adjusted positive predictive value 56.6% and negative predictive value 99.4%. The median change over time was 664 U/ml in cholangiocarcinoma

compared to 6.7 U/ml in primary sclerosing cholangitis alone ($p < 0.0001$). A cutoff of 63.2 U/ml for change in CA 19-9 provided: sensitivity 90%, specificity 98% and positive predictive value 42%.

CA 19-9 is produced by adenocarcinomas of the pancreas, stomach, gall-bladder, colon, ovary, and lung, and it is shed into the circulation. Although numerous studies have addressed the potential utility of CA 19-9 in adenocarcinoma of the colon and rectum, the sensitivity of CA 19-9 was always less than that of the CEA test for all stages of disease. Its use for screening asymptomatic populations has been hampered by a false-positive rate of 15% to 30% in patients with non-neoplastic diseases of the pancreas, liver, and biliary tract. Only a few studies have addressed the use of CA 19-9 in monitoring patients' post-primary therapy. Significant postsurgical decreases are observed for CA 19-9, but these decreases have not been correlated with survival or disease-free interval. In monitoring response to treatment, decreases in CEA have been found to more accurately reflect response to therapy than did decreases of CA 19-9. Progressive increases of the marker may signal disease progression in 25% of the patients who express the CA 19-9 marker, but this monitoring provides only a minimal lead time of 1 to 3 months. Monitoring with CA 19-9 has not been shown to improve the management of patients with colorectal cancer. The serum CA 19-9 level does not add significant information to that provided by CEA, which is currently regarded as the marker of choice for this neoplasm.

Sinakos and colleagues (2011) evaluated the long-term outcomes in Mayo Clinic patients presenting with primary sclerosing cholangitis (PSC) between 2000 and 2010 (n= 73) for incidence of cholagiocarcinoma (CCA). The results showed initial levels of CA 19-9 in patients without CCA were significantly lower than those from patients with CCA ($p < 0.0001$). No factors known to affect CA 19-9 levels were identified in 33% of the patients without CCA; endoscopic treatment and recurrent bacterial cholangitis were associated with levels of CA 19-9 in 26% and 22% of these patients, respectively.

Juntermanns (2011) prospectively analyzed a bile duct tumor database and retrieved records of 238 patinets who underwent surgery between 1999 and 2008. Their findings included that pre-operative CA19-9 serum levels did not show a statically reliable differentiation between benign or malignant dignity. The authors concluded that current diagnostics cannot differentiate malignant from benign tumor masses in the hepatic hilum with required reliability. The authors further concluded that administration of CIK cells, thymus factor, IL-2 and IFN-alpha after AHSCT could improve the immunologic function of patients, and TH1/TH2 ratio may virtually reflect the immune status of patients, but that more information is required to make prognostic assessments of immune reconstruction and the long-term survival rate.

Sarbia et al (1993) investigated 69 adenocarcinomas of the esophagogastric junction and found high rates of antigen expression were found for the “intestinal” markers CA 19-9 (between 55.5% and 100%) and BW 494 (between 42.9 and 86.7%). The authors concluded that these data, in combination with CK-20 expression, PGII, and 2B5 indicate that the distribution of adenocarcinomas with gastric and.or intestinal differentiation at the esophagogastric junction forms a continuum with out clear-cut borders. This study has not been replicated and NCCN guidelines for Esophageal and Esophagogastric Junction Cancers does not include recommendations for CA 19-9 testing for these indications (NCCN, 2011).

The American Society of Clinical Oncology (ASCO)'s update of recommendations for the use of tumor markers in gastrointestinal cancer (Gershon, et al., 2006) stated that for pancreatic cancer, CA 19-9 can be measured every 1 to 3 months for patients with locally advanced or metastatic disease receiving active therapy.

Mucinous carcinoma of the appendix is a rare entity most commonly associated with primary tumors of the appendix and colon, and for which spread is generally confined to the abdominal cavity (Andreopoulou et al, 2007). Imaging assessment of these mucinous lesions is difficult, and recent studies have explored the use of tumor markers as clinical tools in evaluation of mucinous carcinoma of the appendix.

Carmignani et al (2004) evaluated patients with synchronous systemic and intraperitoneal dissemination of appendix cancer greated with cytoreductive surgery and perioperative regional chemotherapy with a mean follow up time of 42.6 months. Results of this study indicated that patients with elevated CEA and CA 19-9 levels had a shorter median survival time (p=0.0083 and p = 0.0193, respectively). In a subsequent study, Carmingnani et al (2004) prospectively recorded tumor markers CEA and CA19-9 within 1 week prior to definitive treatment. The investigators found CEA elevated in 56% of 532 patients and CA19-9 elevated in 67.1% of those patients. They reported that "although the absolute level of tumor marker did not correlate with prognosis, a normal value indicated an improved survival." Their findings included an elevated CEA in 35.2% of 110 patients determined to have recurrent disease and an elevated CA 19-9 in 62.9%, while 68.2% of patients had at least one of the tumor markers elevated.

Current guidelines indicate that for liver transplantation for primary sclerosing cholangitis, stringent efforts should be made to detect superadded cholangiocarcinoma, including measurement of CA 19-9 (Devlin & O'Grady, 1999).

Human chorionic gonadotropin (HCG) is normally produced in increasing quantities by the placenta during pregnancy. Accepted guidelines provide that HCG levels can be used to screen for choriocarcinoma in women who are at high risk for the disease, and to monitor the treatment of trophoblastic disease. The literature states that elevated HCG levels may also indicate the presence of cancers of the testis, ovary, liver, stomach, pancreas, and lung.

Accepted guidelines provide that alpha fetoprotein (AFP) and b-HCG measurements are valuable for determining prognosis and monitoring therapy in patients with non-seminomatous germ cell cancer. Because of the low incidence of elevated AFP and b-HCG levels in early-stage cancer, the literature suggests these markers have no value in screening for testicular cancer. However, the specificity of these markers is such that when determined simultaneously, at least one marker will be positive in 85% of patients with active cancer. The value of AFP and b-HCG as markers is enhanced by a low frequency of false-positive results and by the chemoresponsiveness of testicular cancer. The literature states that only rarely do patients with other types of cancer have elevated levels of AFP. Non-cancerous conditions that can cause elevated AFP levels include benign liver conditions, such as cirrhosis or hepatitis, ataxia telangiectasia, Wiscott-Aldrich syndrome, and pregnancy.

Alpha-fetoprotein is normally produced by a developing fetus. Alpha fetoprotein levels begin to decrease soon after birth and are usually undetectable in the blood of healthy adults, except during pregnancy. According to accepted guidelines, an elevated level of AFP strongly suggests the presence of either primary liver cancer or germ cell cancer of the ovary or testicle. As AFP is an established test for the diagnosis and monitoring of hepatoma, it is used as a screening tool to rule out the presence of a liver neoplasm before considering liver transplantation. This is especially pertinent in cases (e.g., cirrhosis) where there is an increased risk of developing a primary liver tumor.

The estrogen receptor and progesterone receptor are intracellular receptors that are measured directly in tumor tissue. These receptors are polypeptides that bind their respective hormones, translocate to the nucleus, and induce specific gene expression. Most oncologists have used the estrogen receptor and also the progesterone receptor not only to predict the probability of response to hormonal therapy at the time of metastatic disease, but also to predict the likelihood of recurrent disease, and to predict the need for adjuvant hormonal therapy or chemotherapy. Although these latter uses for estrogen and progesterone receptors are commonly accepted by most oncologists, the data on which these conclusions are based are quite controversial.

Neuron-specific enolase (NSE) has been detected in patients with neuroblastoma, small cell lung cancer, Wilms' tumor, melanoma, and cancers of the thyroid, kidney, testicle, and pancreas. However, studies of NSE as a tumor marker have concentrated primarily on patients with neuroblastoma and small cell lung cancer. According to the available literature, measurement of

NSE level in patients with these diseases cannot be correlated to the extent of the disease, the patient's prognosis, or the patient's response to treatment because of the poor sensitivity of this marker.

LASA is a complex marker that measures the amount of sialic acid in serum and can be elevated in serum from patients with any number of different neoplasms. This serum cancer marker has not been widely accepted for use in the detection or prognosis of colorectal carcinoma. There is no practical information concerning outcome and the use of LASA in the medical literature. Although several articles describe the use of LASA in the diagnosis of colorectal cancer and its association with tumor-node-metastasis (TNM) stage, it has been shown that patients with colorectal polyps and colorectal carcinoma both have elevated LASA levels, and that the levels returned to baseline after removal of either polyps or carcinomas.

p53 is a tumor suppressor gene on the short arm of chromosome 17 that encodes a protein that is important in the regulation of cell division. Although the full role of p53 in the normal and neoplastic cell is unknown, there is evidence that the gene product is important in preventing the division of cells containing damaged DNA. p53 gene deletion or mutation is a frequent event along with other molecular abnormalities in colorectal carcinogenesis. The literature on p53 abnormality and prognosis in colorectal cancer suffers from a paucity of reported data and the use of a variety of techniques in assay and statistical analysis in the small numbers of cases analyzed. For these reasons, the literature generally does not recommend p53 analysis as a routine approach to assisting in the management of patients with colorectal cancer.

The ras proto-oncogenes are normal cellular components, which are thought to be important for transduction of signals required for proliferation and differentiation. The ras oncogene family has 3 members: H-ras, K-ras, and N-ras. Ras gene mutations can be found in a variety of tumor types, although the incidence varies greatly. The highest incidences are found in adenocarcinomas of the pancreas (90%), colon (50%), and lung (30%); thyroid tumors (50%), and myeloid leukemia (30%).

Investigators have established an association between some genotypes of K-ras (KRAS) oncogenes and response to treatment with cetuximab or panitumumab (Lievre et al, 2006 and 2008; Di Fiore et al, 2007; Gonçalves et al, 2008; De Roock et al, 2008). Patients whose tumors express specific forms of the KRAS gene exhibit considerably decreased responses to cetuximab and panitumumab. It has been theorized that cetuximab and panitumumab do not target epidermal growth factor receptor (EGFR) associated with these specific KRAS mutations and thus are unable to block their activation. It has been suggested that KRAS genotype be considered as a selection factor for cancer patients who are candidates for treatment with cetuximab or panitumumab.

Karapetis and colleagues (2008) stated that treatment with cetuximab improves overall survival (OS) and progression-free survival (PFS) and preserves the quality of life in patients with colorectal cancer that has not responded to chemotherapy. The mutation status of the K-ras gene in the tumor may affect the response to cetuximab and have treatment-independent prognostic value. These investigators analyzed tumor samples, obtained from 394 of 572 patients (68.9 %) with colorectal cancer who were randomly assigned to receive cetuximab plus best supportive care or best supportive care alone, to look for activating mutations in exon 2 of the K-ras gene. They evaluated if the mutation status of the K-ras gene was associated with survival in the cetuximab and supportive-care groups. Of the tumors evaluated for K-ras mutations, 42.3 % had at least one mutation in exon 2 of the gene. The effectiveness of cetuximab was significantly associated with K-ras mutation status ($p = 0.01$ and $p < 0.001$ for the interaction of K-ras mutation status with OS and PFS, respectively). In patients with wild-type K-ras tumors, treatment with cetuximab as compared with supportive care alone significantly improved OS (median of 9.5 versus 4.8 months; hazard ratio for death, 0.55; 95 % confidence interval [CI], 0.41 to 0.74; $p < 0.001$) and PFS (median of 3.7 months versus 1.9 months; hazard ratio for progression or death, 0.40; 95 % CI, 0.30 to 0.54; $p < 0.001$). Among patients with mutated K-ras tumors, there was no significant difference between those who were treated with cetuximab

and those who received supportive care alone with respect to OS (hazard ratio, 0.98; p = 0.89) or PFS (hazard ratio, 0.99; p = 0.96). In the group of patients receiving best supportive care alone, the mutation status of the K-ras gene was not significantly associated with OS (hazard ratio for death, 1.01; p = 0.97). The authors concluded that patients with a colorectal tumor bearing mutated K-ras did not benefit from cetuximab, whereas patients with a tumor bearing wild-type K-ras did benefit from cetuximab. The mutation status of the K-ras gene had no influence on survival among patients treated with best supportive care alone.

The ASCO's provisional clinical opinion on testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-EGFR monoclonal antibody therapy (Allegra et al, 2009) stated that based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment.

The KRAS oncogene mutation tests are intended to aid in the formulation of treatment decisions for patients who may be candidates for treatment of metastatic epithelial cancers with anti-EGFR therapies such as cetuximab or panitumumab. Several tests for KRAS mutation are currently available in the United States; however, at this time, no KRAS genotype test kits have been approved by the FDA.

At the 2008 Annual Meeting of the American Society of Clinical Oncology (ASCO), data on 540 patients with metastatic colorectal cancer in the randomized, phase III CRYSTAL trial were presented. Among 192 patients with KRAS mutations, there was no improvement in overall responses or PFS from the addition of cetuximab to standard chemotherapy. In the patients with normal KRAS, the 1-year PFS rate was 43% for patients receiving cetuximab versus 25% for those receiving only standard chemotherapy, and the overall response rate was 59% versus 43%, respectively (van Cutsem, 2008). Also at the 2008 ASCO meeting, data from 233 metastatic colorectal cancer patients were presented that confirmed the correlation of KRAS status with patient response to anti-EGFR therapy. No benefit was found after addition of cetuximab to standard chemotherapy with FOLFOX (the combination of fluorouracil, leucovorin, and oxaliplatin) in patients with a mutated KRAS; however, addition of cetuximab to FOLFOX increased both response rate and PFS in patients with a wild-type (i.e., un-mutated) KRAS gene (Bokemeyer, 2008). Response to panitumumab was correlated to KRAS status in a published phase III trial. A total of 427 patients with metastatic colorectal cancer received either panitumumab or best supportive care. Panitumumab exhibited a 17% response rate among patients with normal KRAS, but 0% response among patients with KRAS mutations (Amado, 2008).

A meta-analysis of results from 8 studies involving 817 patients with colorectal cancer found that the presence of KRAS mutation predicted lack of response to treatment with anti-EGFR monoclonal antibodies (e.g., panitumumab or cetuximab), whether as stand-alone therapy or in combination with chemotherapy (Linardou, et al., 2008). This analysis also provided empirical evidence that k-RAS mutations are highly specific negative predictors of response (de-novo resistance) to single-agent EGFR tyrosine-kinase inhibitors in advanced non-small cell lung cancer; and similarly to anti-EGFR monoclonal antibodies alone or in combination with chemotherapy in patients with metastatic colorectal cancer.

The Blue Cross and Blue Shield Association (BCBSA, 2008) Technology Evaluation Center Medical Advisory Panel concluded that use of KRAS mutation analysis meets TEC criteria to predict non-response to anti-EGFR monoclonal antibodies cetuximab and panitumumab to treat metastatic colorectal cancer. The TEC assessment found that the evidence is sufficient to conclude that patients with mutated KRAS tumors in the setting of metastatic colorectal cancer do not respond to anti-EGFR monoclonal antibody therapy. The assessment explained that the data show that the clinical benefit of using EGFR inhibitors in treating metastatic colorectal cancer, either as monotherapy or in combination with other treatment regimens, is not seen in

patients with KRAS-mutated tumors. The assessment found: "This data supports knowing a patient's tumor mutation status before consideration of use of an EGFR inhibitor in the treatment regimen. Identifying patients whose tumors express mutated KRAS will avoid exposing patients to ineffective drugs, avoid exposure to unnecessary drug toxicities, and expedite the use of the best available alternative therapy."

Colorectal cancer guidelines from the National Comprehensive Cancer Network (NCCN, 2010) recommend consideration of reflex BRAF testing in patients with wild type KRAS. The NCCN guidelines explain that several small studies suggest that patients with wild-type KRAS and a BRAF mutation are unlikely to respond to anti-EGFR therapies such as cetuximab and panitumumab. The guidelines explain that patients with a known BRAF mutation are unlikely to respond to anti-EGFR antibodies, although the data are somewhat inconsistent. Studies demonstrate that in patients with metastatic colorectal cancer, about 8 percent have mutations in the BRAF gene. Testing for the BRAF V600E mutation is performed by PCR amplification and direct DNA sequence analysis.

In the United States, bladder malignancy is the 4th commonest cancer in men and the 8th commonest in women. Patients usually present with urinary tract symptoms (e.g., gross or microscopic hematuria or irritative voiding symptoms such as frequency, dysuria, and urgency). Evaluations of these patients usually entail voided-urine cytology, cystoscopy, and upper urinary tract imaging such as intravenous pyelography, renal sonography, or retrograde pyelography. Most newly diagnosed bladder cancers are superficial (i.e., not invading beyond the lamina propria on histological examination), and are known as transitional cell carcinoma (TCC). These superficial bladder cancers are usually managed by transurethral resection. However, the literature shows that approximately 50 - 75% of treated TCC recur. Furthermore, 10 – 15% of TCC progress to muscle-invasive bladder cancer. According to the literature, the prevalence of recurrence after initial treatment as well as the natural history of TCC necessitates long-term follow-up. Following treatment, accepted guidelines provide that patients who have previously been diagnosed with TCC should usually undergo urine cytology/cystoscopy every 3 months in the 1st year, every 6 months in the 2nd year, and once-yearly afterwards.

Currently, urine cytology with confirmatory cystoscopy represents the cornerstone for the identification of bladder tumors. However, the subjectivity and low sensitivity of cytology led to the development of several urine-based tests as adjuncts to cytology/cystoscopy for the diagnosis and follow-up of patients with TCC. These tests include the BTA Stat test (Bard Diagnostic, Redmond, WA), the NMP22 test (Matritech, Newton, MA), the Aura-Tek FDP test (PerImmune, Rockville, MD), and the Vysis UroVysion FISH Test (Vysis, Inc., Downers Grove, IL). They are usually objective, qualitative (BTA Stat and Aura-Tek FDP), or quantitative (NMP22, UroVysion), and have higher sensitivity than cytology, but some have lower specificity. So far, no single bladder tumor marker has emerged as the generally accepted test of choice, and none has been established as a screening tool for bladder malignancy.

Although urine cytology has been shown to be less accurate than urinary biomarker tests, familiarity with the method as well as ease of performance justify the continued routine use of the former by primary care physicians, especially in patients who have no history of bladder malignancy. The urine-based biomarker tests have been shown to be accurate in detecting low-grade bladder tumors. In particular, these tests may be of help in deciding the need for further diagnostic assessment of patients with a history of bladder cancer and negative results on urine cytology. For example, elevated levels of urinary bladder tumor markers in patients with a history of TCC may warrant earlier, rather than delayed, cystoscopic examination. On the other hand, consideration may be given to lengthening the intervals between cystoscopic investigations when values of these tumor markers are normal.

In summary, urine-based bladder tumor marker tests have been shown to be useful as an adjunct to urine cytology and cystoscopy in monitoring for recurrences of bladder cancer, but according to the available literature should not be used as a screening tool for bladder malignancy. The U.S. Preventive Services Task Force (USPSTF, 2004) has concluded that the potential harms of

screening for bladder cancer using available tests, such as microscopic urinalysis, urine dipstick, urine cytology, or such new tests as bladder tumor antigen (BTA) or nuclear matrix protein (NMP22) immunoassay, outweigh any potential benefits.

Svatek et al (2008) examined the role of urinary cathepsin B and L in the detection of bladder urothelial cell carcinoma. These investigators concluded that urinary cathepsin L is an independent predictor of bladder cancer presence and invasiveness in patients with a history of urothelial carcinoma of the bladder. They stated that further evaluation of this marker is necessary before its use as an adjunct to cystoscopy for urothelial carcinoma of the bladder.

Oncotype Dx (Genomic Health, Inc., Redwood City, CA) is a diagnostic laboratory-developed assay that quantifies the likelihood of breast cancer recurrence in women with newly diagnosed, stage I or II, node negative, estrogen receptor positive breast cancer, who will be treated with tamoxifen. The assay analyzes the expression of a panel of 21 genes, and is intended for use in conjunction with other conventional methods of breast cancer analysis. Together with staging, grading, and other tumor marker analyses, Oncotype Dx is intended to provide greater insight into the likelihood of systemic disease recurrence. Clinical studies have evaluated the prognostic significance of the Oncotype Dx multigene assay in breast cancer (Paik, et al., 2004; Esteva, et al., 2003).

There currently is a lack of evidence from prospective clinical studies of the impact of this test on the management of women with breast cancer demonstrating improvements in clinical outcomes, Bast & Hortobagyi (2004) commented that "[b]efore use of the recurrence score [from the Oncotype Dx multigene assay] is applied to general patient care, however, additional studies are needed." The National Cancer Institute is sponsoring a prospective, randomized controlled clinical study, the TAILORx study, using the Oncotype Dx assay to help identify a group of patients with a mid-range risk of recurrence to determine whether treating patients with hormonal therapy only is equivalent to treating them with hormonal therapy in combination with adjuvant chemotherapy.

However, there is indirect evidence of the clinical utility of the Oncotype Dx. Paik et al (2006) used banked tumor samples from previous clinical studies of tamoxifen and adjuvant chemotherapy in early breast cancer to assess the performance of the Oncotype Dx multigene assay in predicting response to adjuvant chemotherapy. These investigators examined tumor samples from subjects enrolled in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B20 trial to determine whether there is a correlation between the recurrence score (RS) determined by Oncotype Dx in tumor samples and subsequent response to adjuvant chemotherapy. A total of 651 patients were assessable (227 randomly assigned to tamoxifen and 424 randomly assigned to tamoxifen plus chemotherapy). The test for interaction between chemotherapy treatment and RS was statistically significant ($p = 0.038$). Patients with high-RS (RS greater than or equal to 31) tumors (ie, high risk of recurrence) had a large benefit from chemotherapy (relative risk, 0.26; 95% confidence interval 0.13 to 0.53; absolute decrease in 10-year distant recurrence rate: mean, 27.6%; standard error, 8.0%). Patients with low-RS (less than 18) tumors derived minimal, if any, benefit from chemotherapy treatment (relative risk, 1.31; 95% confidence interval, 0.46 to 3.78; absolute decrease in distant recurrence rate at 10 years: mean, -1.1%; standard error, 2.2%). The investigators found that patients with intermediate-RS tumors did not appear to have a large benefit, but the investigators concluded that the uncertainty in the estimate cannot exclude a clinically important benefit.

One limitation of the study by Paik et al (2006) is that the NASBP B20 trial was conducted before the advent of important advances in breast cancer chemotherapy, including the introduction of trastuzumab (Herceptin), which has been demonstrated to improve overall and disease-free survival in breast cancer patients with HER2 positive tumors. Current guidelines recommend the use of trastuzumab adjuvant chemotherapy in women with metastatic HER2 positive breast cancer, and women with HER2 positive nonmetastatic breast cancers 1 cm or more in diameter. Thus, the Oncotype Dx score would not influence the decision to use adjuvant trastuzumab in women with HER2 positive tumors 1 cm or more in diameter.

Commenting on an early report of this study by Paik et al, of the Oncotype Dx presented in abstract form, the BlueCross BlueShield Association Technology Evaluation Center assessment stated that “additional studies in different populations are needed to confirm whether risk prediction is sufficiently accurate for physicians and patients to choose with confidence whether to withhold adjuvant chemotherapy.”

The selection criteria for the TailorRx prospective trial of OncotypeDx state that candidates should have negative axillary nodes as determined by a sentinel lymph node biopsy and/or axillary dissection as defined by the American Joint Committee on Cancer 6th Edition Staging System (NCI, 2009). The AJCC 6th Edition criteria redefined isolated tumor cells as node negative (the prior version of the criteria, AJCC 5th Edition, classified isolated tumor cells as node positive). "Isolated tumor cells (single cells or cell deposits) will now be defined as tumor cell deposits no larger than 0.2 mm in diameter that may or may not (but usually do not) show histologic evidence of malignant activity. Pending further information, isolated tumor cells will be classified as node-negative, because it is believed that the unknown benefits of providing treatment for these small lesions would not outweigh the morbidity caused by the treatment itself." (Singletary, et al., 2002). However, the banked tumor samples used in the study by Paik, et al. (2006) to validate the OncotypeDx were classified based on AJCC 5th Ed. criteria. In addition, there is new evidence demonstrating that women with isolated tumor cells are at a significantly increased risk of breast cancer. Investigators from the Netherlands found an association between isolated tumor cells and micrometastases in regional lymph nodes and clinical outcome of breast cancer (de Boer, et al., 2009). These investigators identified all patients in the Netherlands who underwent a sentinel-node biopsy for breast cancer before 2006 and had breast cancer with favorable primary-tumor characteristics and isolated tumor cells or micrometastases in the regional lymph nodes. Patients with node-negative disease were randomly selected from the years 2000 and 2001. The primary end point was disease-free survival. The investigators identified 856 patients with node-negative disease who had not received systemic adjuvant therapy (the node-negative, no-adjuvant-therapy cohort), 856 patients with isolated tumor cells or micrometastases who had not received systemic adjuvant therapy (the node-positive, no-adjuvant-therapy cohort), and 995 patients with isolated tumor cells or micrometastases who had received such treatment (the node-positive, adjuvant-therapy cohort). The median follow-up was 5.1 years. The adjusted hazard ratio for disease events among patients with isolated tumor cells who did not receive systemic therapy, as compared with women with node-negative disease, was 1.50 (95% confidence interval [CI], 1.15 to 1.94); among patients with micrometastases, the adjusted hazard ratio was 1.56 (95% CI, 1.15 to 2.13). Among patients with isolated tumor cells or micrometastases, the adjusted hazard ratio was 0.57 (95% CI, 0.45 to 0.73) in the node-positive, adjuvant-therapy cohort, as compared with the node-positive, no-adjuvant-therapy cohort. The investigators concluded that isolated tumor cells or micrometastases in regional lymph nodes were associated with a reduced 5-year rate of disease-free survival among women with favorable early-stage breast cancer who did not receive adjuvant therapy. In patients with isolated tumor cells or micrometastases who received adjuvant therapy, disease-free survival was improved.

The Medical Advisory Panel of the BlueCross BlueShield Association Technology Evaluation Center (BCBCA, 2014) concluded that use of Oncotype DX to determine recurrence risk for deciding whether to undergo adjuvant chemotherapy in women with unilateral, nonfixed, hormone receptorâ€’positive, lymph nodeâ€’negative breast cancer who will receive hormonal therapy meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria.

The Oncotype Dx has also been promoted for use in women with node-positive, ER-positive breast cancer. An assessment by the BlueCross BlueShield Association (2010) concluded that it has not yet been demonstrated whether use of the Oncotype Dx for selecting adjuvant chemotherapy in patients with lymph-node-positive breast cancer improves health outcomes. The report explained that the evidence for not selecting chemotherapy for women with low RS values is based on low event rates and wide confidence intervals that include the possibility of benefit

from chemotherapy. Because the data allow for a possible benefit of chemotherapy in patients with low RS results, it is unknown if health outcomes would be improved, the same, or worse, if chemotherapy was withheld in these women. The report stated that, due to the lack of clear and sufficient information, there is a need for a second, confirmatory study. The report stated that the Fred Hutchinson Cancer Research Center will conduct a nationwide, NCI-sponsored, Phase III clinical trial to determine the predictive ability of the Oncotype Dx to identify which patients with lymph-node-positive breast cancer will benefit from chemotherapy.

Acceptance of 21-gene recurrence score assays as tools for clinical decision making in women or men with early stage breast cancer is controversial due to the lack of prospective validation studies, nevertheless, 2007 guidelines from an expert panel convened by ASCO on tumor markers in breast cancer concluded that multiparameter gene expression analysis (i.e., Oncotype Dx assay) can be used to predict the risk of recurrence in women with newly diagnosed, node-negative, ER-positive breast cancer. Although it is reasonable to consider the use of a 21-gene recurrence score assay in males, none of the data generated to date have been in men with breast cancer (Gradishar, 2010).

A 2009 abstract that looked at cases of male breast cancer (BC) with Oncotype Dx, concluded, "This large genomic study of male BC reveals a heterogeneous biology as measured by the standardized quantitative oncotype Dx breast cancer assay, similar to that observed in female BC. Some differences, which may reflect the differences in hormone biology between males and females, were noted and deserve further study." (Shak et al, 2009).

The Oncotype Dx has also been promoted for use in colorectal cancer. However, there is a lack of evidence establishing the clinical utility of this test in colorectal cancer.

The results of the Quick and Simple and Reliable Study (QUASAR) were published by Gray et al (2011). The purpose of the QUASAR study was develop quantitative gene expression assays to assess recurrence risk and benefits from chemotherapy in patients with stage II colon cancer. Recurrence score (RS) and treatment score (TS) were calculated from gene expression levels of 13 cancer-related genes and from five reference genes. The results of the study showed risk of recurrence to be significantly associated with RS (95% confidence interval, 1.11 to 1.74; p = 0.004). Recurrence risks at 3 years were 12%, 18%, and 22% for predefined low, intermediate, and high recurrence risk groups, respectively. Continuous RS was associated with risk of recurrence (p=0.006), but there was no trend for increased benefit from chemotherapy at higher TS (p=0.95). The continuous 12-gene RS has been validated in a prospective study for assessment of recurrence risk in patients with stage II colon cancer after surgery. RS was also found to provide prognostic value that complements T stage and mismatch repair.

In February 2007, the Food and Drug Administration (FDA) approved Mammaprint (Agendia, Amsterdam), a DNA microarray-based test used to predict whether women with early breast cancer might face the disease again. The test measures the activity of 70 genes, providing information about the likelihood that cancer will recur. It measures each of these genes in a sample of a woman's breast-cancer tumor and then uses a specific formula to produce a score that determines if the patient is deemed low-risk or high-risk for metastasis. In clinical trials, 1 in 4 women found to be at high risk by Mammaprint had recurrence of their cancer within 5 years. However, there are questions regarding the accuracy of this test. The positive predictive values at 5 and 10 years were 23% and 29%, respectively, while the corresponding negative predictive values were 95% and 90%, respectively.

Mammaprint was tested on 307 patients under the age of 61 years who underwent surgery for stage I or stage II breast cancer, and who have tumor size equal to or less than 5 cm, and lymph node-negative. The study found that Mammaprint more than doubled physicians' ability to predict breast cancer recurrence. Furthermore, a major clinical study is now underway to determine if the test really helps patients avoid needless chemotherapy. The European study will recruit 6,000 patients with early-stage breast cancer.

In reviewing gene expression profiling as a guide for the management of early stage breast cancer, the California Technology Assessment Forum (CTAF, 2007) stated that the Oncotype Dx recurrence score meets CTAF Technology Assessment Criteria 1 through 5 for safety, effectiveness and improvement in health outcomes when used with other tools to inform the decision to use chemotherapy in patients recently diagnosed with invasive breast cancer meeting the following criteria:

- Estrogen or progesterone receptor positive
- Lymph node negative
- Tumor size less than 5 cm.

On the other hand, the CTAF stated that the use of other forms of gene expression profiling, including the 70-gene prognostic signature (Mammaprint), do not meet CTAF Technology Assessment Criteria 3 through 5 for safety, effectiveness and improvement in health outcomes.

More recently, the California Technology Assessment Forum (CTAF) reassessed the Mammaprint, and concluded that it did not meet CTAF criteria (Tice, 2010). The report concluded that "it remains unclear how the 70-gene prognostic signature should be used in managing patients with early stage breast cancer." The report noted that a large, randomized clinical trial (MINDACT) enrolling 6000 patients in Europe will test the hypothesis that use of the 70-gene prognostic score can improve outcomes for women with early stage breast cancer.

Other technology assessments (e.g., CvZ, 2010; Smartt, 2009) and guidelines (e.g., Harris, et al., 2007; NCCN, 2011) have also found insufficient evidence for use of the Mammaprint. ASCO's 2006 update of the breast cancer follow-up and management guidelines in the adjuvant setting stated that the use of CBCs, chemistry panels, bone scans, chest radiographs, liver ultrasounds, computed tomography scans, [18F]fluorodeoxyglucose-positron emission tomography scanning, magnetic resonance imaging, or tumor markers (CEA, CA 15-3, and CA 27.29) is not recommended for routine breast cancer follow-up in an otherwise asymptomatic patient with no specific findings on clinical examination. It stated that careful history taking, physical examination, and regular mammography are appropriate for detecting breast cancer recurrence. Currently, there is a lack of evidence that the use of Mammaprint would influence the decisions that women and their physicians make with regard to adjuvant therapy. The clinical value of this test has yet to be established.

On February 19, 2014, the BlueCross BlueShield Association Technology Evaluation Center Medical Advisory Panel voted that the Mammaprint to determine recurrence risk in women with early stage breast cancer does not meet the TEC criteria (BCBSA, 2014).

A report prepared by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group evaluated the evidence for three gene expression assays on the market; Oncotype Dx, MammaPrint and the Breast Cancer Profiling (BCP or H/I ratio) test, and for gene expression signatures underlying the assays. The report found that, for clinical validity, studies differed according to whether they examined the actual test that is currently being offered to patients or the underlying gene signature. The report found that almost all of the Oncotype Dx evidence was for the marketed test, the strongest validation study being from one arm of a randomized controlled trial (NSABP-14) with a clinically homogeneous population. This study showed that the test, added in a clinically meaningful manner to standard prognostic indices. The MammaPrint signature and test itself was examined in studies with clinically heterogeneous populations (e.g., mix of ER positivity and tamoxifen treatment) and showed a clinically relevant separation of patients into risk categories, but it was not clear exactly how many predictions would be shifted across decision thresholds if this were used in combination with traditional indices.The BCP test itself was examined in one study, and the signature was tested in a variety of formulations in several studies. The report identified one randomized controlled trial that provided high quality retrospective evidence of the clinical utility of Oncotype Dx to predict chemotherapy treatment benefit, but evidence for clinical utility was not found for MammaPrint or the H/I ratio. The report concluded that Oncotype Dx is furthest along the validation pathway,

with strong retrospective evidence that it predicts distant spread and chemotherapy benefit to a clinically relevant extent over standard predictors, in a well-defined clinical subgroup with clear treatment implications. The report concluded that the evidence for clinical implications of using MammaPrint was not as clear as with Oncotype Dx, and the ability to predict chemotherapy benefit does not yet exist. The report concluded that the H/I ratio test requires further validation. For all tests, the relationship of predicted to observed risk in different populations still needs further study, as does their incremental contribution, optimal implementation, and relevance to patients on current therapies.

The EGAPP Working Group (2009) reported that they found adequate evidence regarding the association of the Oncotype Dx Recurrence Score with disease recurrence and adequate evidence for response to chemotherapy. The EWG found adequate evidence to characterize the association of MammaPrint with future metastases, but inadequate evidence to assess the added value to standard risk stratification, and could not determine the population to which the test would best apply. The evidence was inadequate to characterize the clinical validity of the Quest H:I Test. The EGAPP Working Group reported that they found no evidence regarding the clinical utility of the MammaPrint and Quest H:I Ratio tests, and inadequate evidence regarding Oncotype Dx. The EGAPP Working Group noted that these technologies have potential for both benefit and harm.

The Rotterdam Signature 76-gene panel (Veridex, LLC) is a multivariate index assay that is intended to assist in assessing a patient's risk of systemic recurrence of cancer following successful initial treatment of localized node-negative breast cancer with surgery and tamoxifen alone. This multigene assay is intended for use in lymph-node negative breast cancer patients. The Rotterdam Signature panel uses microarray processing to measure cellular concentrations of mRNA in fresh tissue samples. The Rotterdam Signature panel uses the Human Genome U133a GeneChip (Affymetrix, Inc.) to identify patients that have gene expression signatures associated with either a low or high risk of developing metastatic disease. A multicenter study investigated the ability of the Rotterdam 76-gene signature to identify patients at risk of distant metastases within 5 and 10 years of first diagnosis, using frozen tissue samples from 180 patients with node-negative breast cancer who had not received systemic chemotherapy (Foekens, et al., 2006). The Rotterdam 76-gene signature correctly identified 27 out of 30 cases of relapse within 5 years (90% sensitivity) and 75 out of 150 patients who did not relapse (50% specificity). An earlier summary of the same study (Foekens, et al., 2005) reported a hazard ratio for distant metastasis-free survival comparing favorable versus unfavorable signature = 7.41 (95% confidence interval 2.63-20.9); p = 8.5 x 10-6). The hazard ratio of overall survival comparing favorable versus unfavorable signature = 5.45 (95% confidence interval 1.62-18.3); p = .002. There are no published studies that have assessed the clinical utility of the Rotterdam 76-gene signature by monitoring the long-term outcomes of the patients selected and not selected for chemotherapy on the basis of assay results.

The Breast Cancer Gene Expression Ratio (HOXB13:IL17BR, also known as H/I) (AviaraDx, Inc., Carlsbad, CA) is intended to predict the risk of disease recurrence in women with estrogen receptor (ER)-positive, lymph node-negative breast cancer. The Breast Cancer Gene Expression Ratio is based on the ratio of the expression of two genes: the homeobox gene-B13 (HOXB13) and the interleukin- 17B receptor gene (IL17BR). In breast cancers that are more likely to recur, the HOXB13 gene tends to be over-expressed, while the IL-17BR gene tends to be under-expressed. In an 852-patient retrospective study, Ma, et al. (2006) found that the HOXB13:IL17BR ratio (H:I expression ratio) independently predicted breast cancer recurrence in patients with ER-positive, lymph-node negative cancer. The H:I expression ratio was found to be predictive in patients who received tamoxifen therapy as well as in those who did not. Expression of HOXB13, IL17BR, CHDH, estrogen receptor (ER) and progesterone receptor (PR) were quantified by real-time polymerase chain reaction (PCR) in 852 formalin-fixed, paraffin-embedded primary breast cancers from 566 untreated and 286 tamoxifen-treated breast cancer patients. Gene expression and clinical variables were analyzed for association with relapse-free survival (RFS) by Cox proportional hazards regression models. The investigators reported that, in the entire cohort, expression of HOXB13 was associated with shorter RFS (p = .008), and

expression of IL17BR and CHDH was associated with longer RFS ($p < .0001$ for IL17BR and $p = .0002$ for CHDH). In ER-positive patients, the HOXB13:IL17BR index predicted clinical outcome independently of treatment, but more strongly in node-negative patients. In multivariate analysis of the ER-positive node-negative subgroup including age, PR status, tumor size, S phase fraction, and tamoxifen treatment, the two-gene index remained a significant predictor of RFS (hazard ratio = 3.9; 95% confidence interval 1.5 to 10.3; $p = .007$). The clinical value of the Breast Cancer Gene Expression Ratio was also evaluated in a study by Goetz, et al. (2006). That study found that a high H:I expression ratio is associated with an increased rate of relapse and mortality in ER-positive, lymph node-negative cancer patients treated with surgery and tamoxifen. Goetz, et al. (2006) examined the association between the ratio of the HOXB13 to IL17BR expression and the clinical outcomes of relapse and survival in women with ER-positive breast cancer enrolled onto a North Central Cancer Treatment Group adjuvant tamoxifen trial (NCCTG 89-30-52). Tumor blocks were obtained from 211 of 256 eligible patients, and quantitative reverse transcription-PCR profiles for HOXB13 and IL-17BR were obtained from 206 patients. In the node-positive cohort (n = 86), the HOXB13/IL-17BR ratio was not associated with relapse or survival. In contrast, in the node-negative cohort (n = 130), a high HOXB13/IL-17BR ratio was associated with significantly worse RFS [hazard ratio (HR), 1.98; $p = 0.031$], DFS (HR, 2.03; $p = 0.015$), and OS (HR, 2.4; $p = 0.014$), independent of standard prognostic markers.

The Blue Cross and Blue Shield Association Technology Evaluation Center (BCBSA, 2007) announced that its Medical Advisory Panel (MAP) concluded that the use of the Breast Cancer Gene Expression Ratio gene expression profiling does not meet the TEC criteria. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group is currently preparing an evaluation of the H:I ratio test.

In a systematic review on gene expression profiling assays in early-stage breast cancer, Marchionni, et al. (2008) summarized evidence on the validity and utility of 3 gene expression-based prognostic breast cancer tests: Oncotype Dx, MammaPrint, and H/I. The authors concluded that gene expression technologies show great promise to improve predictions of prognosis and treatment benefit for women with early-stage breast cancer. However, more information is needed on the extent of improvement in prediction, characteristics of women in whom the tests should be used, and how best to incorporate test results into decision making about breast cancer treatment.

Guidelines from the American Society for Clinical Oncology (Harris, et al., 2007) found that, in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, the Oncotype Dx assay can be used to predict the risk of recurrence in patients treated with tamoxifen. The ASCO guidelines concluded that Oncotype Dx may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy. The ASCO guidelines found, in addition, that patients with high recurrence scores appear to achieve relatively more benefit from adjuvant chemotherapy than from tamoxifen. ASCO found that there are insufficient data at present to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimens. Guidelines from the American Society for Clinical Oncology (Harris, et al., 2007) concluded that the precise clinical utility and appropriate application for other multiparameter assays, such as the MammaPrint assay, the Rotterdam Signature, and the Breast Cancer Gene Expression Ratio are under investigation. ASCO also found insufficient data to recommend use of proteomic patterns for management of patients with breast cancer.

TargetPrint is a microarray-based gene expression test which offers a quantitative assessment of the patient's level of estrogen receptor (ER), progesterone receptor (PR) and HER2/neu overexpression in breast cancer. The manufacturer states that TargetPrint is offered in conjunction with MammaPrint gene expression profiling to provide the physician an even more complete basis for treatment decisions. The manufacturer states that, as compared to Immunohistochemistry (IHC), TargetPrint provides additional information. Whereas IHC

provides a semi-quantitative positive or negative result, the gene expression result provided by TargetPrint provides data on the absolute level of ER, PR and HER2 gene expression. Published information on the TargetPrint is limited to studies examining its correlation with measurements of ER, PR, and HER2 receptors (Gunven et al, 2011; Gevensleben et al, 2010; Roepman et al, 2009). There is a lack of evidence from published prospective clinical studies that demonstrates that quantification of ER, PR, and HER2 gene expression by TargetPrint alters management such that clinical outcomes are improved.

The Mammostrat is a prognostic immunohistochemistry (IHC) test that measures the risk of breast cancer recurrence in post-menopausal, node-negative, estrogen receptor-expressing breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy. The test analyzes five monoclonal antibody biomarkers and applies a diagnostic algorithm to assess whether patients have a high, moderate, or low risk of recurrence after they have had their breast cancer tumor surgically removed and have been treated with tamoxifen.

Bartlett et al (2010) tested the efficacy of the Mammostrat in a mixed population of cases treated in a single center with breast-conserving surgery and long-term follow-up. Tissue microarrays from a consecutive series of 1,812 women managed by wide local excision and post-operative radiotherapy were collected. Of 1,390 cases stained, 197 received no adjuvant hormonal or chemotherapy, 1,044 received tamoxifen only, and 149 received a combination of hormonal therapy and chemotherapy. Median age at diagnosis was 57 years, 71% were post-menopausal, 23.9% were node-positive and median tumor size was 1.5 cm. Samples were stained using triplicate 0.6 mm2 tissue microarray cores, and positivity for p53, HTF9C, CEACAM5, NDRG1 and SLC7A5 was assessed. Each case was assigned a Mammostrat risk score, and distant recurrence-free survival (DRFS), relapse-free survival (RFS) and overall survival (OS) were analyzed by marker positivity and risk score. Increased Mammostrat scores were significantly associated with reduced DRFS, RFS and OS in ER-positive breast cancer ($p < 0.00001$). In multivariate analyses the risk score was independent of conventional risk factors for DRFS, RFS and OS ($p < 0.05$). In node-negative, tamoxifen-treated patients, 10-year recurrence rates were 7.6 +/- 1.5% in the low-risk group versus 20.0 +/- 4.4% in the high-risk group. Further, exploratory analyses revealed associations with outcome in both ER-negative and un-treated patients. The authors concluded that the Mammostrat can act as an independent prognostic tool for ER-positive, tamoxifen-treated breast cancer and the results of the study revealed a possible association with outcome regardless of node status and ER-negative tumors.

There is insufficient evidence to determine whether the Mammostrat test is better than conventional risk assessment tools in predicting the recurrence of breast cancer. Furthermore, neither NCCN or ASCO have incorporated the test into their guidelines as a diagnostic tool.

The OvaCheck™ (Correlogic Systems, Inc.) is a proteomic analysis of blood for the early detection of ovarian cancer. A similar test, which involved a different molecular pattern, was the subject of a 2002 study of 216 women with ovarian cancer. That study showed that the proteomic test had a specificity of 100% and a sensitivity of 95%, with a positive predictive value of 94% (Petricoin, et al., 2002). While this study showed that a proteomic test detected ovarian cancers even where CA-125 levels were normal, this study included only women who had been detected with ovarian cancer by other means. There is inadequate evidence that this test will be effective for screening women with undetected ovarian cancer.

In addition, there is concern, given the low prevalence of ovarian cancer, that this test is not sufficiently specific for use in screening. The National Cancer Institute explains that even an ovarian cancer test with a specificity of 99% means that 1% of those who did not have cancer would test positive, which is “far too high a rate for commercial use” (NCI, 2004). For a rare disease such as ovarian cancer, which has an approximate prevalence of 1 in 2,500 in the general population, a 99% specificity and 100% sensitivity translates into 25 women falsely identified for every one true cancer found.

The OvaCheck™ test employs electrospray ionization (ESI) type of mass spectrometry using

highly diluted denatured blood samples. This method differs from a matrix-assisted laser desorption ionization (MALDI) analysis of undiluted native sera samples that was used in the Lancet study and is currently under investigation by the National Cancer Institute and Food and Drug Administration (NCI, 2004). The NCI notes that "[t]he class of molecules analyzed by these two approaches, and thus the molecules that constitute the diagnostic patterns, would be expected to be entirely different." Neither the NCI nor FDA has been involved in the design or validation of OvaCheck™ methodology.

As the Ovacheck test is performed as a "home-brewed" test by two national laboratories instead of as a commercially available kit, FDA approval of the OvaCheck test may not be required. The Society for Gynecologic Oncologists (SGO, 2004) has reviewed the literature regarding OvaCheck and concluded that "more research is needed to validate the test's effectiveness before offering it to the public." Similarly, the American College of Obstetricians and Gynecologists (2004) has stated that "more research is needed to validate the test's effectiveness before recommending it to the public."

An assessment of the Ovacheck test and other genomic tests for ovarian cancer prepared for the Agency for Healthcare Research and Quality by the Duke Evidence-Based Practice Center (Myers, et al., 2006) reached the following conclusions: "Genomic test sensitivity/specificity estimates are limited by small sample sizes, spectrum bias, and unrealistically large prevalences of ovarian cancer; in particular, estimates of positive predictive values derived from most of the studies are substantially higher than would be expected in most screening or diagnostic settings. We found no evidence relevant to the question of the impact of genomic tests on health outcomes in asymptomatic women. Although there is a relatively large literature on the association of test results and various clinical outcomes, the clinical utility of changing management based on these results has not been evaluated." Specifically regarding Ovacheck and other proteomic tests for ovarian cancer, the assessment found that, "[a] lthough all studies reported good discrimination for the particular protein profile studied, there were several recurrent issues that limit the ability to draw inferences about potential clinical applicability," in particular technical issues with the assays themselves, variations in analytic methods used among studies, and an unrealistically high prevalence of ovarian cancer in the datasets compared to what would be expected in a normal screening population.

OvaSure is an ovarian cancer screening test that entails the use of 6 biomarkers (leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor and CA-125) to assess the presence of early stage ovarian cancer in high-risk women. Visintin et al (2008) characterized and validated the OvaSure for discriminating between disease-free and ovarian cancer patients. These researchers analyzed 362 healthy controls and 156 newly diagnosed ovarian cancer patients. Concentrations of leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125 were determined using a multiplex, bead-based, immunoassay system. All 6 markers were evaluated in a training set (181 samples from the control group and 113 samples from ovarian cancer patients) and a test set (181 sample control group and 43 ovarian cancer). Multiplex and ELISA exhibited the same pattern of expression for all the biomarkers. None of the biomarkers by themselves was good enough to differentiate healthy versus cancer cells. However, the combination of the 6 markers provided a better differentiation than CA-125. Four models with less than 2% classification error in training sets all had significant improvement (sensitivity 84 % to 98% at specificity 95%) over CA-125 (sensitivity 72% at specificity 95%) in the test set. The chosen model correctly classified 221 out of 224 specimens in the test set, with a classification accuracy of 98.7%. The authors noted that the OvaSure is the first blood biomarker test with a sensitivity of 95.3% and a specificity of 99.4% for the detection of ovarian cancer. Six markers provided a significant improvement over CA-125 alone for ovarian cancer detection. Validation was performed with a blinded cohort. They stated that this novel multiplex platform has the potential for efficient screening in patients who are at high risk for ovarian cancer.

However, the Society of Gynecologic Oncologists (SGO, 2008) released an opinion regarding OvaSure, which stated that additional research is needed before the test should be offered to

women outside the context of a research study. Moreover, SGO stated that it will "await the results of further clinical validation of OvaSure with great interest".

Furthermore, according to the FDA's web site, the FDA sent the Laboratory Corporation of America a warning letter stating that it is illegally marketing OvaSure to detect ovarian cancer. According to the FDA warning letter, their review indicates that this product is a device under section 201(h) of the Food, Drug, and Cosmetic Act (FDCA or Act), 21 U.S.C. 321(h), because it is intended for use in the diagnosis of disease or other conditions, or in the cure, treatment, prevention, or mitigation of disease. The Act requires that manufacturers of devices that are not exempt obtain marketing approval or clearance for their products from the FDA before they may offer them for sale. This helps protect the public health by ensuring that new devices are shown to be both safe and effective or substantially equivalent to other devices already legally marketed in this country for which approval is not required. According to the FDA warning letter, no such determination has been made for OvaSure.

Thrombospondin-1 (THBS-1), an angiogenesis inhibitor, has been identified as a potential monitoring marker in gynecologic malignancies. In a randomized phase III study on the co-expression of angiogenic markers and their associations with prognosis in advanced epithelial ovarian cancer, Secord, et al. (2007) reported that high THBS-1 may be an independent predictor of worse progression-free and overall survival in women with advanced-stage EOC. However, the authors stated, "A larger prospective study is warranted for validation of these findings."

Guanyl cyclase C (GCC) is a receptor protein normally expressed in high concentrations on the luminal surface of the gastrointestinal epithelium. Expression of GCC persists on mucosal cells that have undergone malignant transformation. Thus, GCC has potential use as a marker to determine spread of colorectal cancer to lymph nodes. A retrospective study of 21 patients post surgical resection of colorectal cancer found that all 11 of 21 patients who were free of cancer for 5 years or more were negative for GCC in lymph nodes, whereas all 10 of 21 patients whose cancer returned within 3 years of surgery were positive for GCC. However, the value of the GCC marker test in the management of colorectal cancer needs to be evaluated in prospective clinical outcome studies. A large prospective study is currently being conducted to compare standard histological examination of lymph nodes to the GCC marker test.

Thymidylate synthase is a DNA synthesis related gene. According to Compton (2008), the prognostic value of this promising and potentially clinically applicable molecular marker has been studied in colorectal cancer. Compton found that the independent influence of this marker on prognosis remains unproven. Compton explained that "[v]ariability in assay methodology, conflicting results from various studies examining the same factor, and the prevalence of multiple small studies that lack statistically robust, multivariate analyses all contribute to the lack of conclusive data." Compton concluded that before this marker can be incorporated into clinically meaningful prognostic stratification systems, more studies are required using multivariate analysis, well-characterized patient populations, reproducible and current methodology, and standardized reagents.

In a special report on pharmacogenomics of cancer, the BlueCross and BlueShield Association's Technology Evaluation Center (TEC) (2007) described the results of a meta-analysis on thymidylate synthase protein expression and survival in colorectal cancer that stated low thymidylate synthase expression was significantly associated with better survival, but heterogeneity and possible bias prevented firm conclusions.

Current guidelines from the American Society for Colon and Rectal Surgeons (2004) stated: "In the future, DNA analysis and the intratumoral expression of specific chemical substances", including thymidylate synthase, "may be used routinely to further assess prognosis or response to therapy." In addition, Shankaran, et al. (2008) stated in a review on the role of molecular markers in predicting response to therapy in patients with colorectal cancer, "Although to date no molecular characteristics have emerged as consistent predictors of response to therapy, retrospective studies have investigated the role of a variety of biomarkers, including

microsatellite instability, loss of heterozygosity of 18q, type II transforming growth factor beta receptor, thymidylate synthase, epidermal growth factor receptor, and Kirsten-ras (KRAS)."

Tumour angiogenesis is associated with invasiveness and the metastatic potential of various cancers. Vascular endothelial growth factor (VEGF), the most potent and specific angiogenic factor identified to date, regulates normal and pathologic angiogenesis. An evidence report from Cancer Care Ontario (Welch, et al., 2008) on the use of the VEGF inhibitor bevacizumab in colorectal cancer explained that the increased expression of VEGF has been correlated with metastasis, recurrence, and poor prognosis in many cancers, including colorectal cancer. Guidelines from the National Institute for Health and Clinical Excellence (NICE, 2007) explained that bevacizumab (Avastin) is a recombinant humanised monoclonal IgG1 antibody that acts as an angiogenesis inhibitor. It targets the biological activity of VEGF, which stimulates new blood vessel formation in the tumour. However, neither the FDA approved labeling of bevacizumab or evidence-based guidelines recommend measurement of VEGF to diagnose colorectal cancer or to select patients for treatment. In a special report on pharmacogenomics of cancer, the BlueCross and BlueShield Association's Technology Evaluation Center (TEC) (2007) stated that pre-treatment VEGF levels do not appear to be predictive of response to anti-angiogenic therapy.

PCA3 (DD3) is a gene that has been found to be highly overexpressed in prostate cancer. This gene has been investigated as a potential diagnostic marker for prostate cancer. However, there are no published clinical outcome studies of the effectiveness of the PCA3 gene in screening, diagnosis or management of prostate cancer.

Prostate cancer antigen 3 (PCA3) (Progensa, Gene-Probe, Inc.) encodes a prostate-specific mRNA. It is one of the most prostate cancer-specific genes identified, with over-expression in about 95% of cancers tested. The PCA3 urine assay is an amplified nucleic acid assay, which uses transcription-mediated amplification (TMA) to quantify PCA3 and PSA mRNA in prostate cells found in urine samples. The PCA3 score is calculated as the ratio between PCA3 and PSA mRNA. The main target population of this non-invasive test is men with raised PSA but a negative prostate biopsy. Other target groups include men with a slightly raised PSA, as well as men with signs and symptoms suggestive of prostate cancer.

van Gils and colleagues (2007) stated that the recently discovered PCA3 is a promising prostate cancer marker. These investigators performed a multi-center study to validate the diagnostic performance of the PCA3 urine test established in an earlier single-institution study. The first voided urine after DRE was collected from a total of 583 men with serum PSA levels between 3 and 15 ng/ml who were to undergo prostate biopsies. These researchers determined the PCA3 score in these samples and correlated the results with the results of the prostate biopsies. A total of 534 men (92%) had an informative sample. The area under the receiver-operating characteristic curve, a measure of the diagnostic accuracy of a test, was 0.66 for the PCA3 urine test and 0.57 for serum PSA. The sensitivity for the PCA3 urine test was 65%, the specificity was 66% (versus 47% for serum PSA), and the negative predictive value was 80%. The authors concluded that the findings of this multi-center study validated the diagnostic performance of the PCA3 urine test in the largest group studied thus far using a PCA3 gene-based test.

Marks and associates (2007) examined the potential utility of the investigational PCA3 urine assay to predict the repeat biopsy outcome. Urine was collected after DRE (3 strokes per lobe) from 233 men with serum PSA levels persistently 2.5 ng/ml or greater and at least one previous negative biopsy. The PCA3 scores were determined using a highly sensitive quantitative assay with TMA. The ability of the PCA3 score to predict the biopsy outcome was assessed and compared with the serum PSA levels. The RNA yield was adequate for analysis in the urine samples from 226 of 233 men (i.e., the informative specimen rate was 97%). Repeat biopsy revealed prostate cancer in 60 (27%) of the 226 remaining subjects. Receiver operating characteristic curve analysis yielded an area under the curve of 0.68 for the PCA3 score. In contrast, the area under the curve for serum PSA was 0.52. Using a PCA3 score cutoff of 35, the assay sensitivity was 58% and specificity 72%, with an odds ratio of 3.6. At PCA3 scores of less

than 5, only 12% of men had prostate cancer on repeat biopsy; at PCA3 scores of greater than 100, the risk of positive biopsy was 50%. The authors concluded that in men undergoing repeat prostate biopsy to rule out cancer, the urinary PCA3 score was superior to serum PSA determination for predicting the biopsy outcome. The high specificity and informative rate suggest that the PCA3 assay could have an important role in prostate cancer diagnosis.

Groskopf et al (2007) reported that the PCA3 score is independent of prostate volume and was highly correlated with the risk of positive biopsy. The PCA3 test was performed on 529 men scheduled for prostate biopsy. Overall, the PCA3 score had a sensitivity of 54% and a specificity of 74%. A PCA3 score of less than 5 was associated with a 14% risk of positive biopsy, while a PCA3 score of greater than 100 was associated with a 69% risk of positive biopsy.

Haese et al (2007) presented preliminary results from a European multicenter study of PCA3. Enrolled patients had a PSA level of less than or equal to 2.5 ng/mL, had 1 or 2 previous negative biopsies, and were scheduled for repeat biopsy. The specificity of the PCA3 score (cutoff 35) was found to be 78%, and the sensitivity was 67%. Patients with a PCA3 score of greater than or equal to 35 had a 33% probability of a positive repeat biopsy, compared to a 6% probability for those with a PCA3 score of less than 35.

An assessment by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2008) found that, in general, PCA3 assay results to date are preliminary; interpretation of results has not been standardized and clinical utility studies of decision-making for initial biopsy, repeat biopsy or treatment have not been reported.

Tosoian et al (2010) evaluated the relationship between PCA3 and prostate biopsy results in men in a surveillance program. Urine specimens were obtained from 294 men with prostate cancer enrolled in the Johns Hopkins surveillance program. The follow-up protocol included semi-annual free and total PSA measurements, digital rectal examination and annual surveillance prostate biopsy. Cox proportional hazards regression was used to evaluate the association between PCA3 results and progression on surveillance biopsy (defined as Gleason pattern 4 or 5, more than 2 positive biopsy cores or more than 50% involvement of any core with cancer). Patients with progression on biopsy (12.9%) had a mean PCA3 score similar to that of those without progression (60.0 versus 50.8, $p = 0.131$). Receiver operating characteristics analysis suggested that PCA3 alone could not be used to identify men with progression on biopsy (area under the curve = 0.589, 95% CI 0.496 to 0.683, $p = 0.076$). After adjustment for age and date of diagnosis PCA3 was not significantly associated with progression on biopsy ($p = 0.15$). The authors concluded that in men with low risk prostate cancer who were carefully selected for surveillance the PCA3 score was not significantly associated with short-term biopsy progression. They stated that further analysis is necessary to assess the usefulness of PCA3 in combination with other biomarkers or in selected subsets of patients undergoing surveillance.

While there are studies examining the positive and negative predictive values of the PCA3 urine assay, there is currently a lack of evidence of the effect of this test on management of individuals with or suspected of prostate cancer. The PCA3 urine assay shows promise as a prostate cancer diagnostic tool, however, more research is needed to ascertain the clinical value of this assay for screening and diagnostic purposes.

Donovan et al (2008) from Aureon, the manufacturer of Prostate Px, reported on the development and validation of their systems pathology model for predicting prostate cancer recurrence after prostatectomy. The clinical utility of defining high risk for failure after radical prostatectomy is to decide whether patients require closer follow-up than average or whether adjuvant radiotherapy, hormone therapy, or chemotherapy would be of benefit. In this analysis, the concordance index for the systems pathology approach used by Aureon was 0.83, but was only slightly better than a 10-variable model that used only the usual clinical parameters, with a concordance index of 0.80. The corresponding hazard ratios for clinical failure were 6.37 for the 10-variable clinical model, and 9.11 for the systems pathology approach. In an accompanying editorial, Klein, et al. (2009) questioned the clinical significance of these differences. They noted

that "[a]lthough the difference in concordance indices was statistically significant, the question is whether there is sufficient clinical relevance to justify the extra effort, expense, and clinical expertise needed for the systems approach ... In contemporary clinical practice, a patient with a hazard ratio of 6.37 generated by the model using easily derived, routinely reported clinical and pathological parameters is just as likely to be a candidate for closer monitoring or adjuvant therapy than one with a hazard ratio of 9.11 generated by the systems approach".

Sutcliffe et al (2009) provided an evidence-based perspective on the prognostic value of novel markers in localized prostate cancer and identified the best prognostic model including the 3 classical markers and investigated if models incorporating novel markers are better. Eight electronic bibliographic databases were searched. The reference lists of relevant articles were checked and various health services research-related resources consulted via the internet. The search was restricted to publications from 1970 onwards in the English language. Selected studies were assessed, data extracted using a standard template, and quality assessed using an adaptation of published criteria. Because of the heterogeneity regarding populations, outcomes and study type, meta-analyses were not undertaken and the results are presented in tabulated format with a narrative synthesis of the results. A total of 30 papers met the inclusion criteria, of which 28 reported on prognostic novel markers and 5 on prognostic models. A total of 21 novel markers were identified from the 28 novel marker studies. There was considerable variability in the results reported, the quality of the studies was generally poor and there was a shortage of studies in some categories. The marker with the strongest evidence for its prognostic significance was PSA velocity (or doubling time). There was a particularly strong association between PSA velocity and prostate cancer death in both clinical and pathological models. In the clinical model the hazard ratio for death from prostate cancer was 9.8 (95 % CI 2.8 to 34.3, $p < 0.001$) in men with an annual PSA velocity of more than 2 ng/ml versus an annual PSA velocity of 2 ng/ml or less; similarly, the hazard ratio was 12.8 (95 % CI 3.7 to 43.7, $p < 0.001$) in the pathological model. The quality of the prognostic model studies was adequate and overall better than the quality of the prognostic marker studies. Two issues were poorly dealt with in most or all of the prognostic model studies: (i) inclusion of established markers, and (ii) consideration of the possible biases from study attrition. Given the heterogeneity of the models, they can not be considered comparable. Only 2 models did not include a novel marker, and 1 of these included several demographical and co-morbidity variables to predict all-cause mortality. Only 2 models reported a measure of model performance, the C-statistic, and for neither was it calculated in an external data set. It was not possible to assess whether the models that included novel markers performed better than those without. This review highlighted the poor quality and heterogeneity of studies, which render much of the results inconclusive. It also pinpointed the small proportion of models reported in the literature that are based on patient cohorts with a mean or median follow-up of at least 5 years, thus making long-term predictions unreliable. Prostate-specific antigen velocity, however, stood out in terms of the strength of the evidence supporting its prognostic value and the relatively high hazard ratios. There is great interest in PSA velocity as a monitoring tool for active surveillance but there is as yet no consensus on how it should be used and, in particular, what threshold should indicate the need for radical treatment.

In an editorial on clinically relevant prognostic markers for prostate cancer, Gelmann and Henshall (2009) stated that "[u]ntil we have sufficiently discriminating markers to inform treatment decisions, the problem of whom to treat will continue to grow exponentially as the number of cases of screening-detected low-risk cancer increases".

The CellSearch™ Epithelial Cell Kit, along with the CellSpotter™ Analyzer (Veridex, LLC, Warren, NJ) is a device designed to automate the detection and enumeration of circulating tumor cells (CTCs) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+ and/or 19+) in whole blood in patients with advanced breast cancer. It is intended for use in adjunctively monitoring and predicting cancer disease progression and response to therapy.

The CellSearch Epithelial Cell Kit received FDA 510(k) clearance on January 21, 2004. The FDA concluded that the device is substantially equivalent to immunomagnetic circulating cancer cell selection and enumeration systems. These devices consist of biological probes,

fluorochromes and other reagents, preservation and preparation devices and semi-automated analytical instruments to select and count circulating cancer cells in a prepared sample of whole blood.

The CellSearch Epithelial Cell Kit quantifies CTCs by marking cancerous cells with tiny, protein-coated magnetic balls in whole blood. These cells are stained with fluorescent markers for identification and then dispensed into a cartridge for analysis where a strong magnetic field is applied to the mixture causing the magnetically marked cells to move to the cartridge surface. The cartridge is then analyzed by the CellSpotter Analyzer. A medical professional rechecks the CTCs and the CellSpotter Analyzer tallies the final CTC count.

In a prospective, multicenter study, Cristofanilli et al (2004) used the CellSearch System on 177 patients with measurable metastatic breast cancer for levels of CTCs both before the patients started a new line of therapy and at follow-up. The progression of the disease or the response to treatment was determined with the use of standard imaging studies at the participating centers every nine to twelve weeks. Outcomes were assessed according to levels of CTCs at baseline, before the patients started a new therapy. In the first test, patients with 5 or more CTCs per 7.5 ml of blood compared to a group with fewer than 5 CTCs had a shorter median progression-free survival (2.7 months vs. 7.0 months) and shorter overall survival (10.1 months vs. greater than 18 months). At the follow-up visit, approximately three to four weeks after the initiation of therapy, the percentage of patients with more than 5 CTC was reduced from 49 percent to 30 percent, suggesting a benefit from therapy. The difference in progression-free survival between the two groups remained consistent (2.1 months for women with 5 or more CTCs vs. 7 months for women with less than 5 CTCs). Overall, survival in the women with more than 5 CTCs was 8.2 months compared to greater than 18 months in the cohort with less than 5 CTCs. Cristofanilli concluded that the number of CTCs before treatment was an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer. However, Cristofanilli also concluded that the results may not be valid for patients who do not have measurable disease or for those starting a new regimen of hormone therapy, immunotherapy, or both. He states, “The prognostic implications of an elevated level of circulating tumor cells for patients with metastatic disease who are starting a new treatment may be an opportunity to stratify these patients in investigational studies”. Furthermore, the study did not address whether patients with an elevated number of circulating tumor cells might benefit from other therapies. Thus, this minimally invasive assay requires further evaluation as a prognostic marker of disease progression and response to therapy.

The clinical application of quantifying CTCs in the peripheral blood of breast cancer patients remains unclear. Published data in the peer-reviewed medical literature are needed to determine how such measurements would guide treatment decisions and whether these decisions would result in beneficial patient outcomes (Kahn, et al., 2004; Abeloff, et al., 2004). An assessment of CellSearch by AETSA (2006) concluded "In the current stage of development of this technology, there is no evidence that it provides any advantage over existing technology for CTC identification or indeed any additional clinical use." Guidelines from the American Society for Clinical Oncology (Harris, et al., 2007) found: "The measurement of circulating tumor cells (CTCs) should not be used to make the diagnosis of breast cancer or to influence any treatment decisions in patients with breast cancer. Similarly, the use of the recently U.S. Food and Drug Administration (FDA)-cleared test for CTC (CellSearch Assay) in patients with metastatic breast cancer cannot be recommended until further validation confirms the clinical value of this test."

Although studies relate circulating tumor cells to prognostic indicators (see, e.g., Cohen, et al., 2008; De Giorgi, et al., 2009), there are a lack of published prospective clinical studies demonstrating that measurement of CTCs alters management such that clinical outcomes are improved. Such clinical outcome studies are currently ongoing. Current guidelines from the National Comprehensive Cancer Network (NCCN) and the American Society for Clinical Oncology (ASCO) make no recommendation for use of circulating tumor cells.

Estrogen and progestin receptors are important prognostic markers in breast cancer, and the

higher the percentage of overall cells positive as well as the greater the intensity, the better the prognosis. Estrogen and progesterone receptor positivity in breast cancer cells is an indication the patient may be a good candidate for hormone therapy. HER-2/neu is an oncogene. Its gene product, a protein, is over-expressed in approximately 20 to 30% of breast cancers. The over-expressed protein is present in unusually high concentration on the surface of some malignant breast cancer cells, causing these cells to rapidly proliferate. It is important because these tumors are susceptible to treatment with Herceptin (trastuzumab), which specifically binds to this over-expressed protein. Herceptin blocks these protein receptors, inhibiting continued replication and tumor growth.

There are additional tests that may be used in breast cancer cases, such as DNA ploidy, Ki-67 or other proliferation markers. However, most authorities believe that HER-2/neu, estrogen and progesterone receptor status are the most important to evaluate first. The other tests do not have therapeutic implications and, when compared with grade and stage of the disease, are not independently significant with respect to prognosis.

Harris et al (2007) updated ASCO's recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast cancer. Thirteen categories of breast tumor markers were considered, 6 of which were new for the guideline. The following categories showed evidence of clinical utility and were recommended for use in practice: CA 15-3, CA 27.29, CEA, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, urokinase plasminogen activator, plasminogen activator inhibitor 1, and certain multi-parameter gene expression assays. Not all applications for these markers were supported, however. The following categories demonstrated insufficient evidence to support routine use in clinical practice: DNA/ploidy by flow cytometry, p53, cathepsin D, cyclin E (fragments or whole length), proteomics, certain multi-parameter assays, detection of bone marrow micrometastases, and circulating tumor cells (e.g., CellSearch assay). These guidelines found present data insufficient to recommend measurement of Ki67, cyclin D, cyclin E, p27, p21, thymidine kinase, topoisomerase II, or other markers of proliferation to assign patients to prognostic groups. The guidelines also found insufficient data to recommend assessment of bone marrow micrometastases for management of patients with breast cancer.

The PathFinderTG (RedPath Integrated Pathology, Pittsburgh, PA), also known as topographic genotyping, is described by the manufacturer as a quantitative genetic mutational analysis platform for resolving “indeterminate, atypical, suspicious, equivocal and non-diagnostic specimen” diagnoses from pathology specimens (RedPath, 2007). The manufacturer states that PathFinder TG "focuses on acquired mutational damage rather than inherited genetic predisposition for certain diseases, although there are certain NIH recommended inherited conditions for which we do test." The manufacturer states that the temporal sequence of acquired mutational damage revealed by the PathFinderTG test is an earlier demonstration of tumor biological aggressiveness than current staging systems that rely on the depth of invasion already achieved by the tumor. Available published evidence for topographic genotyping focuses on retrospective analyses of pathology specimens examining correlations of test results with tumor characteristics (e.g., Saad, et al., 2008; Lin, et al., 2008; Finkelstein, et al., 2003; Pollack, et al., 2001; Riberio, et al., 1998; Kounelis, et al., 1998; Finkelstein, et al., 1998; Holst, et al., 1998; Jones, et al., 1997; Holst, et al., 1997; Pricolo, et al., 1997; Przygodzki, et al., 1997; Finkelstein, et al., 1996; Kanbour-shakir, et al., 1996; Ribeiro, et al., 1996; Pryzgodzki, et al., 1996; Safatle-Ribeiro, et al., 1996; Papadaki, et al., 1996; Przygodzki, et al., 1996; Pricolo, et al., 1996; Finkelstein, et al., 1994). There are no prospective clinical outcome studies on the use of topographic genotyping in guiding patient management. Current evidence-based guidelines from leading medical professional organizations and public health agencies do not include recommendations for topographic genotyping. In a review on molecular analysis of pancreatic cyst fluid, Shen and colleagues (2009) stated that a large study with validation of PathFinderTG molecular testing of pancreatic fluid will be needed before a firm conclusion can be drawn.

A systematic evidence review of the PathFinderTG prepared for the Agency for Healthcare Research and Quality (Trikalinos, et al., 2010) found that most studies on loss-of-heterozygosity

based topographic genotyping with PathfinderTG were excluded because they only described the molecular profile of different tumors, without assessing the ability of the method to help make diagnosis, prognosis or treatment guidance. The review found no studies that directly measured whether using loss-of-heterozygosity based topographic genotyping with PathfinderTG improves patient-relevant clinical outcomes. The review reported that eligible studies on the diagnostic and prognostic ability of loss-of-heterozygosity based topographic genotyping with PathfinderTG were small in sample sizes and had overt methodological limitations. The review reported that important characteristics of their designs were not clearly reported. The report noted that loss-of-heterozygosity based topographic genotyping with PathfinderTG is claimed to be particularly useful in cases where conventional pathology is unable to provide a conclusive diagnosis. However, the included studies were not designed to address this question. Therefore, it is unclear if the findings of the reviewed studies are directly applicable to patients with the same cancers but with inconclusive diagnosis.

Carcinoma of unknown primary (CUP) is a biopsy-proven metastatic solid tumor with no primary tumor identified and represents approximately 2% to 4% of all cancer cases. The diagnosis of CUP is made following inconclusive results from standard tests (e.g., biopsy, immunochemistry and other blood work, chest x-rays, and occult blood stool test). The absence of a known primary tumor presents challenges to the selection of appropriate treatment strategies. As a result, patients have a poor prognosis, and fewer than 25% survive 1 year from the time of diagnosis. A variety of tissue-biopsy testing techniques currently are used to determine the origin of the CUP, including immunochemistry; histological examination of specimens stained with eosin and hematoxylin, and electron microscopy. These techniques definitively identify the type of carcinoma in less than 20% to 30% of CUP.

Gene expression profiling is a technique used to identify the genetic makeup of a tissue sample by characterizing the patterns of mRNA transcribed, or "expressed", by its DNA. Specific patterns of gene expression, reflected in unique configurations of mRNA, are associated with different tumor types. By comparing the gene expression profile (GEP) of an unknown tumor to the profiles of known primary cancers ("referent" profiles), it may be possible to determine the type of tumor from which the CUP originated.

In July 2008, the FDA cleared for marketing the Pathwork Tissue of Origin test (Pathwork Diagnostics, Sunnyvale, CA), a gene expression profiling test that uses microarray processing to determine the type of cancer cells present in a tumor of unknown origin. The test uses the PathChip (Affymetrix Inc., Santa Clara, CA), a custom-designed gene expression array, to measure the expression from 1,668 probe sets to quantify the similarity of tumor specimens to 15 common malignant tumor types, including: bladder, breast, colorectal, gastric, germ cell, hepatocellular, kidney, non-small cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid. The degree of correspondence between the tissue sample's GEP and a referent profile is quantified and expressed as a probability-based score.

A multi-center, clinical validation study reported on comparisons of diagnoses based on GEP from 477 banked tissue samples of undifferentiated and poorly differentiated metastases versus standard of care pathology based diagnoses. Comparison of the GEP based diagnoses versus pathology based diagnoses yielded an 89% agreement and the concurrence was greater than 92% for 8 out of 15 types of primary tumors. The overall accuracy of the test was approximately 95% and 98% for positive and negative determinations, respectively (Monzon, et al., 2007).

Gene expression profiling is a promising technology in the management of CUP; however, there is insufficient evidence of its clinical utility compared to that achieved by expert pathologists using current standards of practice. A draft clinical guideline on metastatic malignant disease of unknown origin by the National Institute for Clinical Excellence (NICE, 2010) recommends against using gene expression profiling (e.g., Pathwork TOT, CupPrint, Theros CancerTypeID, miRview Mets) to identify primary tumors in patients with CUP. The guideline explained that currently there is no evidence that gene-expression based profiling improves the management or

changes the outcomes for patients with CUP. Guidelines on occult primary from the National Comprehensive Cancer Network (NCCN, 2010) state that, while gene expression profiling looks promising, "prospective clinical trials are necessary to confirm whether this approach can be used in choosing treatment options which would improve the prognosis of patients with occult primary cancers."

A technology assessment prepared for the Agency for Healthcare Research and Quality (Meleth, et al., 2013) found that the clinical accuracy of the PathworkDx, miRview, and CancerTypeID are similar, ranging from 85 percent to 88 percent, and that the evidence that the tests contribute to identifying a tumor of unknown origin was moderate. The assessment concluded that we do not have sufficient evidence to assess the effect of the tests on treatment decisions and outcomes. The assessment noted that most studies of these tests were funded wholly or partially by the manufacturers of these tests, and that the most urgent need in the literature is to have the clinical utility of the tests evaluated by research groups that have no evidence conflict of interest.

The presence of breast tumor cells in axillary lymph nodes is a key prognostic indicator in breast cancer. During surgery to remove breast tumors, patients often undergo biopsy of the sentinel (i.e., first) node(s) that receive lymphatic fluid from the breast. Excised sentinel lymph nodes are currently evaluated post-operatively by formalin-fixed paraffin-embedded Hematoxylin and Eosin (H&E) histology and IHC. GeneSearch™ Breast Lymph Node (BLN) assay (Veridex, LLC, Warren, NJ) is a novel method to examine the extracted sentinel lymph nodes for metastases and can provide information during surgery within 30 to 40 minutes from the time the sentinel node is removed, potentially avoiding a second operation for some patients. The GeneSearch BLN assay received FDA pre-market approval on July 16, 2007 as a qualitative in vitro diagnostic test for the rapid detection of metastases larger than 0.2 mm in nodal tissue removed from sentinel lymph node biopsies of breast cancer patients. GeneSearch BLN assay uses real time reverse transcriptase polymerase chain reaction (RT-PCR) to detect the gene expression markers, mammaglobin (MG) and cytokeratin 10 (CI19), which are abundant in breast tissue but scarce in lymph node cells. In the clinical trial conducted by Veridex, which was submitted to the FDA, the sensitivity of the GeneSearch BLN Assay was reported to be 87.6% and the specificity was 94.2% (Julian, et al., 2008). According to the product labeling,"The GeneSearch™ Breast Lymph Node (BLN) assay may be used in conjunction with sentinel lymph node biopsy for a patient who has been counseled on use of this test and has been informed of its performance. False positive results may be associated with increased morbidity. False negative and inconclusive test results may be associated with delayed axillary node dissection. Clinical studies so far are inconclusive about a benefit from treatment based on findings of breast cancer micro-metastases in sentinel lymph nodes."

Blumencranz et al (2007) compared the GeneSearch BLN assay with results from conventional histologic evaluation from 416 patients at 11 clinical sites and reported that the GeneSearch BLN assay detected 98% of metastases greater than 2 mm in size and 57% of metastasis less than 0.2 mm. False positives were reported in 4% of the cases. However, there were several limitations of this study, including the lack of a description of patient recruitment, inadequate descriptions of several analyses performed, substantial variations in test performance across sites, and ad hoc comparison of the assay to other intra-operative techniques.

Viale et al (2008) analyzed 293 lymph nodes from 293 patients utilizing the GeneSearch BLN assay. Using histopathology as the reference standard, the authors reported that the BLN assay correctly identified 51 of 52 macro-metastatic and 5 of 20 micro-metastatic sentinel lymph nodes (SLNs), with a sensitivity of 98.1% to detect metastases larger than 2 mm, 94.7% for metastases larger than 1 mm, and 77.8% for metastases larger than 0.2 mm. The overall concordance with histopathology was 90.8%, with a specificity of 95.0%, a positive predictive value of 83.6%, and a negative predictive value of 92.9%. When the results were evaluated according to the occurrence of additional metastases to non-SLN in patients with histologically positive SLNs, the assay was positive in 33 (91.7%) of the 36 patients with additional metastases and in 22 (66.6%) of the 33 patients without further echelon involvement. The authors concluded that the sensitivity of the GeneSearch BLN assay is comparable to that of the histopathologic

examination of the entire SLN by serial sectioning at 1.5 to 2 mm.

Although treatment for metastases larger than 2.0 mm is widely accepted as beneficial, clinical studies have not yet provided data for a consensus on benefit from treatment based on very small breast cancer metastases (between 0.2 mm and 2.0 mm) in SLNs. False positive results may be associated with increased morbidity, usually due to effects of axillary node dissection surgery. Patients who undergo axillary lymph node dissection (ALND) have significantly higher rates of increased swelling in the upper arm and forearm (lymphedema), pain, numbness, and motion restriction about the shoulder when compared with patients who undergo only sentinel lymph node dissection (SLND). False negative and inconclusive test results may be associated with delayed axillary node dissection. Clinical studies so far are inconclusive regarding a benefit from treatment based on findings of breast cancer micro-metastases in SLNs. Preliminary data suggest that the GeneSearch BLN assay has high specificity and moderate sensitivity when only macro-metastases are included in the analysis. The clinical significance of micro-metastases is still being debated in the literature, thus, the failure of the GeneSearch BLN assay to perform adequately in the detection of micro-metastases is of unknown significance.

A systematic evidence review by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2007) determined that the use of the GeneSearch BLN assay to detect sentinel node metastases in early stage breast cancer does not meet the TEC criteria. The assessment stated, "There are several operational issues that add difficulty to the use of the GeneSearch assay, including the need for fresh specimens (rather than putting them in formalin for permanent fixation), the learning curve involved in reducing both the percentage of invalid results (from about 15% initially to 4 - 8% for more experienced technicians) and the time to perform the test compared to alternative intra-operative techniques (which take less than 15 minutes)." Furthermore, the assessment stated "The GeneSearch assay also provides less information for staging than other intra-operative procedures, since it cannot distinguish between micro- and macro-metases. Nor can it indicate the location of the metastasis (inside or outside the node). Post-operative histology is therefore required in all cases. It is less crucial when frozen section histology is performed, since pathologists can judge the size of the metastasis and its location from this test, although distortion is possible. To summarize, the data available is inadequate to assess the clinical utility of the GeneSearch assay compared to either post-operative histology alone or to the alternative intra-operative tests such as imprint cytology and frozen section histology. In addition, the balance of benefits versus harms may require higher specificity to avoid unnecessary ALNDs and their sequelae, whereas the GeneSearch design emphasizes sensitivity."

Thus, there is insufficient evidence to make a conclusion about the effectiveness of the GeneSearch BLN assay. The FDA is requiring the manufacturer to conduct two post-approval studies. The primary objective of the first study is to estimate the positive predictive concordance between the GeneSearch BLN assay and histology as routinely practiced and the objectives of the second clinical study are (i) determine the assay turn-around-time from the time of node removal to the report of the assay result to the surgeon and (ii) determine whether the assay result was or was not received in time to make an intra-operative decision and (iii) collect data in relation to other surgical procedures during the sentinel lymph node dissection/breast surgery to determine if the assay turn-around-time resulted in longer surgery time.

Provista Life Sciences (Phoenix, AZ) has developed a laboratory test called the Biomarker Translation Test, or the BT Test, which is a test score based on a multi-protein biomarker analysis (i.e., IL-2, -6,-8,-12, TNFa, EGF, FGF, HGF, VEGF) and medical profile of an individual's risk factors for breast cancer. It is intended to be used as an adjunctive test along with other breast cancer detection modalities, however, there are no published studies of the effectiveness of this test.

Bcl-2 (B-cell CLL/lymphoma 2; BCL2) is a proto-oncogene whose protein product, bcl-2, suppresses programmed cell death (apoptosis), resulting in prolonged cellular survival without increasing cellular proliferation. Dysregulation of programmed cell death mechanisms plays a

role in the pathogenesis and progression of cancer as well as in the responses of tumors to therapeutic interventions. Many members of the Bcl-2 family of apoptosis-related genes have been found to be differentially expressed in various malignancies (Reed, 1997).

Salgia (2008) reviewed the evidence for detection of Bcl-2 in lung cancer. The author observed that Bcl-2 over-expression has been reported in 22 to 56% of lung cancers with a higher expression in squamous cell carcinoma as compared to adenocarcinoma histology. The author concluded, however, that the association of Bcl-2 expression and prognosis in non-small cell lung cancer is unclear. Multiple reports have demonstrated that Bcl-2-positive lung cancers are associated with a superior prognosis compared to those that are Bcl-2 negative. However, other studies have failed to demonstrate any survival impact with bcl-2 positivity, while over-expression has also been associated with a poorer outcome. A meta-analysis that included 28 studies examining the prognostic influence of Bcl-2 in non-small cell lung cancer concluded that over-expression of Bcl-2 was associated with a significantly better prognosis in surgically resected (hazard ratio 0.5, 95% CI 0.39-0.65).

Compton (2008) recently reviewed the evidence on the Bcl-2 oncogene and other tumor markers in colon cancer. Compton explained that Bcl-2 is a gene related to aptosis/cell suicide. Bcl-2 over-expression leading to inhibition of cell death signaling has been observed as a relatively early event in colorectal cancer development. The author concluded that the independent influence of the Bcl-2 oncogene on prognosis remains unproven, and explained that the variability in assay methodology, conflicting results from various studies examining the same factor, and the prevalence of multiple small studies that lack statistically robust, multivariate analyses all contribute to the lack of conclusive data. Compton concluded that before the Bcl-2 oncogene and certain other tumor markers can be incorporated into clinically meaningful prognostic stratification systems,"more studies are required using multivariate analysis, well-characterized patient populations, reproducible and current methodology, and standardized reagents."

Compton (2008) reviewed the evidence for intratumor microvessel density (MVD) and antibodies against CD31 in colorectal cancer. The author explained that intratumoral MVD is a reflection of tumor-induced angiogenesis. Microvessel density has been independently associated with shorter survival in some, but not all studies. A meta-analysis of all studies relating MVD expression to prognosis concluded that at least some of the variability could be explained by the different methods of MVD assessment. The author noted that there was a significant inverse correlation between immunohistochemical expression and survival when MVD was assessed using antibodies against CD31 or CD34, but not factor VIII. The author concluded, however, that there is a need for evaluation of MVD in large studies of prognostic factors using multivariate analysis; however, standard guidelines for staining, evaluation, and interpretation of MVD are lacking.

In a review, Hayes (2008) reviewed the evidence for assessing angiogenesis factors in breast cancer. The author noted that, in an early report, MVD count (as indicated by IHC staining for endothelial cells, such as factor VIII-related antigen or CD31) was a statistically significant independent predictor of both disease-free and overall survival in women with both node-negative and node-positive breast cancer. The author noted, however, that subsequent data are conflicting, with some studies confirming and others refuting the initial findings. The author stated that, "As with many of the other tumor marker studies, evaluation of angiogenesis is complicated by technical variation, reader inconsistency, and potential interaction with therapy."

Burgdorf (2006) reviewed the use of CD31 in acquired progressive lymphangioma. The author stated that special staining techniques reveal that the cells are variably positive for CD31, but that the staining patterns are too variable to be of diagnostic importance.

Some authorities have stated that CD31 staining may be useful for diagnosing angiosarcomas (Schwartz, 2008; Carsi & Sim, 2008; Fernandez & Schwartz, 2007; McMains & Gourin, 2007). CD31 immunostaining can help confirm that the tumor originates from blood vessels.

The topoisomerase II alpha gene (TOP2A) is located adjacent to the HER-2 oncogene at the chromosome location 17q12-q21 and is either amplified or deleted (with equal frequency) in a great majority of HER-2 amplified primary breast tumors and also in tumors without HER-2 amplification. Recent experimental as well as numerous, large, multi-center trials suggest that amplification (and/or deletion) of TOP2A may account for both sensitivity or resistance to commonly used cytotoxic drugs (e.g., anthracyclines) depending on the specific genetic defect at the TOP2A locus. An analysis of TOP2A aberrations in the Danish Breast Cancer Cooperative Group trial 89D (Nielsen, et al., 2008) suggested a differential benefit of adjuvant chemotherapy in patients with primary breast cancer, favoring treatment with epirubicin in patients with TOP2A amplifications, and perhaps deletions; however, the authors concluded that, "Additional studies are needed to clarify the exact importance of TOP2A deletions on outcome, but deletions have proven to be associated with a very poor prognosis."

The National Comprehensive Cancer Network (NCCN, 2008) guideline on breast cancer does not address the use of TOP2A testing.

Ghoneim et al (2008) explained that thrombospondin-1 (TSP-1) is a member of a family of five structurally related extracellular glycoproteins that plays a major role in cell-matrix and cell to cell interactions. Due to its multifunctional nature and its ability to bind to a variety of cell surface receptors and matrix proteins, TSP-1 has been identified as a potential regulator of angiogenesis and tumor progression. Data collected by Secord, et al. (2007) suggested that high THBS-1 levels may be an independent predictor of worse progression-free and overall survival in women with advanced-stage epithelial ovarian cancer. However, a phase II clinical trail (Garcia, et al., 2008) of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer reported that levels of TSP-1 were not associated with clinical outcome.

In a review on multidrug resistance in acute leukemia, List and Spier (1992) explained that the mdr1 gene or its glycoprotein product, P-glycoprotein, is detected with high frequency in secondary acute myeloid leukemia (AML) and poor-risk subsets of acute lymphoblastic leukemia. Investigations of mdr1 regulation in normal hematopoietic elements have shown a pattern that corresponds to its regulation in acute leukemia, explaining the linkage of mdr1 to specific cellular phenotypes. Therapeutic trials are now in progress to test the ability of various MDR-reversal agents to restore chemotherapy sensitivity in high-risk acute leukemias.

In a phase III multi-center randomized study to determine whether quinine would improve the survival of adult patients with de novo AML, Soary, et al. (2003) reported that neither mdr1 gene or P-glycoprotein expression influenced clinical outcome.

A phase I/II study of the MDR modulator Valspodar (PSC 833, Novartis Pharma) combined with daunorubicin and cytarabine in patients with relapsed and primary refractory acute myeloid leukemia (Gruber, et al., 2003) reported that P-glycoprotein did not give an obvious improvement to the treatment results.

Motility-related protein (MRP-1) is a glycoprotein with a sequence identical to that of CD9, a white blood cell differentiation antigen. The level of MRP-1/CD9 expression has been found in investigational studies to inhibit cell motility and low MRP-1/CD9 expression may be associated with the metastatic potential of breast cancer (Miyake, et al., 1995). CD9 immuno-expression is also being investigated as a potential new predictor of tumor behavior in patients with squamous cell carcinoma of the head and neck (Mhawech, et al., 2004) as well as other tumors (e.g., urothelial bladder carcinoma, colon cancer, lung cancer); however, prospective studies are needed to determine the clinical role of MRP-1/CD9 expression in tumors.

The National Comprehensive Cancer Network's guideline on occult primary tumors includes placental alkaline phosphatase (PLAP) as a useful marker to assist in identifying germ cell seminoma and non-seminoma germ cell tumors in unknown primary cancer (NCCN, 2009).

Myeloperoxidase (MPO), a blood protein, is a major component of azurophilic granules of

neutrophils. Myeloperoxidase analysis has been used to distinguish between the immature cells in acute myeloblastic leukemia (cells stain positive) and those in acute lymphoblastic leukemia (cells stain negative). The National Comprehensive Cancer Network guidelines on acute myeloid leukemia (AML) include MPO analysis in the classification of AML (NCCN, 2008).

Matsuo et al (2003) examined the prognostic factor of the percentage of MPO-positive blast cells for AML. Cytochemical analysis of MPO was performed in 491 patients who were registered to the Japan Adult Leukemia Study Group (AML92 study). Patients were divided into two groups using the percentage of MPO-positive blast (high [> or = 50%] and low [< 50%]). Complete remission rates were 85.4% in the former and 64.1% in the latter (p = 0.001). The OS and the disease-free survival (DFS) were significantly better in the high MPO group (48.3 versus 18.7% for OS, and 36.3 versus 20.1% for DFS, p < 0.001, respectively). Multi-variate analysis showed that both karyotype and the percentage of MPO-positive blast cells were equally important prognostic factors. The high MPO group still showed a better survival even when restricted to the intermediate chromosomal risk group or the patients with normal karyotype (p < 0.001). The OS of patients with normal karyotype in the high MPO group was almost equal to that of the favorable chromosomal risk group. The authors concluded that the percentage of MPO-positive blast cells is a simple and highly significant prognostic factor for AML patients, and especially useful to stratify patients with normal karyotype.

The most commonly used marker for hepatocellular carcinoma (HCC) is the AFP level. Des-gamma-carboxy prothrombin (DCP) (also known as "prothrombin produced by vitamin K absence or antagonism II" [PIVKA II]) has also shown promise in the diagnosis of HCC (Toyoda, et al., 2006; Ikoma, et al., 2002; Nomura, et al., 1996; Liebman, et al., 1984). In one series of 76 patients with HCC, this marker was elevated in 69 patients with a mean serum concentration of 900 mcg/L. Much lower mean values were seen in patients with chronic active hepatitis, metastatic disease to the liver, and normal subjects (10 and 42 mcg/L and undetectable, respectively) (Liebman, et al., 1984). Elevations in serum levels of DCP are less frequent in tumors less than 3 cm in size (Nakamura, et al., 2006; Weitz & Liebman, 1993;). Aoyagi, et al. (1996) and Weitz & Liebman (1993) reported that abnormal prothrombin levels do not correlate well with serum AFP.

Toyoda at al (2006) measured AFP, lens culinaris agglutinin A-reactive fraction of AFP (AFP-L3), and DCP for the evaluation of tumor progression and prognosis of patients with HCC (n = 685) at the time of initial diagnosis. Positivity for AFP > 20 ng/dL, AFP-L3 > 10% of total AFP, and/or DCP > 40 mAU/mL was determined. In addition, tumor markers were measured after treatment of HCC. Of the 685 patients, 337 (55.8%) were positive for AFP, 206 (34.1%) were positive for AFP-L3, and 371 (54.2%) were positive for DCP. In a comparison of patients positive for only 1 tumor marker, patients positive for AFP-L3 alone had a greater number of tumors, whereas patients positive for DCP alone had larger tumors and a higher prevalence of portal vein invasion. When patients were compared according to the number of tumor markers present, the number of markers present clearly reflected the extent of HCC and patient outcomes. The number of markers present significantly decreased after treatment. The authors concluded that tumor markers AFP-L3 and DCP appeared to represent different features of tumor progression in patients with HCC and that the number of tumor markers present could be useful for the evaluation of tumor progression, prediction of patient outcome, and treatment efficacy.

The National Comprehensive Cancer Network's guideline on HCC (NCCN, 2008) does not include measurement of DCP among the surveillance test options for HCC. According to NCCN guidelines, proposed surveillance for the early detection of HCC among high-risk populations (e.g., chronic hepatitis C virus-infected patients) includes liver ultrasonography every 3 to 6 months and evaluation of alkaline phosphatase, albumin, and AFP. The guidelines stated, "It is not yet clear if early detection of hepatocellular cancer with routine screening improves the percentage of patients detected with disease at a potentially curative stage, but high-risk chronic hepatitis C virus - infected patients should be considered for ongoing recurrent screening until these issues have been resolved. The level of des-gamma-carboxy-prothrombin protein induced by vitamin K absence (PIVKA-II) is also increased in many patients with hepatocellular

carcinoma. However, as is true with AFP, PIVKA-II may be elevated in patients with chronic hepatitis." Furthermore, according to Sherman (2008), DCP has not been adequately studied as a screening test for HCC and cannot be recommended at this time.

Researchers at Matritech (Newton, MA) have detected the presence of nuclear matrix protein (NMP) in the blood of women at the early stage of breast cancer, which is absent in the blood of healthy women, as well as those with fibroadenoma. NMP66 has been selected as a marker for further development and clinical trials of a test for use in the detection and monitoring of women with, or at risk for, breast cancer have been initiated (Wright and McGechan, 2003). However, there are no published studies on the effectiveness of NMP66 testing at this time.

HERmark testing has been proposed for a number of indications, including use to predict response to trastuzumab in the treatment of metastatic breast cancer. Monogram, the manufacturer of the HERmark test, claims that the test can provide a more precise and quantitative measurement of the HER2 gene than IHC and fluorescent in-situ hybridization (FISH) tests. The HERmark provides a quantitative measurement of HER2 total protein and HER2 homodimer levels, while conventional methods are an indirect measure of the HER2 gene, the manufacturer claims. The HERmark test will be offered as a CLIA-validated assay through Monogram's CAP-certified clinical laboratory. Other proposed indications for HERmark include determining the prognosis for breast cancer, and predicting treatment results in cancers other than breast cancer (e.g., ovarian prostate, head and neck, etc.). There are no current recommendations from leading medical professional organizations for the use of HERmark testing for breast cancer.

Noon et al (2010) stated that renal cell carcinoma (RCC) is the ype of kidney cancer and f ollows an unpredictable disease cours searchers reviewed 2 critical genes associated with disease p op53 and murine double minute 2 (MDM2) -- and provided a comp and critical analysis of th e literature regarding these Information was compiled by searching the PubMed databas ere published or e-published up to April 1, 2009. Search te renal cancer, renal cell carcinoma, p53, and MDM2. Full articles and dary data were examined; and, when appropriate, references we re chitional material. All studies that described assessment of p53 in renal cancer were i ncluded. The authors concluded that inpression, but not p53 mutation, is associated with redu ced ovemore rapid disease progression in RCC. There also was evidence regulation is associated with decreased disease-specific survival. of RCC stood out as unusual and will requi re further inves increased p53 expression is tightly linked with increased ion; and (ii) p atients who have tumors that display increaDM2 expression may have the poorest overall survival. Becausevidence to support the conclusion that p53 mutation is associatsurvival, it seemed clear that increased p53 expression in Rpendent of mutation. The authors stated that further investigation of the mechanisms leading to increased p53/MDM2 expression in RCC may lead to improved prognostication and to the identification of novel therapeutic interventions.

The OVA1 Test (Vermillion Inc. and Quest Diagnostics) is a serum test that is intended to help physicians determine if a woman is at risk for a malignant pelvic mass prior to biopsy or exploratory surgery, when the physician’s independent clinical and radiological evaluation does not indicate malignancy. The OVA1 Test employs an in vitro diagnostic multivariate index (IVDMIA) that combines the results of five immunoassays to produce a numerical score indicating a women's likelihood of malignancy. The OVA1 Test is intended to help physicians assess if a pelvic mass is benign or malignant in order to help determine whether to refer a woman to a gynecologic oncologist for surgery. The OVA1 Test was cleared by the FDA for use in women who meet the following criteria: over age 18, ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. The intended use of the OVA1 Test is an aid to further assess the likelihood that malignancy is present when the physician’s independent clinical and radiological evaluation does not indicate malignancy. According to the product labeling, the OVA1 Test is not intended as a screening or stand-alone diagnostic assay. There is a lack of evidence in the peer-reviewed published medical literature on the OVA1 Test.

In an editorial on the OVA1 Test, Muller (2010) stated that the role of OVA1 in a generalized

practice setting and cost-benefit ratio has yet to be determined. Furthermore, the ACOG Committee Opinion on the role of the obstetrician-gynecologist in the early detection of epithelial ovarian cancer (2011) noted that the clinical utility of the OVA1 Test "is not yet established".

The ColonSentry test (GeneNews, Toronto, Canada) measures the expression of seven genes, which serve as biomarkers to detect colorectal cancer. Interpretation of the status of these seven biomarkers is intended to assist physicians in identifying patients who have an increased current risk. According to the manufacturer, individuals assessed as having an increased current risk of colorectal cancer should consider having a colonoscopy. Individuals assessed as having a decreased current risk of colorectal cancer should discuss with their doctor further screening, including repeating ColonSentry at regular intervals. There is a lack of evidence in the peer-reviewed published medical literature on the effectiveness of colorectal cancer screening with ColonSentry. No current evidence-based guidelines from medical professional organizations or public health agencies recommend ColonSentry for colorectal cancer screening.

Prostate Px (Aureon) uses a prostate cancer patient's biopsy tissue to provide an assessment of disease severity and disease recurrence. Clinical data is integrated with an analysis of each patient's cancer using tissue histology and molecular biomarkers, such as androgen receptor, associated with disease progression. Although the manufacturer states that the results of the Prostate Px can be used in decision-making, there is a lack of evidence of the clinical utility of this test in altering the management of patients such that clinical outcomes are improved.

Post-Op Px is a prognostic test that utilizes a patented systems pathology approach to analyze prostatectomy tissue by combining cellular, molecular and clinical information to provide a thorough and more accurate picture of each patient's individual risk of prostate cancer recurrence. (Aureon, 2010). Donovan et al (2011) evaluated the performance of a systems-based risk assessment tool with standard defined risk groups and the 10 year postoperative normogram for predicting disease progression. The systems model was found to be more accurate than standard risk groups both to predict significant disease progression ($p < 0.001$) and for predicting prostate-specific antigen recurrence ($p < 0.001$). However, this study has not been replicated in the peer-reviewed literature.

An assessment by the BlueCross BlueShield Association Technology Evaluation Center (2010) concluded that tumor-cell epidermal growth factor receptor (EGFR) mutation analysis to predict response to erlotinib (Tarceva) in patients with advanced non-small cell lung cancer (NSCLC) meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria. Furthermore, guidelines from the National Comprehensive Cancer Network (NCCN, 2010) recommend EGFR testing for the following histologic subtypes of NSCLC: (i) adenocarcinoma, (ii) large cell, and (iii) NSCLC not otherwise specified. Epidermal growth factor receptor testing is not recommended for squamous cell carcinoma.

Messick et al (2010) evaluated carcinoembryonic antigen cellular adhesion molecule-7 (CEACAM-7) expression in rectal cancer as a predictive recurrence factor. A single-institution colorectal cancer database and a frozen tissue biobank were queried for rectal cancer patients. CEACAM-7 messenger RNA (mRNA) expression from normal rectal mucosa and rectal cancers was analyzed using quantitative real-time polymerase chain reaction (PCR). Expression-level differences among normal tissue, disease-free survivors, and those that developed recurrence were analyzed. A total of 84 patients were included in the study, which consisted of 37 patients with non-recurrent disease (median follow-up of 170 months), 29 patients with recurrent disease, and 18 patients with stage IV disease. CEACAM-7 expression was decreased 21-fold in rectal cancers compared with normal mucosa ($p = 0.002$). The expression levels of CEACAM-7 were relatively decreased in tumors that developed recurrence compared with non-recurrence, significantly for stage II patients (14-fold relative decrease, $p = 0.002$). For stages I-III, disease-free survival segregates were based on relative CEACAM-7 expression values ($p = 0.036$), specifically for stage II ($p = 0.018$). The authors concluded that CEACAM-7 expression is significantly decreased in rectal cancer. Expression differences between long-term survivors and

those with recurrent disease introduce a potential tumor marker to define a subset of patients who benefit most from adjuvant therapy. Moreover, they stated that additional study and validation are needed before CEACAM-7 can be applied in clinical settings.

Castro et al (2010) assessed the potential value of cofilin (CFL1) gene (main member of the invasion/metastasis pathway) as a prognostic and predictive NSCLC biomarker. Meta-analysis of tumor tissue microarray was applied to examine expression of CFL1 in archival lung cancer samples from 111 patients, and its clinicopathologic significance was investigated. The robustness of the finding was validated using another independent data set. Finally, the authors assayed in vitro the role of CFL1 levels in tumor invasiveness and drug resistance using 6 human NSCLC cell lines with different basal degrees of CFL1 gene expression. Cofilin levels in biopsies discriminated between good and bad prognosis at early tumor stages (IA, IB, and IIA/B), where high CFL1 levels are correlated with lower overall survival rate ($p < 0.0001$). Biomarker performance was further analyzed by IHC, hazard ratio ($p < 0.001$), and receiver-operating characteristic curve (area = 0.787; $p < 0.001$). High CFL1 mRNA levels and protein content are positively correlated with cellular invasiveness (determined by Matrigel Invasion Chamber System) and resistance (2-fold increase in drug 50 % growth inhibition dose) against a list of 22 alkylating agents. Hierarchical clustering analysis of the CFL1 gene network had the same robustness for stratified NSCLC patients. The authors concluded that these findings indicated that the CFL1 gene and its functional gene network can be used as prognostic biomarkers for NSCLC and could also guide chemotherapeutic interventions. Moreover, prospective, large-scale, randomized clinical trials are needed to establish the role of CFL1 as a prognostic and drug resistance marker for NSCLC.

The *Early*CDT-Lung (Oncimmune, De Soto, KS) test measures antibodies to 6 tumor-associated antigens: p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1, and SOX2. Elevation of any one of the panel of immuno-biomarkers above a predetermined cut-off value suggests that a tumor might be present. The test is designed to be used in conjunction with diagnostic imaging. High-risk individuals with a positive *Early*CDT-Lung would have additional testing such as a CT scan or the test would be used as a follow-up test for indeterminate lung nodules identified by CT.

Deeb et al (2004) stated that E-cadherin (E-cad) and epidermal growth factor receptor (EGFR) are important cell adhesion and signaling pathway mediators. Theyr reported the results of a study which aimed to assess their expression in lung adenocarcinoma (AdC) and squamous cell carcinoma (SCC) and their association with clinicopathologic variables. Two to three cores from 130 resectable lung cancers (stages I-IIIA) were arrayed into three blocks using a Beecher system. Markers expression and coexpression were analyzed against clinicopathologic variables (age, gender, smoking status, performance status, weight loss, histology, grade, stage, and lymph node involvement) and patient survival. For E-cad, 65 cases (55%) were positive (+), 53 (45%) were negative (-); and for EGRF, 43 cases (34%) were (+), and 83 (66%) were (-). There was no significant association between E-cad or EGFR, and any of the clinicopathologic variables except for an association between EGFR(+) and SCC histologic type. Both negative and cytoplasmic staining of E-cad correlated with shorter patient survival with P=0.008 and 0.002, respectively. EGFR expression did not correlate with patient survival, but, patients with E-cad(-)/EGFR(+) phenotype had poorer survival than those with E-cad(+)/EGFR(-) (P=0.026). The authors concluded that lung AdC and SCC may be stratified based on expression of E-cad and EGFR with the E-cad(-)/EGFR(+) expression having a worse disease outcome.

Yoshida et al (2011) report that a subset of lung cancers harbors an EML4-ALK (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase) gene fusion, and they examined 15 lung adenocarcinomas with reverse-transcriptase polymerase chain reaction-proven EML4-ALK fusion transcripts and 30 ALK-negative cases. Positive rearrangement signals (splits or isolated 3' signals) were identified in 13 to 78% (mean ± SD, 41% ± 19%) of tumor cells in the ALK-positive cohort and in 0 to 15% (mean ± SD, 6% ± 4%) of cells in the ALK-negative cohort. Sensitivity was at 93% and specificity at 100%. The only false-negative tumor having only 13% CISH-positive cells displayed predominantly (76%) isolated 5' signals unaccompanied by 3' signals. FISH showed largely similar signal profiles, and the results were completely

concordant with CISH.The authors stated that they have successfully introduced CISH for diagnosing EML4-ALK-positive lung adenocarcinoma. This method allows simultaneous visualization of genetics and tumor cytomorphology and facilitates the molecular evaluation and could be applicable in clinical practice to detect lung cancer that may be responsive to ALK inhibitors.

Ellis et al (2011) conducted a systematic review and a consensus meeting of Canadian lung cancer oncologists and pathologists to make recommendations on the use of biomarkers in NSCLC. The articles were reviewed by pairs of oncologists and pathologists to determine eligibility for inclusion. Ten oncologists and pathologists reviewed and summarized the literature at a meeting attended by 37 individuals. The findings included that there is some evidence that histology is prognostic for survival as well as evidence from multiple randomized clinical trials to recommend the following: histologic subtype is predictive of treatment efficacy and for some agents toxicity. Immunohistochemistry testing should be performed on NSCLC specimens that cannot be classified accurately with conventional H&E staining. As EGFR mutations are predictive of benefit from tyrosine kinase inhibitors, diagnostic NSCLC samples should be routinely tested for EGFR-activating mutations. Clinical data on K-RAS mutations are inconsistent, therefore testing is not recommended. There is insufficient evidence to recommend other biomarker testing. No biomarkers to date reliably predict improved efficacy for anti-VEGF therapy. The authors concluded that outine assessment for EML4/ALK mutations is not recommended at present, although emerging data suggest that it may become valuable in the near future.

Boyle et al (2011) reported the sensitivity and specificity of an panel of 6 tumor-related antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin) in patients with lung cancer. Three cohorts of patients with newly diagnosed lung cancer were identified: group 1 (n = 145), group 2 (n = 241) and group 3 (n = 269). Patients were individually matched by gender, age and smoking history to a control individual with no history of malignant disease. Serum samples were obtained after diagnosis but before any anticancer treatment. Autoantibody levels were measured against the panel of 6 tumor-related antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1 and SOX2). Assay sensitivity was tested in relation to demographic variables and cancer type/stage. The autoantibody panel demonstrated a sensitivity/specificity of 36 %/91 %, 39 %/89 % and 37 %/90 % in groups 1, 2 and 3, respectively, with good reproducibility. There was no significant difference between different lung cancer stages, indicating that the antigens included covered the different types of lung cancer well. The authors concluded that the assay confirms the value of an autoantibody panel as a diagnostic tool and offers a potential system for monitoring patients at high-risk of lung cancer.

There is insufficient evidence of the effectiveness of the *Early*CDT-Lung as a screening test for the early detection of lung cancer. Systematic screening for lung cancer is not unequivocally recommended by any major professional organization. The USPSTF (2004) concluded that current evidence was insufficient to recommend for, or against, screening for lung cancer. Whether earlier detection of lung cancer will translate to a mortality benefit remains unclear.

Shanmugan and co-workers (2010) stated that mucin 4 (MUC4) is aberrantly expressed in colorectal adenocarcinomas (CRCs) but its prognostic value is unknown. Archival tissue specimens collected from 132 CRC patients who underwent surgical resection without pre-surgery or pos-tsurgery therapy were evaluated for expression of MUC4 by using a mouse monoclonal antibody and horseradish peroxidase. MUC4 expression levels were correlated with clinicopathologic features and patient survival. Survival was estimated by both uni-variate Kaplan-Meier and multi-variate Cox regression methods. In both normal colonic epithelium and CRCs, MUC4 staining was localized primarily in the cytoplasm. The optimal immunostaining cut-off value (greater than or equal to 75 % positive cells and an immunostaining score greater than or equal to 2.0), which was derived by using the bootstrap method, was used to categorize CRCs into groups of high expression (33 of 132 patients; 25 %) or low expression (99 of 132 patients; 75 %). Patients who had early stage tumors (stages I and II) with high MUC4

expression had a shorter disease-specific survival (log-rank; p = 0.007) than patients who had with low expression. Patients who had advanced-stage CRCs (stages III and IV) did not demonstrate such a difference (log-rank; p = 0.108). Multi-variate regression models that were generated separately for patients with early stage and advanced-stage CRC confirmed that increased expression of MUC4 was an independent indicator of a poor prognosis only for patients who had early stage CRCs (HR 3.77; 95 % CI: 1.46 to 9.73). The authors stated that aftr validating these findings in larger retrospective and prospective studies, a stage-based anayses could establish the utility of MUC4 as a prognostic molecular marker of early stage CRC.

Ratner et al (2010) stated that ovarian cancer (OC) is the single most deadly form of women's cancer, typically presenting as an advanced disease at diagnosis in part due to a lack of known risk factors or genetic markers of risk. The KRAS oncogene and altered levels of the microRNA (miRNA) let-7 are associated with an increased risk of developing solid tumors. In this study, these researchers investigated a hypothesized association between an increased risk of OC and a variant allele of KRAS at rs61764370, referred to as the KRAS-variant, which disrupts a let-7 miRNA binding site in this oncogene. Specimens obtained were tested for the presence of the KRAS-variant from non-selected OC patients in 3 independent cohorts, 2 independent ovarian case-control studies, and OC patients with hereditary breast and ovarian cancer syndrome (HBOC) as well as their family members. The results indicated that the KRAS-variant is associated with more than 25 % of non-selected OC cases. Furthermore, these researchers found that it is a marker for a significant increased risk of developing OC, as confirmed by 2 independent case-control analyses. Lastly, they determined that the KRAS-variant was present in 61 % of HBOC patients without BRCA1 or BRCA2 mutations, previously considered uninformative, as well as in their family members with cancer. These findings supported the hypothesis that the KRAS-variant is a genetic marker for increased risk of developing OC, and they suggested that the KRAS-variant may be a new genetic marker of cancer risk for HBOC families without other known genetic abnormalities.

Hollestelle et al (2011) noted that recently, a variant allele in the 3'UTR of the KRAS gene (rs61764370 T>G) was shown to be associated with an increased risk for developing non-small cell lung cancer, as well as OC, and was most enriched in OC patients from HBOC families. This functional variant has been shown to disrupt a let-7 miRNA binding site leading to increased expression of KRAS in vitro. In the current study, these investigators genotyped this KRAS-variant in breast cancer index cases from 268 BRCA1 families, 89 BRCA2 families, 685 non-BRCA1/BRCA2 families, and 797 geographically matched controls. The allele frequency of the KRAS-variant was found to be increased among patients with breast cancer from BRCA1, but not BRCA2 or non-BRCA1/BRCA2 families as compared to controls. As BRCA1 carriers mostly develop ER-negative breast cancers, these researchers also examined the variant allele frequency among indexes from non-BRCA1/BRCA2 families with ER-negative breast cancer. The prevalence of the KRAS-variant was, however, not significantly increased as compared to controls, suggesting that the variant allele not just simply associates with ER-negative breast cancer. Subsequent expansion of the number of BRCA1 carriers with breast cancer by including other family members in addition to the index cases resulted in loss of significance for the association between the variant allele and mutant BRCA1 breast cancer. In this same cohort, the KRAS-variant did not appear to modify breast cancer risk for BRCA1 carriers. More importantly, results from the current study suggested that KRAS-variant frequencies might be increased among BRCA1 carriers, but solid proof requires confirmation in a larger cohort of BRCA1 carriers.

ProOnc TumorSourceDx test is designed to identify tissue or origin for metastastic tumor. It identifies 25 possible classes of tissue origin corresponding to 17 distinct tissues and organs. It requires only 48 microRNAs to identify tissue of origin based on microRNA expression levels. However, there is insufficient evidence regarding its clinical value as tumor markers.

Cocco and associates (2010) examined the expression of serum amyloid A (SAA) in endometrial endometrioid carcinoma and evaluated its potential as a serum biomarker. SAA gene and protein expression levels were evaluated in endometrial endometrioid carcinoma and normal endometrial

tissues, by real-time PCR, IHC, and flow cytometry. SAA concentration in 194 serum samples from 50 healthy women, 42 women with benign diseases, and 102 patients including 49 grade 1, 38 grade 2, and 15 grade 3 endometrial endometrioid carcinoma was also studied by a sensitive bead-based immunoassay. SAA gene expression levels were significantly higher in endometrial endometrioid carcinoma when compared with normal endometrial tissues (mean copy number by real-time PCR = 182 versus 1.9; p = 0.001). IHC revealed diffuse cytoplasmic SAA protein staining in poorly differentiated endometrial endometrioid carcinoma tissues. High intra-cellular levels of SAA were identified in primary endometrial endometrioid carcinoma cell lines evaluated by flow cytometry, and SAA was found to be actively secreted in vitro. SAA concentrations (microg/ml) had medians of 6.0 in normal healthy women and 6.0 in patients with benign disease (p = 0.92). In contrast, SAA values in the serum of endometrial endometrioid carcinoma patients had a median of 23.7, significantly higher than those of the healthy group (p = 0.001) and benign group (p = 0.001). Patients harboring G3 endometrial endometrioid carcinoma were found to have SAA concentrations significantly higher than those of G1/G2 patients. The authors concluded that SAA is not only a liver-secreted protein, but is also an endometrial endometrioid carcinoma cell product. SAA is expressed and actively secreted by G3 endometrial endometrioid carcinoma, and it is present in high concentration in the serum of endometrial endometrioid carcinoma patients. SAA may represent a novel biomarker for endometrial endometrioid carcinoma to monitor disease recurrence and response to therapy. They stated that additional studies are needed to validate these findings.

Monzon et al (2009) stated that malignancies found in unexpected locations or with poorly differentiated morphologies can pose a significant challenge for tissue of origin determination. Current histologic and imaging techniques fail to yield definitive identification of the tissue of origin in a significant number of cases. The aim of this study was to validate a predefined 1,550-gene expression profile for this purpose. Four institutions processed 547 frozen specimens representing 15 tissues of origin using oligonucleotide microarrays were used in this study. Half of the specimens were metastatic tumors, with the remainder being poorly differentiated and undifferentiated primary cancers chosen to resemble those that present as a clinical challenge. In this blinded multi-center validation study the 1,550-gene expression profile was highly informative in tissue determination. The study found overall sensitivity (positive percent agreement with reference diagnosis) of 87.8 % (95 % CI: 84.7 % to 90.4 %) and overall specificity (negative percent agreement with reference diagnosis) of 99.4 % (95 % CI: 98.3 % to 99.9 %). Performance within the subgroup of metastatic tumors (n = 258) was found to be slightly lower than that of the poorly differentiated and undifferentiated primary tumor subgroup, 84.5 % and 90.7 %, respectively (p = 0.04). Differences between individual laboratories were not statistically significant. The authors concluded that this study represents the first adequately sized, multi-center validation of a gene-expression profile for tissue of origin determination restricted to poorly differentiated and undifferentiated primary cancers and metastatic tumors. These results indicate that this profile should be a valuable addition or alternative to currently available diagnostic methods for the evaluation of uncertain primary cancers.

Monzon and Koen (2010) stated that tumors of uncertain or unknown origin are estimated to constitute 3 % to 5 % of all metastatic cancer cases. Patients with these types of tumors show worse outcomes when compared to patients in which a primary tumor is identified. New molecular tests that identify molecular signatures of a tissue of origin have become available. The authors reviewed the literature on existing molecular approaches to the diagnosis of metastatic tumors of uncertain origin and discuss the current status and future developments in this area. Published peer-reviewed literature, available information from medical organizations (NCCN), and other publicly available information from tissue-of-origin test providers and/or manufacturers were used in this review. The authors concluded that molecular tests for tissue-of-origin determination in metastatic tumors are available and have the potential to significantly impact patient management. However, available validation data indicate that not all tests have shown adequate performance characteristics for clinical use. Pathologists and oncologists should carefully evaluate claims for accuracy and clinical utility for tissue-of-origin tests before using test results in patient management. The personalized medicine revolution includes the use of molecular tools for identification/confirmation of the site of origin for metastatic tumors, and in

the future, this strategy might also be used to determine specific therapeutic approaches.

Anderson and Weiss (2010) noted that pathologists use various stains to identify the site of tissue of origin for metastatic tumors that are poorly or undifferentiated cancers of unknown or uncertain origin. Although clinicians believe that immunostains contribute greatly to the probable primary site among 3 or more possibilities, objective evidence has not been convincingly presented. This meta-analysis reviews the objective evidence supporting this practice and summarizes the performance reported in 5 studies published between 1993 and 2007. A literature search was conducted to identify IHC performance studies published since 1990 that were masked, included more than 3 tissues types, and used more than 50 specimens. The 5 studies found in this search were separated into 2 subgroups for analysis: those, which included only metastatic tumors (n = 368 specimens) and the blended studies, which combined primary tumors and metastases (n = 289 specimens). The meta-analysis found that IHCs provided the correct tissue identification for 82.3 % (95 % CI: 77.4 % to 86.3 %) of the blended primary and metastatic samples and 65.6 % (95 % CI: 60.1 % to 70.7 %) of metastatic cancers. This difference is both clinically and statistically significant. The authors concluded that this literature review confirms that there is still an unmet medical need in identification of the primary site of metastatic tumors. It establishes minimum performance requirements for any new diagnostic test intended to aid the pathologist and oncologist in tissue of origin determination.

According to the manufacturer, the Caris Life Sciences molecular profiling test, Caris Target Now, examines the genetic and molecular changes unique to a patient's tumor so that treatment options may be matched to the tumor's molecular profile. The manufacturer states that the Caris Target Now test is performed after a cancer diagnosis has been established and the patient has exhausted standard of care therapies or if questions in therapeutic management exist. Using tumor samples obtained from a biopsy, the tumor is examined to identify biomarkers that may have an influence on therapy. Using this information, Caris Target Now is intended to provide information on the drugs that will be more likely to produce a positive response. The manufacturer states that Caris Target Now can be used with any solid cancer such as lung cancer, breast cancer, and prostate cancer.

There is insufficient evidence to support the use of Caris Target Now molecular profiling. A study (Von Hoff, et al., 2010) compared the progression-free survival (PFS) of patients with refractory metastatic cancers using a treatment regimen selected by Caris Target Now molecular profiling of the patient's tumor with the PFS for the most recent regimen on which the patient had experienced progression. The investigators prespecified that a molecular profiling approach would be deemed of clinical benefit for the individual patient who had a PFS ratio (defined as a ratio of PFS on molecular profiling-selected therapy to PFS on prior therapy) of greater than or equal to 1.3. In 86 patients who had molecular profiling attempted, there was a molecular target detected in 84 (98%). Sixty-six of the 84 patients were treated according to molecular profiling results. Eighteen (27%) of 66 patients had a PFS ratio of greater than or equal 1.3 (95% confidence interval 17% to 38%). Therefore, the null hypothesis (that less than or equal to 15% of this patient population would have a PFS ratio of greater than or equal to 1.3) was rejected. The authors concluded that, in 27% of patients, the molecular profiling approach resulted in a longer PFS on an molecular profiling-suggested regimen than on the regimen on which the patient had just experienced progression. An accompanying editorial (Doroshow, 2010) noted that the trial had a number of significant limitations, including uncertainty surrounding the achievement of time to progression (the study’s primary endpoint), and a lack of a randomized design for this trial.

Kumaresan et al (2010) reviewed 1034 cases of morphologically difficult prostate cancer, which were divided into benign (585), malignant (399) and suspicious (50) and evaluated using CoA racemase (P504S) and HMWCK (34betaE12). Forty nine suspicious cases were resolved by using both markers whereas 1 case was resolved by further support with CD68. The original diagnosis was changed in 15 of 50 suspicious cases from benign to malignant, one case from benign to high grade PIN, and in one case from malignant to benign. The authors concluded that a combination of HMWCK and AMACR is of value in combating morphologically suspicious

cases and that although the sensitivity and specificity of HMWCK and AMACR in this study were high, “it should be used with caution, keeping in mind all their pitfalls and limitations.”

Murray et al (2010) studied P504S expressing circulating prostate cells as a marker for prostate cancer. The authors stated that PSA is the only biomarker routinely used in screening. This study aimed to develop a system to test the presence of circulating prostate cells in men without a diagnosis of prostate cancer in realation with age, serum PSA levels and prostate biopsy by determining the co-expression of several markers such as CD82, HER-2 and matrix metalloproteinase 2 MMP-20. The results indicated that among 409 men screened for prostate cancer 16.6% were positive for circulating prostate cells. The authors concluded that the study of circulating prostate cells with various markers could be a useful complementary screening test for prostate cancer in men with increased PSA level.

Prolaris (Myriad Genetics, Salt Lake City, UT) uses archived tumor specimens as the mRNA source, reverse transcriptase polymerase chain reaction amplification, and a low density RTPCR array platform. Prolaris is used to quantify expression levels of 31 cell cycle progression (CCP) genes and 15 housekeeper genes to generate a CCP score. An assessment by the BlueCross BlueShield Association Technology Evaluation Center (TEC) concluded that direct evidence is insufficient to establish the analytic validity, clinical validity, or clinical utility of the Prolaris test.

The NCCN Task Force issued a report in November of 2011 which updated their position regarding molecular markers for diagnosis, prognosis, prediction, and companion diagnostic markers (Febbo et. al., 2011). As a result of these recommendations, use of MGMT, IDH mutation and 1p/19q codeletion are now established for glioma. Also, use of ALK gene fusion has been established for non-small cell lung cancer. The updated NCCN guidelines have not yet established the efficacy of ColoPrint, CIMP, LINE-1 hypomethylation, or Immune cells for colon cancer. Similarly, the efficacy of FLT3-TKD mutation, WT1 mutation, RUNX1 mutation, MLL-PTD, IDH1 mutation, IDH2 R172, and IDH2 codon 140 mutation has not been established for use in acute myeloid leukemia.

The DecisionDx-UM test determines the molecular signature of a patient's tumor. The DecisionDx-UM test is also known as the gene expression profile test (GEP) for uveal melanoma. The results of the test provide knowledge regarding the risk of near term metastasis (5 years). Tumors with a Class 1 signature are associated with a good prognosis and a low potential to spread (or metastasize), while tumors with a Class 2 signature have a high potential to spread. There is a lack of adequate published evidence on the clinical utility of this test. Current guidelines on melanoma from the National Comprehensive Cancer Network make no recommendation for this test.

Guidelines on thyroid carcinoma from the National Comprehensive Cancer Network (NCCN, 2013) state: "Molecular diagnostics to detect individual mutations (eg, BRAF, RET/PTC, RAS, PAX8/PPAR gamma) or pattern recognition approaches using molecular classifiers may be useful in the evaluation of FNA samples that are indeterminate. For the 2013 update, the NCCN Panel added recommendations to consider molecular diagnostics for the evaluating FNA results that are suspicious for 1) follicular or Hürthle cell neoplasms; or 2) follicular lesions of undetermined significance (see Nodule Evaluation in the NCCN Thyroid Carcinoma algorithm). (63,64) Rather than proceeding to immediate surgical resection to obtain a definitive diagnosis in these categories, patients can be followed with observation if the application of a specific molecular diagnostic test results in a predicted risk of malignancy that is comparable to the rate seen in cytologically benign thyroid FNAs (approximately 5% or less)." For support, the guidelines reference validation studies of the Afirma Thyroid FNA Analysis (Alexander, et al., 2012; Chudova, et al., 2010). These studies demonstrate that this molecular diagnostic meets NCCN threshold of predicting malignancy of 5 percent or less (i.e., a negative predictive value of 95 percent), allowing physicians to observe an indeterminate thyroid nodule in lieu of surgery.

Quest Diagnostics offers a molecular test panel designed to help physicians determine if a thyroid

gland is cancerous and requires surgical removal. The test includes mutations associated with four gene markers indicated by the American Thyroid Association for the clinical management of indeterminate thyroid biopsies. According to Quest Diagnostics, the Quest Diagnostics Thyroid Cancer Mutation Panel aids in detecting cancer in thyroid biopsies which are found to be indeterminate for cancer by current cytology test methods. Approximately 15% to 20% of these biopsies, which are collected by fine needle aspiration (FNA), produce indeterminate results. An unclear result may increase the risk that a physician, in an abundance of caution, will biopsy additional tissue using a larger needle or surgically remove part or all of a thyroid suspected of having cancer that is later diagnosed as healthy. About 300,000 thyroid FNA biopsy procedures are performed annually in the United States. The panel identifies mutations of the molecular markers BRAF V600E, RAS, RET/PTC, and PAX8/PPAR gamma, which are associated with papillary and follicular thyroid cancer, two common forms of the disease. The manufacturer states that practice guidelines from the American Thyroid Association recommend that physicians consider these markers as aids in clinical management of patients with indeterminate biopsy test results. Results of a Quest Diagnostics study found that 90 of 149 FNA specimens, or about 60%, had mutations of one or more of the four markers tested by the new panel (Reitz, et al., 2014). The authors of the study stated that the presence of the four markers was generally mutually exclusive, suggesting potential value in a hierarchical screening strategy for samples with limited tissue. According to the American Cancer Society, about two tests in every 10 may need to be repeated because the sample does not contain enough cells for testing.

The Alberta Provincial Thoracic Tumour Team's clinical practice guideline on "Non-small cell lung cancer stage IV" (2011) stated that "First-line monotherapy with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib is recommended for patients with EGFR mutation-positive NSCLC. Testing for EGFR mutations should take place for all eligible patients with advanced NSCLC and adenocarcinoma histology who are being considered for first-line therapy with gefitinib, irrespective of their gender, ethnicity, and smoking status".

Gao et al (2012) stated that gefiinib and erlotinib are 2 similar small molecules of selective and reversible epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), which have been approved for second-line or third-line indication in previously treated advanced NSCLC patients. The results of comparing the EGFR-TKI with standard platinum-based doublet chemotherapy as the first-line treatment in advanced NSCLC patients with activated EGFR mutation were still controversial. A meta-analysis was performed to derive a more precise estimation of these regimens. Finally, 6 eligible trials involved 1,021 patients were identified. The patients receiving EGFR-TKI as front-line therapy had a significantly longer PFS than patients treated with chemotherapy [median PFS was 9.5 versus 5.9 months; HR = 0.37; 95 % CI: 0.27 to 0.52; $p < 0.001$]. The overall response rate (ORR) of EGFR-TKI was 66.60 %, whereas the ORR of chemotherapy regimen was 30.62 %, which was also a statistically significant favor for EGFR-TKI [relative risk (RR) = 5.68; 95 % CI: 3.17 to 10.18; $p < 0.001$]. The OS was numerically longer in the patients received EGFR-TKI than patients treated by chemotherapy, although the difference did not reach a statistical significance (median OS was 30.5 versus 23.6 months; HR = 0.94; 95 % CI: 0.77 to 1.15; $p = 0.57$). Comparing with first-line chemotherapy, treatment of EGFR-TKI achieved a statistical significantly longer PFS, higher ORR and numerically longer OS in the advanced NSCLC patients harboring activated EGFR mutations, thus, it should be the first choice in the previously untreated NSCLC patients with activated EGFR mutation.

The BCBS TEC's assessment on "Multi-Analyte Testing for the Evaluation of Adnexal Masses" (2013) concluded that OVA1 does not meet TEC criteria. It noted that "evidence regarding the effect of OVA1 and ROMA and effects on health outcomes is indirect, and based on studies of diagnostic performance of the tests in patients undergoing surgery for adnexal masses. Although the studies show improvements in sensitivity and worsening of specificity with the use of the tests in conjunction with clinical assessment, there are problems in concluding that this results in improved health outcomes. The clinical assessment performed in the studies is not well characterized. Although OVA1 improves sensitivity, specificity declines so much that most patients test positive. ROMA does not improve the sensitivity of testing to a great extent.

Underlying these issues is some uncertainty regarding the benefit of initial treatment by a gynecologic oncologist beyond the need for reoperation is some cases".

Krijgsman et al (2012) noted that classification of breast cancer into molecular subtypes maybe important for the proper selection of therapy, as tumors with seemingly similar histopathological features can have strikingly different clinical outcomes. Herein, these researchers reported the development of a molecular subtyping profile (BluePrint), which enables rationalization in patient selection for either chemotherapy or endocrine therapy prescription. An 80-Gene Molecular Subtyping Profile (BluePrint) was developed using 200 breast cancer patient specimens and confirmed on 4 independent validation cohorts (n = 784). Additionally, the profile was tested as a predictor of chemotherapy response in 133 breast cancer patients, treated with T/FAC neoadjuvant chemotherapy. BluePrint classification of a patient cohort that was treated with neoadjuvant chemotherapy (n = 133) shows improved distribution of pathological Complete Response (pCR), among molecular subgroups compared with local pathology: 56 % of the patients had a pCR in the Basal-type subgroup, 3 % in the MammaPrint low-risk, luminal-type subgroup, 11 % in the MammaPrint high-risk, luminal-type subgroup, and 50 % in the HER2-type subgroup. The group of genes identifying luminal-type breast cancer is highly enriched for genes having an Estrogen Receptor binding site proximal to the promoter-region, suggesting that these genes are direct targets of the Estrogen Receptor. Implementation of this profile may improve the clinical management of breast cancer patients, by enabling the selection of patients who are most likely to benefit from either chemotherapy or from endocrine therapy.

Furthermore, there is no information regarding BluePrint/molecular subtyping from NCCN's clinical practice guideline on "Breast cancer" (Version 2.2013).

Yu and colleagues (2012) stated that the excision repair cross-complementation group 1 (ERCC1) plays an essential role in DNA repair and has been linked to resistance to platinum-based anticancer drugs among advanced NSCLC patients. These investigators examined if ERCC1 Asn118Asn and C8092A genetic variants are associated with treatment response of platinum chemotherapy. They performed a meta-analysis using 10 eligible cohort studies (including 11 datasets) with a total of 1,252 NSCLC patients to summarize the existing data on the association between the ERCC1 Asn118Asn and C8092A polymorphisms and response to platinum regiments. Odds ratio or hazard ratio with 95 % CI were calculated to estimate the correlation. These researchers found that neither ERCC1 C8092A polymorphism nor Asn118Asn variant is associated with different response of platinum-based treatment among advanced NSCLC patients. Additionally, these 2 genetic variants are not related to treatment response in either Caucasian patients or Asian patients. The authors concluded that the findings of this meta-analysis indicated that the ERCC1 Asn118Asn and C8092A polymorphisms may not be good prognostic biomarkers for platinum-based chemotherapy in patients with stage III-IV NSCLC.

Wang et al (2012) performed a meta-analysis by using 20 eligible studies to examine polymorphisms of ERCC1, GSTs, TS and MTHFR in predicting clinical outcomes (response rate, OS and toxicity) of gastric cancer (GC) patients treated with platinum/5-Fu-based chemotherapy. The association was measured using random/fixed effect odds ratios (ORs) or hazard ratios (HRs) combined with their 95 % CIs according to the studies' heterogeneity. Statistical analysis was performed with the software STATA 9.0 package. No significant association was found between response rate and genetic polymorphism in TS, MTHFR, ERCC1, GSTM1 and GSTP1. However, response rate was higher in GSTT1 (+) genotype compared with GSTT1 (-) genotype (T-/T+: OR = 0.67, 95 % CI: 0.47 to 0.97). With regard to long-term outcomes, these researchers observed a significant longer OS in TS 3R/3R [(2R2R+2R3R)/3R3R: HR = 1.29, 95 % CI: 1.02 to 1.64] and GSTP1 GG/GA [(GG+AG)/AA: HR = 0.51, 95 % CI: 0.39 to 0.67] genotypes. In addition, significant association was demonstrated between toxicity and genetic polymorphism in TS, MTHFR and GSTP1 in included studies. The authors concluded that polymorphisms of ERCC1, GSTs, TS and MTHFR were closely associated with clinical outcomes of GC patients treated with platinum/5-Fu-based chemotherapy. Moreover, they state that studies with large sample size using the method of multi-variant analyses may help us to give more persuasive data on the putative association in

future.

In a meta-analysis, Gong and colleagues (2012) examined if RRM1 expression is associated with the clinical outcome of gemcitabine-containing regimen in advanced NSCLC. An electronic search was conducted using the databases PubMed, Medline, EMBASE, Cochrane library and CNKI, from inception to May, 2011. A systemic review of the studies on the association between RRM1 expression in advanced NSCLC and clinical outcome of gemcitabine-containing regimen was performed. Pooled odds ratios (OR) for the response rate, weighted median survival and time to progression were calculated using the software Revman 5.0. The search strategy identified 18 eligible studies (n = 1,243). Response rate to gemcitabine-containing regimen was significantly higher in patients with low/negative RRM1 (OR = 0.31, 95 % CI: 0.21 to 0.45, $p < 0.00001$). Non-small cell lung cancer SCLC patients with low/negative RRM1 who were treated with gemicitabine-containing regimen survived 3.94 months longer (95 % CI: 2.15 to 5.73, $p < 0.0001$) and had longer time to progression for 2.64 months (95 % CI: 0.39 to 4.89, $p = 0.02$) than those with high/positive RRM1. The authors concluded that low/negative RRM1 expression in advanced NSCLC was associated with higher response rate to gemcitabine-containing regimen and better prognosis. Moreover, they stated that large phase III randomized trials are needed to identify whether RRM1 detection is clinically valuable for predicting the prognosis and sensitivity to gemcitabine-containing regimen in advanced NSCLC.

Friboulet et al (2013) stated that the ERCC1 protein is a potential prognostic biomarker of the effectiveness of cisplatin-based chemotherapy in (NSCLC. Although several ongoing trials are evaluating the level of expression of ERCC1, no consensus has been reached regarding a method for evaluation.

Besse et al (2013) noted that somatic ERCC1 and ribonucleotide reductase M1 (RRM1) expression levels have been extensively explored as markers of DNA repair capacity in tumor cells. Although low ERCC1 and/or RRM1 expression is generally associated with sensitivity to platinum, the results published in retrospective and prospective studies are not always consistent. These researchers examined the function of these 2 biomarkers as well as the tools available for their assessment and the associated technical issues. Their prognostic and predictive values were summarized and considered in terms of customizing systemic therapy according to biomarker (ERCC1 and RRM1) expression levels. The authors discussed why the use of both markers should at this point be restricted to clinical research.

Trock et al (2012) noted that hyper-methylation of genes such as glutathione-S-transferase P1 (GSTP1) and adenomatous polyposis coli (APC) occurs with high frequency in prostate tumor tissue but is much less common in the benign prostate; however, the potential value of gene methylation biomarkers as an adjunct to biopsy histopathology has had little study. When measured in histologically benign prostate biopsy tissue, APC gene hyper-methylation was found to have high negative-predictive value (NPV) and high sensitivity. GSTP1 hype-rmethylation was found to have lower performance than APC. These investigators evaluated the performance of DNA methylation biomarkers in the setting of repeat biopsy in men with an initially negative prostate biopsy but a high index of suspicion for missed prostate cancer. They prospectively evaluated 86 men with an initial histologically negative prostate biopsy and high-risk features. All men underwent repeat 12-core ultrasonography-guided biopsy. DNA methylation of GSTP1 and APC was determined using tissue from the initially negative biopsy and compared with histology of the repeat biopsy. The primary outcome was the relative NPV of APC compared with GSTP1, and its 95 % CI. On repeat biopsy, 21/86 (24 %) men had prostate cancer. APC and GSTP1 methylation ratios below the threshold (predicting no cancer) produced a NPV of 0.96 and 0.80, respectively. The relative NPV was 1.2 (95 % CI: 1.06 to 1.36), indicating APC has significantly higher NPV. Methylation ratios above the threshold yielded a sensitivity of 0.95 for APC and 0.43 for GSTP1. Combining both methylation markers produced a performance similar to that of APC alone. APC methylation patterns were consistent with a possible field effect or occurrence early in carcinogenesis. The authors concluded that APC methylation provided a very high NPV with a low percentage of false-negatives, in the first prospective study to evaluate performance of DNA methylation markers in a clinical cohort of

men undergoing repeat biopsy. They stated that the potential of APC methylation to reduce unnecessary repeat biopsies warrants validation in a larger prospective cohort.

In a systematic review and meta-analysis, Yu and colleagues (2013) examined the association between GSTP1 Ile105Val polymorphism and prostate cancer (PCa) in different inheritance models. A total of 13 eligible studies were pooled into this meta-analysis. There was significant association between the GSTP1 Ile158Val variant genotypes and PCa for Ile/Ile versus Val/Val comparison [odds ratio (OR) = 0.705; I 2 = 63.7 %; 95 % CI: 0.508 to 0.977], Ile/Val versus Val/Val comparison (OR = 0.736; I 2 = 8.0 %; 95 % CI: 0.613 to 0.883), and dominant model (OR = 0.712; I 2 = 45.5 %; 95 % CI: 0.555 to 0.913). However, no associations were detected for other genetic models. In the stratified analysis by ethnicity, significant associations between GSTP1 Ile105Val polymorphism and PCa risk were also found among Caucasians (Ile/Ile versus Val/Val comparison OR = 0.818, I 2 = 0.0 %, 95 % CI: 0.681 to 0.982; Ile/Val versus Val/Val comparison OR = 0.779, I 2 = 0.0 %, 95 % CI: 0.651 to 0.933; and dominant model OR = 0.794, I 2 = 0.0 %, 95 % CI: 0.670 to 0.941), while there were no associations found for other genetic models. However, no associations were found in Asians and African-Americans for all genetic models when stratified by ethnicity. The authors concluded that the findings of this meta-analysis indicated that GSTP1 Ile105Val polymorphisms contributed to the PCa susceptibility. However, they stated that a study with the larger sample size is needed to further evaluate gene-environment interaction on GSTP1 Ile105Val polymorphisms and PCa risk.

An UpToDate review on "Screening for breast cancer" (Fletcher, 2013) does NOT mention the use of carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6). Also, the NCCN's clinical practice guideline on "Breast Cancer" (Version 2.2013) does NOT mention the use of carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6).

An UpToDate review on “Ductal carcinoma in situ: Treatment and prognosis” (Collins et al, 2013) states that “A DCIS Recurrence Score utilizing a multigene assay has been developed and a prospective evaluation of this assay was performed using tumors from 327 patients who participated in the aforementioned E5194 trial. In a preliminary analysis, patients were stratified by recurrence score into three groups that were associated the following risks of an ipsilateral breast event (DCIS or invasive breast cancer) or invasive breast cancer:

- Low (less than 39) -- 12 and 5 %, respectively
- Intermediate (39 to 54) -- 25 and 9 %, respectively
- High (greater than or equal to 55) -- 27 and 19 %, respectively

These results suggest that the DCIS score may help select patients who should undergo adjuvant radiation. However, further validation of these results is required before the multigene assay can become a part of clinical practice. It is also worth noting that a 12 percent risk of an ipsilateral breast event at 10 years in the lowest risk category may not be low enough to justify the routine omission of post-excision RT”.

The NCCN’s clinical practice guideline on multiple myeloma (MM) (Version 2.2013) stated that “Further understanding of the molecular subtypes of MM is emerging from the application of high-throughput genomic tools such as gene expression profiling (GEP). With the currently available novel treatment approaches, a majority of patients with MM can now anticipate long-term disease control. However, patients with cytogenetically and molecularly defined high-risk disease do not receive the same benefit from current approaches as low-risk patients. GEP is a powerful and fast tool with the potential to provide additional prognostic value to further define risk-stratification, help therapeutic decisions, and inform novel drug design and development. At the present time, standardized testing for GEP is not available and there is inadequate data to determine how this prognostic information should be used to direct patient management”. The NCCN guideline does not include a specific recommendation for the use of the MyPRS test in risk-stratification or determining prognosis in the clinical management of patients with MM.

Oxnard et al (2013) stated that the identification of oncogenic driver mutations underlying

sensitivity to EGFR and anaplastic lymphoma kinase tyrosine kinase inhibitors has led to a surge of interest in identifying additional targetable oncogenes in NSCLC. A number of new potentially oncogenic gene alterations have been characterized in recent years, including BRAF mutations, HER2 insertions, phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide gene (PIK3CA) mutations, fibroblast growth factor receptor 1 (FGFR1) amplifications, discoidin domain receptor 2 (DDR2) mutations, ROS1 re-arrangements, and RET re-arrangements. These investigators discussed the techniques used to discover each of these candidate oncogenes, the prevalence of each in NSCLC, the pre-clinical data supporting their role in lung cancer, and data on small molecular inhibitors in development.

Shin and colleagues (2013) evaluated inhibitory effects of bevacizumab on VEGF signaling and tumor growth in-vitro and in-vivo, and assessed phosphorylation of VEGF receptor 2 (VEGFR2) and downstream signaling in endothelial cells as pharmacodynamic markers using phospho-flow cytometry. These researchers also validated markers in patients with mCRC treated with bevacizumab-based chemotherapy. In in-vitro studies, bevacizumab inhibited proliferation of human umbilical vein endothelial cells in association with reduced VEGF signaling. Notably, bevacizumab inhibited VEGF-induced phosphorylation of VEGFR-2, Akt, and extra-cellular signal-regulated kinase (ERK). In-vivo, treatment with bevacizumab inhibited growth of xenografted tumors and attenuated VEGF-induced phosphorylation of Akt and ERK. The median percentages of VEGFR2 + pAkt + and VEGFR2 + pERK + cells, determined by phospho-flow cytometry, were approximately 3-fold higher in mCRC patients than in healthy controls. Bevacizumab treatment decreased VEGFR2 + pAkt + cells in 18 of 24 patients on day 3. The authors concluded that bevacizumab combined with chemotherapy decreased the number of VEGFR2 + pAkt + cells, reflecting impaired VEGFR2 signaling. Together, these data suggested that changes in the proportion of circulating VEGFR2 + pAkt + cells may be a potential pharmacodynamic marker of the effectiveness of anti-angiogenic agents, and could prove valuable in determining drug dosage and administration schedule.

Janku et al (2013) noted that despite development of new therapies, metastatic colorectal cancer (mCRC) largely remains an incurable disease. Approximately 2 to 6 % of colorectal cancers harbor NRAS mutations. The anti-VEGF antibody bevacizumab is a backbone of most therapeutic regimens; however, biomarkers predicting its activity are not known. These investigators reported 2 cases of mCRC with a Q61K NRAS mutation that had a favorable response to bevacizumab and the histone deacetylase inhibitor valproic acid. In contrast, none of 10 patients with wild-type NRAS or unknown NRAS status and mutated KRAS (NRAS and KRAS mutations are mutually exclusive) responded to the same regimen. The authors concluded that these results suggested that NRAS mutation merits further investigation as a potential biomarker predicting the efficacy of bevacizumab-based treatment.

Carmignani et al (2004a) conducted a study to report the role of combined treatments, including cytoreductive surgery and perioperative regional chemotherapy, in patients with synchronous systemic and intraperitoneal dissemination of appendix cancer. Study subjects were treated with cytoreductive surgery and perioperative regional chemotherapy and statistical analysis of variables utilized survival as an end point and included demographic characteristics, prior surgical score (PSS), tumor marker levels, peritoneal cancer index (PCI), and completeness of cytoreduction (CC). With a mean follow-up of 42.6 months, median survival time (MST) for 15 patients was 28 months and 5-year survival rate was 29.4%. Female patients had a longer MST than male patients (p=0.0199) and survival was better in patients with PSS 0 and 1 (p=0.0277). Patients with elevated CEA and CA 19-9 levels had a shorter MST (p=0.0083 and p=0.0193, respectively) while PCI and CC comparisons did not show significant differences. The morbidity rate (n=2) was 13.3% and the mortality (n=2) rate was also 13.3%. The authors concluded that "acceptable morbidity and mortality and a 29.4% 5-year survival rate allows cytoreductive surgery and regional chemotherapy to be considered as a treatment option for selected patients with synchronous systemic and intraperitoneal dissemination of appendix cancer."

Carmignani et al (2004b) in a further publication regarding gastrointestinal cancer, stated that carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) tumor markers have

found selected clinical application. The authors remarked that the use of these tumor markers in mucinous epithelial tumors of the appendix has not been previously determined. Thus, the authors conducted a study in which, in patients with peritoneal dissemination of a mucinous epithelial malignancy of the appendix, tumor markers CEA and CA 19-9 were prospectively recorded preoperatively within 1 week prior to definitive treatment and if the appendiceal tumor recurred, the tumor marker was determined. The primary endpoint was the accuracy of these two tumor markers in the management of this disease for these two specific clinical situations. CEA was elevated in 56% of 532 patients and CA 19-9 was elevated in 67.1% of these patients. Although the absolute level of tumor marker did not correlate with prognosis, a normal value indicated an improved survival. CEA was elevated in 35.2% of 110 patients determined to have recurrent disease and CA 19-9 was elevated in 62.9%. At least one of the tumor markers was elevated in 68.2% of patients. An elevated CEA tumor marker at the time of recurrence indicated a reduced prognosis and both CEA and CA 19-9 tumor markers were elevated in a majority of these patients. This should be a valuable diagnostic tool previously underutilized in this group of patients. These tumor markers were also of benefit in the assessment of prognosis in that a normal level indicated an improved prognosis. At the time of a reoperative procedure, CEA and CA 19-9 tumor markers gave information regarding the progression of disease and have practical value in the management of epithelial appendiceal malignancy with peritoneal dissemination.

Andreopoulou et al (2007) stated that mucinous carcinoma of the appendix is a rare entity with a distinct natural history that poses diagnostic and therapeutic challenges and that mucinous peritoneal carcinomatosis is most commonly associated with primary tumors of the appendix and colon. The authors stated that usually the spread remains confined to the abdominal cavity and that imaging assessment of these mucinous lesions is difficult, while tumor markers (CEA and CA19.9) may be surrogates for extent of disease.

Recruitment for large scale studies given the rare nature of mucinous appendiceal carcinoma would be challenging. However, available evidence does illustrate a benefit to use of CA 19-9 in patients with mucinous appendiceal carcinoma.

Rasamny et al (2012) stated that cyclin D1 and FADD (Fas-associated protein with death domain) regulate the cell cycle and apoptosis, respectively, and are located on chromosome 11q13, which is frequently amplified in head and neck squamous cell carcinoma (HNSCC). This study evaluated these proteins as predictors of clinical outcomes for HNSCC. A total of 222 patients with upper aero-digestive HNSCC were included in this study. Patients with tumors that were strongly positive for cyclin D1 and FADD had reduced OS ($p = 0.003$ and $p < 0.001$), disease-specific survival (DSS; $p = 0.039$ and $p < 0.001$), and DFS ($p = 0.026$ and $p < 0.001$) survival, respectively. Together, the 2 markers effectively stratified OS ($p < 0.001$), DSS ($p < 0.001$), and DFS ($p = 0.002$). Strong FADD staining correlated with greater alcohol consumption and varied significantly with primary tumor site: 56 % of hypopharynx tumors expressed high levels of FADD but only 7 % of glottis tumors. Using Cox regression analysis, FADD and N stage were significant independent predictors of DSS and DFS, whereas cyclin D1, FADD, and N stage were independently significant for OS. The authors concluded that cyclin D1 and FADD may have utility as predictors of long-term outcomes for patients with HNSCC. Moreover, they stated that further study is needed to determine if these proteins predict response to different treatment approaches or assist in selecting patients for multi-modality therapy.

O'Sullivan and colleagues (2012) examined if the RNA assay uRNA® and its derivative Cxbladder® have greater sensitivity for the detection of bladder cancer than cytology, NMP22™ BladderChek™ and NMP22™ ELISA, and whether they are useful in risk stratification. A total of 485 patients presenting with gross hematuria but without a history of urothelial cancer were recruited prospectively from 11 urology clinics in Australasia. Voided urine samples were obtained before cystoscopy. The sensitivity and specificity of the RNA tests were compared to cytology and the NMP22 assays using cystoscopy as the reference. The ability of Cxbladder to distinguish between low grade, stage Ta urothelial carcinoma and more advanced urothelial carcinoma was also determined. uRNA detected 41 of 66 urothelial carcinoma cases (62.1 % sensitivity, 95 % confidence interval [CI]: 49.3 to 73.8) compared with NMP22 ELISA (50.0 %,

95 % CI: 37.4 to 62.6), BladderChek (37.9 %, 95 % CI: 26.2 to 50.7) and cytology (56.1 %, 95 % CI: 43.8 to 68.3). Cxbladder, which was developed on the study data, detected 82 %, including 97 % of the high grade tumors and 100 % of tumors stage 1 or greater. The cut-offs for uRNA and Cxbladder were pre-specified to give a specificity of 85 %. The specificity of cytology was 94.5 % (95 % CI: 91.9 to 96.5), NMP22 ELISA 88.0 %, (95 % CI: 84.6 to 91.0) and BladderChek 96.4 % (95 % CI: 94.2 to 98.0). Cxbladder distinguished between low-grade Ta tumors and other detected urothelial carcinoma with a sensitivity of 91 % and a specificity of 90 %. The authors concluded that uRNA and Cxbladder showed improved sensitivity for the detection of urothelial carcinoma compared to the NMP22 assays. Stratification with Cxbladder provides a potential method to prioritize patients for the management of waiting lists.

An UpToDate review on "Clinical presentation, diagnosis, and staging of bladder cancer" (Lotan and Choueiri, 2013) does not mention the use of mRNA biomarkers/PCR testing as a management tool for bladder cancer. Furthermore, NCCN's clinical practice guideline on "Bladder cancer" (Version 1.2014) does not mention the use of mRNA biomarkers/PCR testing as a management tool for bladder cancer.

Oncotype Dx Prostate Cancer Assay (Genomic Health, Redwood City, o quantify expression l evels of 12 cancer-related and 5 reference gen a Genomic Prostate Score (GPS). In the final analysis, the cell cycle progression (CCP) score (median 1.03, interquartile range 0.41–1.74) and GPS (range 0–100) are combined in proprietary algorithms with clinical risk criteria (PSA, Gleason grade, tumor stage) to generate new risk categories (i.e., reclassification) intended to reflect biological indolence or aggressiveness of individual lesions, and thus inform management decisions. An assessment by the BlueCross BlueShield Technology Evaluation Center (TEC, 2014) concluded that direct evidence is insufficient to establish the analytic validity, clinical validity, or clinical utility of the Oncotype Dx Prostate.

Glossary of Terms:

a2-PAG	Pregnancy-associated alpha2 glycoprotein
BCM	Breast cancer mucin
BTA	Bladder tumor antigen
CA19-9	Cancer antigen 19-9
CA50	Cancer antigen 50
CA72-4	Cancer antigen 72-4
CA195	Cancer antigen 195
CA242	Cancer antigen 242
CA549	Cancer antigen 549
CA-SCC	Squamous cell carcinoma
CAM17-1	Monoclonal antimucin antibody 17-1
CAM26	Monoclonal antimucin antibody 26
CAM29	Monoclonal antimucin antibody 29
CAR3	Antigenic determinant recognized by monoclonal antibody AR3
DU-PAN-2	Sialylated carbohydrate antigen DU-PAN-2
FDP	Fibrin/fibrinogen degradation products
GCC	Guanylyl cyclase C

MCA	Mucin-like carcinoma-associated antigen
NMP22	Nuclear matrix protein22
NSE	Neuron-specific enolase
PLAP	Placental alkaline phosphatase
PNA-ELLA	Peanut lectin-bonding assay
SLEX	Sialylated Lewis X-antigen
SLX	Sialylated SSEA-1 antigen
SPAN-1	Sialylated carbonated antigen SPAN-1
ST-439	Sialylated carbonated antigen ST-439
TAG12	Tumor-associated glycoprotein 12
TAG72	Tumor-associated glycoprotein 72
TAG72.3	Tumor-associated glycoprotein 72.3
TATI	Tumor-associated trypsin inhibitor
TNF-a	Tumor necrosis factor alpha
TPA	Tissue polypeptide antigen

CPT Codes / HCPCS Codes / ICD-9 Codes

Prostate-specific antigen (PSA):

CPT codes covered if selection criteria are met:

84152

84153

84154

HCPCS codes covered if selection criteria are met:

G0103 Prostate cancer screening; prostate specific antigen test (PSA)

HCPCS codes not covered for indications listed in the CPB:

S3721 Prostate cancer antigen 3 (PCA3) testing

ICD-9 codes covered if selection criteria are met:

185 Malignant neoplasm of prostate

233.4 Carcinoma in situ of prostate

236.5 Neoplasm of uncertain behavior of prostate

790.93 Elevated prostate specific antigen (PSA)

V10.46 Personal history of malignant neoplasm of prostate

V76.44 Special screening for malignant neoplasm of prostate

Carcinoembryonic antigen (CEA):

CPT codes covered if selection criteria are met:

82378

ICD-9 codes covered if selection criteria are met:

153.0 - 154.1	Malignant neoplasm of colon and rectum
230.3	Carcinoma in situ of colon
V10.05	Personal history of malignant neoplasm of large intestine
V10.06	Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus

ICD-9 codes not covered for indications listed in the CPB:

162.2 - 162.9	Malignant neoplasm of bronchus and lung
174.0 - 175.9	Malignant neoplasm of breast
217	Benign neoplasm of breast
231.2	Carcinoma in situ of bronchus and lung
233.0	Carcinoma in situ of breast
238.3	Neoplasm of uncertain behavior of breast
239.3	Neoplasm of unspecified nature of breast
V76.0	Special screening for malignant neoplasm of respiratory organs
V76.10	Breast screening for malignant neoplasms, unspecified
V76.19	Other screening breast examination for malignant neoplasms
V76.41	Special screening for malignant neoplasm of rectum
V76.51	Special screening for malignant neoplasm of colon

Other ICD-9 codes related to the CPB:

795.81	Elevated carcinoembryonic antigen [CEA]

Adenomatous polyposis coli (APC):

CPT codes covered if selection criteria are met:

81201 - 81203

ICD-9 covered if selection criteria are met:

211.3	Benign neoplasm of colon
238.1	Neoplasm of uncertain behavior of connective and other soft tissue
V18.51	Family history, colonic polyps [familial adenomatous polyposis]

Afirma Thyroid FNA analysis:

No specific code

ICD-9 covered if selection criteria are met:

237.4	Neoplasm of uncertain behavior of other and unspecified endocrine glands [indeterminate thyroid nodules]

FISH assay of the BCR/ABL gene:

CPT codes covered if selection criteria are met:

81206 - 81208

88271

88275

ICD-9 codes covered if selection criteria are met:

200.10 - 200.18	Lymphosarcoma [Lymphoblastic lymphoma]
204.00 - 204.02	Acute lymphoid leukemia
205.00 â€“ 205.02	Myeloid leukemia, acute
205.10 - 205.12	Chronic myeloid leukemia

Cancer antigen 125 (CA 125):

CPT codes covered if selection criteria are met:

86304

ICD-9 codes covered if selection criteria are met:

183.0	Malignant neoplasm of ovary
199.1	Malignant neoplasm without specification of site, other
236.2	Neoplasm of uncertain behavior of ovary
V10.43	Personal history of malignant neoplasm of ovary
V16.41	Family history of malignant neoplasm of ovary

ICD-9 codes not covered for indications listed in the CPB:

V76.41	Special screening for malignant neoplasm of rectum
V76.46	Special screening for malignant neoplasm of ovary
V76.51	Special screening for malignant neoplasm of colon

Other ICD-9 codes related to the CPB:

795.82	Elevated cancer antigen 125 [CA 125]

Serial measurements of CA 15-3 (also known as CA 27-29 or Truquant RIA):

CPT codes covered if selection criteria are met:

86300

ICD-9 codes covered if selection criteria are met:

174.0 - 174.9	Malignant neoplasm of the female breast
233.0	Carcinoma in situ of breast
V10.3	Personal history of malignant neoplasm of breast

ICD-9 codes not covered for indications listed in the CPB:

V76.10	Breast screening, unspecified

CA 19-9:

CPT codes covered if selection criteria are met:

86301

ICD-9 codes covered if selection criteria are met:

151.0 - 151.9	Malignant neoplasm of stomach [not covered for gastro-esophageal cancer]
153.5	Malignant neoplasm of appendix vermiformis [mucinous appendiceal carcinoma]
155.1	Malignant neoplasm of intrahepatic bile ducts [cholangiocarcinoma]
156.0 - 156.9	Malignant neoplasm of gallbladder and extrahepatic bile ducts

157.0 - 157.9	Malignant neoplasm of pancreas
230.2	Carcinoma in situ of stomach
230.8	Carcinoma in situ of liver and biliary system [covered for gallbladder and bile duct]
230.9	Carcinoma in situ of other and unspecified digestive organs
V10.04	Personal history of malignant neoplasm of stomach
V10.09	Personal history of malignant neoplasm of gastrointestinal tract, other
V49.83	Awaiting organ transplant status [liver transplant]

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

150.0 - 150.9	Malignant neoplasm of esophagus
153.0 - 154.1	Malignant neoplasm of colon and rectum
155.0, 155.2	Malignant neoplasm of liver [primary and not specified as primary or secondary]
174.0 - 175.9	Malignant neoplasm of the breast
179 - 182.8	Malignant neoplasm of uterus
230.3	Carcinoma in situ of colon
230.8	Carcinoma in situ of liver and biliary system [not covered for liver]
233.0	Carcinoma in situ of breast
577.2	Cyst and pseudocyst of pancreas

Other ICD-9 codes related to the CPB:

576.1	Cholangitis [primary sclerosing] [only covered for persons undergoing liver transplantation]

Cardioembryonic antigen cellular adhesion modecule-7 (CEACAM-7):

There is no specific code for Cardioembryonic antigen cellular adhesion molecule-7 (CEACAM-7)

ICD-9 codes not covered if selection criteria are met:

154.0 - 154.8	Malignant neoplasm of rectum, rectosigmoid junction and anus
230.4	Carcinoma in situ of digestive organs (rectum)
V10.06	Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus

Caris Target Now Molecular Profiling Test:

No specific code

Human epidermal growth factor receptor 2 (HER2) evaluation:

CPT codes covered if selection criteria are met:

83950

ICD-9 codes covered if selection criteria are met:

150.0 - 150.9	Malignant neoplasm of esophagus
151.0 - 151.9	Malignant neoplasm of stomach
174.0 - 175.9	Malignant neoplasm of breast [see criteria]

IGH@ (Immunoglobulin heavy chain locus):

CPT codes covered if selection criteria are met:

81261

ICD-9 codes covered if selection criteria are met:

200.00 - 202.98	Malignant neoplasm of lymphatic and hematopoietic tissue [non- hodgkin lymphoma, code range includes hairy-cell leukemia]

IGK@ (Immunoglobulin kappa light chain locus):

CPT codes covered if selection criteria are met:

81264

ICD-9 codes covered if selection criteria are met:

200.00 - 202.98	Malignant neoplasm of lymphatic and hematopoietic tissue [non-hodgkin lymphoma]
277.30 - 277.39	Amyloidosis [systemic light chain]

Serial measurements of human chorionic gonadotropin (HCG):

CPT codes covered if selection criteria are met:

84702

ICD-9 codes covered if selection criteria are met:

181	Malignant neoplasm of placenta (e.g., choriocarcinoma)
183.0	Malignant neoplasm of ovary
186.0 - 186.9	Malignant neoplasm of testis
196.1	Secondary malignant neoplasm of intrathoracic lymph nodes [mediastinal nodes]
233.39	Carcinoma in situ of other and unspecified female genital organs [germinal cell tumors (teratocarcinoma and embryonal cell carcinoma) of the ovaries]
233.6	Carcinoma in situ of other and unspecified male genital organs
236.1	Neoplasm of uncertain behavior of placenta
630	Hydatidiform mole
V10.43	Personal history of malignant neoplasm of ovary
V10.47	Personal history of malignant neoplasm of testis

Serial measurements of AFP to diagnose germ cell tumors or the diagnosis and monitoring of hepatocellular carcinoma:

CPT codes covered if selection criteria are met:

82105

ICD-9 codes covered if selection criteria are met:

155.0 - 155.2	Malignant neoplasm of the liver and intrahepatic bile ducts
183.0	Malignant neoplasm of ovary
186.0 - 186.9	Malignant neoplasm of testis
196.1	Secondary malignant neoplasm of intrathoracic lymph nodes [mediastinal nodes]
230.8	Carcinoma in situ of liver and biliary system

233.39	Carcinoma in situ of other and unspecified female genital organs [germ cell tumors]
233.6	Carcinoma in situ of other and unspecified male genital organs
V10.43	Personal history of malignant neoplasm of ovary
V10.47	Personal history of malignant neoplasm of testis
V76.49	Special screening for malignant neoplasm of other sites
V76.89	Special screening for malignant neoplasm, other

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

181	Malignant neoplasm of placenta (e.g., choriocarcinoma)
236.1	Neoplasm of uncertain behavior of placenta
630	Hydatidiform mole

Serial measurements of AFP and HCG together to diagnose and monitor testicular cancer:

CPT codes covered if selection criteria are met:

82105

84702

ICD-9 codes covered if selection criteria are met:

186.0 - 186.9	Malignant neoplasm of testis
233.6	Carcinoma in situ of other and unspecified male genital organs
V76.45	Special screening for malignant neoplasm of testis

Measurement of estrogen and progesterone receptors and steroid receptor status:

CPT codes covered if selection criteria are met:

84233

84234

ICD-9 codes covered if selection criteria are met:

174.0 - 175.9	Malignant neoplasm of breast
233.0	Carcinoma in situ of breast

Other ICD-9 codes related to the CPB:

627.2	Symptomatic menopausal or female climacteric states
V49.81	Asymptomatic postmenopausal status (age-related) (natural)

TCB@ (T cell antigen receptor, beta):

CPT codes covered if selection criteria are met:

81340

81341

ICD-9 codes covered if selection criteria are met:

204.00 - 204.92	Lymphoid leukemia [T-cell prolymphocytic leukemia]

K-ras (KRAS) with BRAF reflex testing:

CPT codes covered if selection criteria are met:

81210

81275

Other CPT codes related to the CPB:

88363

Other HCPCS codes related to the CPB:

J9055	Injection, cetuximab, 10 mg [to predict non-response to cetuximab (Erbitux) and panitumumab (Vectibix) in the treatment of anal adenocarcinoma]
J9303	Injection, panitumumab, 10 mg [to predict non-response to cetuximab (Erbitux) and panitumumab (Vectibix) in the treatment of anal adenocarcinoma]

ICD-9 codes covered if selection criteria are met:

152.0 - 152.9	Malignant neoplasm of small intestine, including duodenum [small bowel adenocarcinoma]
153.0 - 154.1	Malignant neoplasm of colon, rectum and rectosigmoid junction [metastatic colorectal cancer]
154.2 - 154.3	Malignant neoplasm of anal canal and anus [anal adenocarcinoma]
162.2 - 162.9	Malignant neoplasm of trachea, bronchus, and lung
211.4	Benign neoplasm of rectum and anal canal [if KRAS nonmutated] [Lynch syndrome (HNPCC)]
230.4	Carcinoma in situ of rectum [if KRAS nonmutated] [Lynch syndrome (HNPCC)]

MLH1, MSH2, MSH6:

CPT codes covered if selection criteria are met:

81292 - 81294

81295 - 81297

81298 - 81300

ICD-9 codes covered if selection criteria are met:

153.0 - 153.9	Malignant neoplasm of colon [Lynch syndrome (HNPCC)] [all persons with Stage 2 colon cancer]
154.0 - 154.8	Malignant neoplasm of rectum, rectosigmoid junction, and anus [Lynch syndrome (HNPCC)] [all persons with Stage 2 colon cancer] [under age 50]
211.4	Benign neoplasm of rectum and anal canal [under age 50]
230.4	Carcinoma in situ of rectum [under age 50]

ALK Gene Fusion:

No specific code

ICD-9 codes covered if selection criteria are met:

162.2 - 162.9	Malignant of neoplasm of bronchus and lung [non-small-cell lung cancer]

ALK Gene Rearrangement:

No specific code

ICD-9 codes covered if selection criteria are met:

200.70 - 200.78 Large cell lymphoma [B cell]

202.70 - 202.78 Peripheral T-cell lymphoma

238.77 Post-transplant lymphoproliferative disorder (PTLD)

ALK Translocations :

No specific code

Other CPT codes related to CPB:

81401

ICD-9 codes covered if selection criteria are met:

162.2 - 162.9 Malignant of neoplasm of bronchus and lung [non-small-cell lung cancer]

Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1):

CPT codes covered if selection criteria are met:

85415

ICD-9 codes covered if selection criteria are met:

174.0 - 175.9 Malignant neoplasm of breast [node negative]

233.0 Carcinoma in situ of breast

CD 117 (c-kit):

CPT codes covered if selection criteria are met:

88184

+ 88185

ICD-9 codes covered if selection criteria are met:

[for determining eligibility for treatment with Gleevac]

150.0 - 159.9 Malignant neoplasm of esophagus

205.10 - 205.12 Chronic myeloid leukemia

CD 20:

CPT codes covered if selection criteria are met:

88184

+ 88185

ICD-9 codes covered if selection criteria are met:

[for determining eligibility for treatment with Rituxan]

200.00 - 202.98 Lymphosarcoma and reticulosarcoma and other specified malignant tumors of lymphatic tissue, hodgkin's disease, and other malignant neoplasms of lymphoid and histiocytic tissue

204.10 - 204.12 Chronic lymphoid leukemia

CD 25:

CPT codes covered if selection criteria are met:

88184

+ 88185

ICD-9 codes covered if selection criteria are met:

[for determining eligibility for treatment with Ontak]

202.10 - 202.18 Mycosis fungoides

202.20 - 202.28 Sezary's disease

202.70 - 202.78 Peripheral T-cell lymphoma

CD 31:

No specific code

Other CPT codes related to the CPB:

88342

88343

Other HCPCS codes related to the CPB:

G0461 Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain

G0462 each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)

ICD-9 codes covered if selection criteria are met:

171.0 - 171.9 Malignant neoplasm of connective and other soft tissue [angiosarcoma]

CD 33:

CPT codes covered if selection criteria are met:

88184

+ 88185

ICD-9 codes covered if selection criteria are met:

[for determining eligibility for treatment with Mylotarg]

204.00 - 204.02 Acute lymphoid leukemia

205.00 - 205.02 Acute myeloid leukemia

206.00 - 206.02 Acute monocytic leukemia

207.00 - 207.02 Acute erythremia and erythroleukemia

208.00 - 208.02 Acute leukemia of unspecified cell type

CD 52:

CPT codes covered if selection criteria are met:

88184

+ 88185

ICD-9 codes covered if selection criteria are met:

[for determining eligibility for treatment with Campath]

200.20 - 200.48 Burkitt's tumor or lymphoma, marginal zone lymphoma, or mantle cell lymphoma

200.70 - 200.78 Large cell lymphoma

202.00 - 202.88 Other malignant neoplasms of lymphoid and histiocytic tissue

204.10 - 204.12 Chronic lymphoid leukemia

Cyclin D1:

CPT codes covered if selection criteria are met:

81401

ICD-9 codes covered if selection criteria are met:

200.40 - 200.48	Mantle cell lymphoma [diagnosing and predicting disease recurrence]

ICD-9 codes not covered if selection criteria are met:

173.02, 173.12, 173.22, 173.32, 173.42	Squamous cell carcinoma of lip, eyelid including canthus, ear and external auditory canal, face scalp and skin of neck

Fas-Associated Protein with Death Domain FADD:

No specific code

ICD-9 codes not covered if selection criteria are met:

173.02, 173.12, 173.22, 173.32, 173.42	Squamous cell carcinoma of lip, eyelid including canthus, ear and external auditory canal, face scalp and skin of neck

Prostate PX, Post-op PX:

Other CPT codes related to the CPB:

88305

88313

88323

88342

88343

88347

Other HCPCS codes related to the CPB:

G0461	Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462	each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)

ICD-9 codes not covered for indications listed in the CPB:

185	Malignant neoplasm of prostate

NRAS mutation:

Other CPT codes related to the CPB:

81404

Ras oncogenes (except KRASand BRAF):

There is no specific code for ras oncogenes

Epidermal growth factor receptor (EGFR) Testing:

CPT codes covered if selection criteria are met:

81235

Other CPT codes related to the CPB:

88271

88275

88291

88313

88323

88342

88343

88358

88360

88361

88381

Other HCPCS codes related to the CPB:

G0461	Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462	each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)
J8565	Gefitinib (Iressa), oral, 250 mg

ICD-9 codes covered if selection criteria are met:

162.2 - 162.9	Malignant neoplasm of bronchus and lung [non small cell lung cancer]

ICD-9 codes not covered if selection criteria are met:

191.0 - 191.9	Malignant neoplasm of brain [Glioblastoma multiforme]
233.7	Carcinoma in situ of bladder [urothelial carcinoma]
233.9	Carcinoma in situ of other and unspecified urinary organs (ureter, renal pelvis) [urothelial carcinoma]

ROS-1:

No specific code

ICD-9 codes covered if selection criteria are met:

162.2 - 162.9	Malignant neoplasm of bronchus and lung [non small cell lung cancer]

ZAP-70:

CPT codes covered if selection criteria are met:

88184

+ 88185

ICD-9 codes covered if selection criteria are met :

204.10 - 204.12	Chronic lymphoid leukemia [assessing prognosis and need for aggressive therapy]

Oncotype Dx:

Other CPT codes related to the CPB:

88360

88361

88367

88368

HCPCS codes covered if selection criteria are met:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment [not covered for Mammaprint, Rotterdam Signature 76-gene Panel, or Breast Cancer Gene Expression Ratio (HOXB13:IL17BR), or TargetPrint Gene Expression]

ICD-9 codes covered if selection criteria are met:

174.0 - 174.9	Malignant neoplasm of female breast [except node positive - see criteria]
175.0 - 175.9	Malignant neoplasm of male breast

ICD-9 codes not covered for indications listed in the CPB:

153.0 - 154.1	Malignant neoplasm of colon and rectum
185	Malignant neoplasm of prostate
230.3	Carcinoma in situ of colon
233.0	Carcinoma in situ of breast [ductal carcinoma in situ (DCIS)]
233.4	Carcinoma in situ of prostate
V10.05	Personal history of malignant neoplasm of large intestine
V10.06	Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus

Other ICD-9 codes related to the CPB:

V86.0	Estrogen receptor positive status [ER+]
V86.1	Estrogen receptor negative status [ER-]

T-cell receptor gamma chain gene rearrangement:

CPT codes covered if selection criteria are met:

81342

ICD-9 codes covered if selection criteria are met:

202.10 - 202.18	Mycosis fungoides
204.00 - 204.92	Lymphoid leukemia [T-cell prolymphocytic leukemia]

Myeloperoxidase (MPO) immunostaining FLT3-ITD, CEBPA mutation, NPM1 mutation, and KIT mutation:

CPT codes covered if selection criteria are met:

81244

81245

83876

ICD-9 codes covered if selection criteria are met:

205.00 - 205.02	Acute myeloid leukemia

PML/RARA:

CPT codes covered if selection criteria are met:

81315 - 81316

ICD-9 codes covered if selection criteria are met:

205.00 - 205.02	Myeloid leukemia, acute

Placental alkaline phosphatase (PLAP):

CPT codes covered if selection criteria are met:

84080

ICD-9 codes covered if selection criteria are met:

183.0	Malignant neoplasm of ovary
186.0 - 186.9	Malignant neoplasm of testes
233.39	Carcinoma in situ of other and unspecified female genital organs [germ cell tumors]
233.6	Carcinoma in situ of other and unspecified male genital organs
V10.43	Personal history of malignant neoplasm of ovary
V10.47	Personal history of malignant neoplasm of testis

Bladder tumor antigen (BTA) Stat Test, the nuclear matrix protein (NMP22) test, the fibrin/fibrinogen degradation products (Aura-Tek FDP) test, or the UroVysion fluorescent in situ hybridization (FISH) test:

CPT codes covered if selection criteria are met:

85362 - 85380

86294

86386

88120

88121

88365

ICD-9 codes covered if selection criteria are met:

188.0 - 188.9	Malignant neoplasm of bladder
233.7	Carcinoma in situ of bladder
V10.51	Personal history of malignant neoplasm of bladder

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

599.70 - 599.72	Hematuria
V76.3	Special screening for malignant neoplasm of bladder

ImmunoCyte:

No specific code

ICD-9 codes covered if selection criteria are met:

188.0 - 188.9	Malignant neoplasm of bladder [as an adjunct to cystoscopy or cytology in monitoring]

ICD-9 codes not covered for indications listed in the CPB:

V76.3	Special screening for malignant neoplasm of bladder [diagnosis or screening in asymptomatic persons]

Janus Kinase 2 (JAK2) mutations:

CPT codes covered if selection criteria are met:

81270

ICD-9 codes covered if selection criteria are met:

205.10 - 205.12	Chronic myeloid leukemia
238.4	Polycythemia vera
238.71	Essential thrombocythemia
238.76	Myelofibrosis with myeloid metaplasia (Primary myelofibrosis)
238.79	Other lymphatic and hematopoietic tissues (Chronic myeloproliferative disease)
289.83	Myelofibrosis

BRAF mutation analysis:

CPT codes covered if selection criteria are met:

81210

ICD-9 codes covered if selection criteria are met:

153.0 - 153.9	Malignant neoplasm of colon [Lynch syndrome (HNPCC)]
154.0 - 154.8	Malignant neoplasm of rectum, rectosigmoid junction, and anus [Lynch syndrome (HNPCC)]
171.5	Malignant neoplasm of connective and other soft tissue of abdomen [gastrointestinal stromal tumors]
172.0 - 172.9	Melanoma of skin [for consideration of Vemurafenib, Dabrafenib and Trametinib]
202.40 - 202.48	Leukemic reticuloendotheliosis

ICD-9 codes not covered if selection criteria are met:

162.2 - 162.9	Malignant neoplasm of bronchus, and lung
193	Malignant neoplasm of thyroid gland

Assaying for loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) or deleted in colon cancer (DCC) protein (18q-LOH/DCC) for colorectal cancer:

There is no specific code for 18q-LOH/DCC

ICD-9 codes not covered for indications listed in the CPB:

153.0 - 154.1	Malignant neoplasm of colon, rectum, and rectosigmoid junction

OvaCheck test:

There is no specific code for OvaCheck

ICD-9 codes not covered for indications listed in the CPB:

183.0	Malignant neoplasm of ovary
V76.46	Special screening for malignant neoplasm of ovary

Ovasure:

There is no specific code for Ovasure

Other CPT codes related to the CPB:

82985

83520

84146

84305

86304

CellSearch assay:

CPT codes not covered for indications listed in the CPB:

86152 - 86153

88346

88361

Cofilin (CFL1):

There is no specific code for cofilin (CFL1)

162.2 - 162.9	Malignant neoplasm of lung [non-small cell lung carcinoma]

ColonSentry:

There is no specific code for ColonSentry

ICD-9 codes not covered for indications listed in the CPB:

V76.41	Special screening for malignant neoplasms; rectum
V76.51	Special screening for malignant neoplasms; colon

Early CDT-Lung Test:

CPT codes not covered for indications listed in the CPB:

83520

Mammaprint:

HCPCS codes not covered for indications listed in the CPB:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment

Mammostrat:

There is no specific code for Mammostrat

Mucin 4 expression:

CPT codes not covered for indications listed in the CPB:

88313

ICD-9 codes not covered for indications listed in the CPB:

153.0 - 154.1	Malignant neoplasm of colon, rectum and rectosigmoid junction

Microarray-based gene expression profile testing:

Other CPT codes related to the CPB:

81406

OVA1:

There is no specific code for OVA1

CPT codes not covered for indications listed in the CPB:

81503

Pathwork Tissue of Origin Test:

CPT codes not covered for indications listed in the CPB:

81504

PreOvar Test for the KRAS-variant [to determine ovarian cancer risk]:

No specific code

ICD-9 codes not covered for indications listed in the CPB:

183.0	Malignant neoplasm of ovary
V10.43	Personal history of malignant neoplasm of ovary

ProOnc Tumor Source Dx Test:

No specific code

Rotterdam Signature 76-gene Panel:

HCPCS codes not covered for indications listed in the CPB:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment

Serum amyloid A:

CPT codes not covered for indications listed in the CPB:

88342

88343

G0461

G0462

ICD-9 codes not covered for indications listed in the CPB:

182.0 - 182.8	Malignant neoplasm of corpus uteri, isthmus and uterus
V10.42	Personal history of malignant neoplasm of uterus

Breast Cancer Gene Expression Ratio (HOXB13:IL17BR):

HCPCS codes not covered for indications listed in the CPB:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment

Panexia:

CPT codes not covered for indications listed in the CPB:

81406

ICD-9 codes not covered for indications listed in the CPB:

185	Malignant neoplasm of prostate

PTEN gene expression:

CPT codes covered if selection criteria are met:

81321 - 81323

ICD-9 codes covered if selection criteria are met:

759.6	Other congenital hamartoses, NEC [Cowden syndrome]

ICD-9 codes not covered for indications listed in the CPB:

162.2 - 162.9	Malignant neoplasm of bronchus and lung [non-small cell lung cancer]

GeneSearch Breast Lymph Node (BLN) assay:

There is no specific code for GeneSearch Breast Lymph Node (BLN) assay

Thymidylate synthase:

There is no specific code for Thymidylate synthase

Other CPT codes related to the CPB:

88342

88343

88360

88361

Other HCPCS codes related to the CPB:

G0461	Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462	each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)

Topographic genotyping (PathfinderTG):

No specific code

Biomarker Translation (BT):

No specific code

ICD-9 codes not covered for indications listed in the CPB:

174.0 - 175.9	Malignant neoplasm of breast

HE4:

CPT codes not covered for indications listed in the CPB:

81500

86305

Other CPT codes related to the CPB:

86316

ICD-9 codes not covered for indications listed in the CPB:

183.0	Malignant neoplasm of ovary

HERmark:

There is no specific code for HERmark

ICD-9 codes not covered for indications listed in the CPB:

174.0 - 175.9	Malignant neoplasm of breast
233.0	Carcinoma in situ of breast

Vascular endothelial growth factor (VEGF):

No specific code

Other CPT codes related to the CPB:

83520

TargetPrint Gene Expression:

Other CPT codes related to the CPB:

88360

88361

88367

88368

HCPCS codes not covered for indications listed in the CPB:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment

ICD-9 codes not covered for indications listed in the CPB:

174.0 - 174.9	Malignant neoplasm of female breast
175.0 - 175.9	Malignant neoplasm of male breast

Other ICD-9 codes related to the CPB:

V86.0	Estrogen receptor positive status [ER+]
V86.1	Estrogen receptor negative status [ER-]

CK5, CK14, p63 and Racemase P504S:

Other CPT codes related to the CPB:

88342

88343

Other HCPCS codes related to the CPB:

G0461	Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462	each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)

ICD-9 codes not covered for indications listed in the CPB:

185	Malignant neoplasm of prostate

EML4-ALK:

Other CPT codes related to the CPB:

88381

ICD-9 codes not covered for indications listed in the CPB:

162.2 - 162.9	Malignant neoplasm of bronchus and lung [non-small-cell lung cancer]

Coloprint, CIMP, Line-1 hypomethylation and immune cells:

There are no specific codes for Coloprint, CIMP, Line-1 hypomethylation and immune cells

ICD-9 codes not covered for indications listed in the CPB:

153.0 - 154.1	Malignant neoplasm of colon and rectum

WT1 mutation, RUNX1 mutation, MLL-PTD mutation, IDH2 R172, and IDH2 codon 140 mutation:

Des-gamma-carboxyl prothrombin (DCP):

CPT codes not covered for indications listed in the CPB:

83951

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

155.0, 155.2	Malignant neoplasm of the liver [primary or unspecified as primary or secondary]
230.8	Carcinoma in situ of liver and biliary system

Experimental and investigational circulating tumor markers:

CPT codes not covered for indications listed in the CPB:

0006M

0007M

0008M

82387

84275

86316

88342

88343

G0461

G0462

There are no specific codes for the circulating tumor markers listed below:

Affirma Thyroid FNA Analysis; anti-VEGF antibody bevacizumab; BluePrint molecular subtyping profile for breast cancer; Catalytic subunit alpha polypeptide gene (PIK3CA); C-Met expression; DCIS Recurrence Score; Glutathione-S-transferase P1 (GSTP1); Phosphatidylinositol-4,5-bisphosphonate 3-kinase; Proveri prostate cancer assay (PPCA); Ribonucleotide reductase subunit M1 (RRM1); ROS1 re-arrangements; vascular endothelial growth factor receptor 2 (VEGFR2); VeriStrat for non-small cell lung cancer and other indications; BreastNext Next-Gen Cancer Panel; CancerNext Next-Gen Cancer Panel; ColoNext Next-Gen Cancer Panel; Ovanext Next-Gen Cancer Panel; Mirinform thyroid test; Previstage GCC; Prostate core mitotic test; Prolaris; Oncovue; BREVAgen; UroCor cytology assay (DD23 and P53); CXBladder Test;

The above policy is based on the following references:

1. Lein M, Stephan C, Jung K, et al. Relation of free PSA/total PSA in serum for differentiating between patients with prostatic cancer and benign hyperplasia of the prostate: Which cutoff should be used? Cancer Invest. 1998;16(1):45-49.
2. Littrup PJ. Future benefits and cost-effectiveness of prostate carcinoma screening. American Cancer Society. Cancer. 1997;80(9):1864-1870.
3. Gao X, Porter AT, Grignon DJ, et al. Diagnostic and prognostic markers for human prostate cancer. Prostate. 1997;31(4):264-281.
4. Duffy MJ. PSA as a marker for prostate cancer: A critical review. Ann Clin Biochem. 1996;33(Pt 6):511-519.
5. No authors listed. Ovarian cancer: Screening, treatment, and followup. NIH Consens Statement. 1994;12(3):1-30.
6. Watine J, Charet JC. Are the ATS (American Thoracic Society) and the ERS (European Respiratory Society) correct in not recommending routine tfumor marker assays for screening, staging, or evaluation of non-small cell lung cancer? Rev Mal Respir. 1999;16(2):139-149.
7. Seifert JK Morris DI. Indicators of recurrence following cryotherapy for hepatic metastases from colorectal cancer. Br J Surg. 1999;86(2):234-240.
8. Lagautriere F, Valvano L, Chaazl M, et al. Prognostic factors in colorectal adenocarcinoma. Ann Ital Chir. 1998;69(4):491-497.

9. Hamm CM, Cripps C. Carcinoembryonic antigen in metastatic colorectal cancer. Clin Invest Med. 1998;21(4-5):186-191.
10. Jessup JM, Loda M. Prognostic markers in rectal carcinoma. Semin Surg Oncol. 1998;15(2):131-140.
11. Mazurek A. Niklinski J. Laudanski T. et al. Clinical tumour markers in ovarian cancer. Eur J Cancer Prev. 1998;7(1):23-35.
12. American Society of Clinical Oncology. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. J Clin Oncol. 1996;14(10):2843-2877.
13. American Society of Clinical Oncology. 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. Adopted on November 7, 1997 by the American Society of Clinical Oncology. J Clin Oncol. 1998;16(2):793-795.
14. Harrison LE, Guillem JG, Paty P, et al. Preoperative carcinoembryonic antigen predicts outcomes in node-negative colon cancer patients: A multivariate analysis of 572 patients. J Am Coll Surg. 1997;185(1):55-59.
15. Lamerz R, Stieber P, Fateh-Moghadam A. Serum marker combinations in human breast cancer. In Vivo. 1993;7(6B):607-613.
16. American College of Physicians. Screening for ovarian cancer: Recommendations and rationale. Ann Intern Med. 1994 J;121(2):141-142.
17. Carlson KJ. Screening for ovarian cancer. Ann Intern Med. 1994;121(2):124-132
18. Mogenson O. Prognostic value of CA 125 in advanced ovarian cancer. Gynecol Oncol. 1992;44:207-212.
19. Patsner B, Mann WJ, Vissicchio M, Loesch M. Comparison of serum CA-125 and lipid-associated sialic acid (LASA-P) in monitoring patients with invasive ovarian adenocarcinoma. Gynecol Oncol. 1988;30(1):98-103.
20. Makar AP, Kristensen GB, Bormer OP, Trope CG. CA 125 measured before a second-look laparotomy us an independent prognostic factor for survival in patients with epithelial ovarian cancer. Gynecol Oncol. 1992;45(3):323-328.
21. Werner M, Faser C, Silverberg M. Clinical utility and validation of emerging biochemical markers for mammary adenocarcinoma. Clin Chem. 1993;39(11 Pt 2):2386-2396.
22. Bates SE. Clinical Applications of serum tumor markers. Ann Intern Med. 1991;115(8):623-638.
23. Steinberg W. The clinical utility of the CA19-9 tumor associated antigen. Am J Gastroenterol. 1990;85:350-355.
24. Devlin J, O'Grady J. Indications for referral and assessment in adult liver transplantation: A clinical guideline. British Society of Gastroenterology. Gut. 1999;45 Suppl 6:VI1-VI22.
25. Jorgensen LG,, Osterlind K, Hansen HH, Cooper EH. Serum neuron specific enolase (NSE) is a determinant of response duration in small cell lung cancer. Br J Cancer. 1992;66(3):594-598.
26. Johnson PW, Joel SP, Love S, et al. Tumour markers for prediction of survival and monitoring of remission in small cell lung cancer. Br J Cancer. 1993;67(4):760-766.
27. Wobbes T, Thomas CM, Segers MF, Nagengast FM. Evaluation of seven tumor markers in the treatment sera of patients with gastric carcinoma. Cancer. 1992;69(8):2036-2041.
28. Hayes DF. Serum tumor markers for breast cancer. Anticancer Drugs. 1995;6(Suppl 2):26-27.
29. Coveney EC, Geraghty JG, Sherry F, et al. The clinical value of CEA and CA 15-3 in breast cancer management. Int J Biol Markers. 1995;10(1):35-41.
30. Sharma S, Zippe CD, Pandrangi L, et al. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA Stat. J Urol. 1999;162(1):53-57.
31. Pirtskalaishvili G, Konety BR, Getzenberg RH. Update on urine-based markers for bladder cancer. How sensitive and specific are the new noninvasive tests? Postgrad Med. 1999;106(6):85-86, 91-94.
32. Sawczuk IS, Lee B. The mechanism and clinical applications of the NMP22 tumor marker immunoassay: A review. Am Clin Lab. 1999;18(3):24-26.
33. Burchardt M, Burchardt T, Shabsigh A, et al. Current concepts in biomarker technology for

bladder cancer. Clin Chem. 2000;46(5):595-605.
34. Grocela JA, McDougal WS. Utility of nuclear matrix protein (NMP22) in the detection of recurrent bladder cancer. Urol Clin North Am. 2000;27(1):47-51.
35. Brown FM. Urine cytology. Is it still the gold standard for screening? Urol Clin North Am. 2000;27(1):25-37.
36. Bast RC Jr, Ravdin P, Hayes DF, et al. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: Clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol. 2001;19(6):1865-1878.
37. Smith RA, Cokkinides V, von Eschenbach AC, et al. American Cancer Society guidelines for the early detection of cancer. CA Cancer J Clin. 2002;52(1):8-22.
38. Morabito A, Magnani E, Gion M, et al. Prognostic and predictive indicators in operable breast cancer. Clin Breast Cancer. 2003;3(6):381-390.
39. Duffy MJ, van Dalen A, Haglund C, et al. Clinical utility of biochemical markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines. Eur J Cancer. 2003;39(6):718-727.
40. Bubendorf L, Grilli B, Sauter G, et al. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. Am J Clin Pathol. 2001;116(1):79-86.
41. Sarosdy MF, Schellhammer P, Bokinsky G, et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol. 2002;168(5):1950-1954.
42. Kruger S, Mess F, Bohle A, Feller AC. Numerical aberrations of chromosome 17 and the 9p21 locus are independent predictors of tumor recurrence in non-invasive transitional cell carcinoma of the urinary bladder. Int J Oncol. 2003;23(1):41-48.
43. Society of Gynecologic Oncologists (SGO). Society of Gynecologic Oncologists statement regarding OvaCheck™. Position Statements. Chicago, IL: SGO; February 7, 2004. Available at: http://www.sgo.org/policy/position_statement.cfm. Accessed June 4, 2004.
44. National Cancer Institute (NCI). Questions and answers: OvaCheck ™ and NCI/FDA Ovarian Cancer Clinical Trials using proteomics technology. NCI News. Bethesda, MD: NCI; March22, 2004.Available at: http://www.cancer.gov/newscenter/pressreleases/ProteomicsOvarian. Accessed June 4, 2004.
45. Permanente Medical Group. Ovacheck. Permanente Medical Group Physician Home Pages. San Francisco, CA: Kaiser Permanente; 2004. Available at: http://www.permanente.net/kaiser/pages/f21815.html. Accessed June 4, 2004.
46. Sullivan MG. Validity testing indefinitely delays Ovacheck release. FDA raises regulatory issues. Ob.Gyn.News Online. 2004;39(7):5. Available at: http://www.imng.com/IMNG/obgyn/. Accessed June 4, 2004.
47. U.S. Food and Drug Administration (FDA), Center for Devices and Radiologic Health (CDRH), Office of In Vitro Diagnostic Device Evaluation and Safety (OVID). Letter to Laboratory Corporation of America. Re: Ovacheck - Ovarian Cancer Screen. Rockville, MD: FDA; March 2, 2004. Available at: http://www.fda.gov/cdrh/oivd/letters/030204-labcorp.html. Accessed June 4, 2004.
48. U.S. Food and Drug Administration (FDA), Center for Devices and Radiologic Health (CDRH), Office of In Vitro Diagnostic Device Evaluation and Safety (OVID). Letter to Quest Diagnostics. Re: Ovacheck - Ovarian Cancer Screen. Rockville, MD: FDA; March 2, 2004. Available at: http://www.fda.gov/cdrh/oivd/letters/030204-labcorp.html. Accessed June 4, 2004.
49. Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet. 2002;359(9306):572-577.
50. U.S. Preventive Services Task Force (USPSTF). Screening for bladder cancer in adults. In: Guide to Clinical Preventive Services. Report of the U.S. Preventive Services Task Force. 3rd ed. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); June 2004.
51. American College of Obstetricians and Gynecologists (ACOG). The role of the generalist obstetrician-gynecologist in the early detection of ovarian cancer. ACOG Committee Opinion No. 280. Washington, DC: ACOG; December 2002.

52. National Cancer Institute (NCI). Ovarian Cancer (PDQ): Screening. Health Professional Version. Bethesda, MD: NCI; updated February 20, 2004. Available at: http://www.cancer.gov/cancertopics/pdq/screening/ovarian/healthprofessional. Accessed September 22, 2004.
53. U.S. Preventive Services Task Force (USPSTF). Screening for ovarian cancer. In: Guide to Clinical Preventive Services. Report of the U.S. Preventive Services Task Force. 3rd ed. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); May 2004.
54. American College of Obstetricians and Gynecologists (ACOG). Position of the American College of Obstetricians and Gynecologists Committee on Gynecologic Practice Regarding OvaCheck. Washington, DC: ACOG; February 25, 2004.
55. Andrews E. Ovacheck: Breakthrough or also ran? Nat Rev Med. 2004;1(5).
56. Check E. Proteomics and cancer: Running before we can walk? Nature. 2004;429(6991):496-497.
57. Diamandis EP. OvaCheck: Doubts voiced soon after publication. Nature. 2004;430(7000):611.
58. Gandini O, Luci L, Stigliano A, et al. Is DD3 a new prostate-specific gene? Anticancer Res. 2003;23:305-308.
59. Schalken JA, Hessels D, Verhaegh G. New targetes for therapy in prostate cancer: Differential display code 3 (DD3/PCA3), a highly prostate cancer specific gene. Urology. 2003;62(suppl 5A):34-43.
60. Bussemakers MJG, van Bokhoven A, Verheagh GW, et al. DD3: A new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res. 1999;59:5975-5979.
61. de Kok JB, Verhaegh GW, Roelofs RW et al. DDS (PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res. 2002;62:2695-2698.
62. Hessels D, Klein Gunnewiek JM, van Oort I, et al. DD3 (PCA3) based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol. 2003;44:8-16.
63. Abeloff MD, Armitage JO, Niederhuber JE, et al. Clinical Oncology. 3rd ed. Philadelphia, PA. Churchill Livingstone; 2004: 2369.
64. U.S. Food and Drug Administration (FDA), Center for Devices and Radiologic Health (CDRH). CellSearch™ Epithelial Cell Kit / CellSpotter™ Analyzer. 510(k) no. K031588. Rockville, MD: FDA; January 21, 2004.
65. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781-791.
66. Kahn HJ, Presta A, Yang LY, et al. Enumeration of circulating tumor cells in the blood of breast cancer patients after filtration enrichment: Correlation with disease stage. Breast Cancer Res Treat. 2004;86(3):237-247.
67. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004;10(20):6897-6904.
68. Veridex, LLC. CellSearch™ [website]. Warren, NJ: Veridex; 2005. Available at: http://www.veridex.com/. Accessed December 17, 2004.
69. Muller V, Pantel K. Bone marrow micrometastases and circulating tumor cells: Current aspects and future perspectives. Breast Cancer Res. 2004;6(6):258-261.
70. Gilbey AM, Burnett D, Coleman RE, Holen I. The detection of circulating breast cancer cells in blood. J Clin Pathol. 2004;57(9):903-911.
71. Ring A, Smith IE, Dowsett M. Circulating tumour cells in breast cancer. Lancet Oncol. 2004 Feb;5(2):79-88.
72. Pantel K, Muller V, Auer M, et al. Detection and clinical implications of early systemic tumor cell dissemination in breast cancer. Clin Cancer Res. 2003;9(17):6326-6334.
73. Baker M, Gillanders WE, Mikhitarian K, et al. The molecular detection of micrometastatic breast cancer. Am J Surg. 2003;186(4):351-358.
74. Weigelt B, Bosma AJ, Hart AA, et al. Marker genes for circulating tumour cells predict survival in metastasized breast cancer patients. Br J Cancer. 2003;88(7):1091-1094.
75. Glas A S, Roos D, Deutekom M, et al. Tumor markers in the diagnosis of primary bladder cancer: A systematic review. J Urol. 2003;169(6):1975-1982.
76. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated,

node-negative breast cancer. N Engl J Med. 2004;351(27):2817-2826.
77. Esteva FJ, Sahin AA, Coombes K, et al. Multi-gene RT-PCR assay for predicting recurrence in node negative breast cancer patients -- M.D. Anderson Clinical Validation Study [abstract]. Breast Cancer Res Treat. 2003;82 (Suppl 1):S11.
78. Bast RC Jr, Hortobagyi GN. Individualized care for patients with cancer - a work in progress. N Engl J Med. 2004;351(27):2865-2867.
79. Kamisawa T, Fukayama M, Koike M, et al. Carcinoma of the ampulla of Vater: Expression of cancer-associated antigens inversely correlated with prognosis. Am J Gastroenterol. 1988;83(10):1118-1123.
80. Matorras R, Rodriguez-Escuderoi FJ, Diez J, et al. Monitoring endometrial adenocarcinoma with a four tumor marker combination. CA 125, squamous cell carcinoma antigen, CA 19.9 and CA 15.3. Acta Obstet Gynecol Scand. 1992;71(6):458-464.
81. Dorandeu A, Raoul JL, Siriser F, et al. Carcinoma of the ampulla of Vater: prognostic factors after curative surgery: A series of 45 cases. Gut. 1997;40:350-355.
82. Abrams RA, Grochow LB, Chakravarthy A, et al. Intensified adjuvant therapy for pancreatic and periampullary adenocarcinoma: Survival results and observations regarding patterns of failure, radiotherapy dose and CA19-9 levels. Int J Radiat Oncol Biol Phys. 1999;44(5):1039-1046.
83. Kau SY, Shyr YM, Su CH, et al. Diagnostic and prognostic values of CA 19-9 and CEA in periampullary cancers. J Am Coll Surg. 1999;188(4):415-420.
84. Cherchi PL, Dessole S, Ruiu GA, et al. The value of serum CA 125 and association CA 125/CA 19-9 in endometrial carcinoma. Eur J Gynaecol Oncol. 1999;20(4):315-317.
85. Rojas MP, Telaro E, Russo A, et al. Follow-up strategies for women treated for early breast cancer. Cochrane Database Syst Rev. 2000;(4):CD001768.
86. Grossfeld GD, Litwin MS, Wolf JS Jr, et al. Evaluation of asymptomatic microscopic hematuria in adults: The American Urological Association best practice policy--part II: Patient evaluation, cytology, voided markers, imaging, cystoscopy, nephrology evaluation, and follow-up. Urology. 2001;57(4):604-610.
87. Segal R, Lukka H, Klotz LH, et al.; Cancer Care Ontario Practice Guidelines Initiative Genitourinary Cancer Disease Site Group. Surveillance programs for early stage non-seminomatous testicular cancer: A practice guideline. Can J Urol. 2001;8(1):1184-1192.
88. Levine M, Moutquin JM, Walton R, Feightner J. Chemoprevention of breast cancer. A joint guideline from the Canadian Task Force on Preventive Health Care and the Canadian Breast Cancer Initiative's Steering Committee on Clinical Practice Guidelines for the Care and Treatment of Breast Cancer. CMAJ. 2001;164(12):1681-1690.
89. U.S. Preventive Services Task Force. Chemoprevention of breast cancer: Recommendations and rationale. Ann Intern Med. 2002;137(1):56-58.
90. Jeffery GM, Hickey BE, Hider P. Follow-up strategies for patients treated for non-metastatic colorectal cancer. Cochrane Database Syst Rev. 2007;(1):CD002200.
91. Garcia-Barcina M, Martin Bueno AE, de Bujanda Fernandez Pierola L, et al. Tumour markers in certain cancer locations. D-02-03. Vitoria-Gasteiz, Spain: Basque Office for Health Technology Assessment, Health Department Basque Government (OSTEBA); 2002.
92. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. Ann Intern Med. 2003;139(1):46-50.
93. Scottish Intercollegiate Guidelines Network (SIGN). Epithelial ovarian cancer. A national clinical guideline. SIGN Pub. No. 75. Edinburgh, Scotland: Scottish Intercollegiate Guidelines Network (SIGN); October 2003.
94. Baron TH, Mallery JS, Hirota WK, et al. The role of endoscopy in the evaluation and treatment of patients with pancreaticobiliary malignancy. Gastrointest Endosc. 2003;58(5):643-649.
95. Riley RD, Burchill SA, Abrams KR, et al. A systematic review and evaluation of the use of tumour markers in paediatric oncology: Ewing's sarcoma and neuroblastoma. Health Technol Assess. 2003;7(5):1-162
96. Society for Surgery of the Alimentary Tract (SSAT). Operative treatment for chronic pancreatitis. Manchester, MA: SSAT; 2004.

97. Institute for Clinical Systems Improvement (ICSI). Breast cancer treatment. ICSI Healthcare Guidelines. Bloomington, MN: ICSI; September 2004.
98. Fradet Y, Saad F, Aprikian A, et al. uPM3, a new molecular urine test for the detection of prostate cancer. Urology. 2004;64(2):311-315; discussion 315-316.
99. Tinzl M, Marberger M, Horvath S, Chypre C. DD3PCA3 RNA analysis in urine--a new perspective for detecting prostate cancer. Eur Urol. 2004;46(2):182-186; discussion 187.
100. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: Update 2003. J Clin Oncol. 2004;22(2):330-353.
101. Otchy D, Hyman NH, Simmang C, et al. Practice parameters for colon cancer. Dis Colon Rectum. 2004;47(8):1269-1284.
102. Saad F. UPM3: Review of a new molecular diagnostic urine test for prostate cancer. Can J Urol. 2005;12 Suppl 1:40-43; discussion 99-100.
103. Bast RC Jr, Badgwell D, Lu Z, et al. New tumor markers: CA125 and beyond. Int J Gynecol Cancer. 2005;15 Suppl 3:274-281.
104. Garner EI. Advances in the early detection of ovarian carcinoma. J Reprod Med. 2005;50(6):447-453.
105. Fields MM, Chevlen E. Ovarian cancer screening: A look at the evidence. Clin J Oncol Nurs. 2006;10(1):77-81.
106. Paik et al. Gene Expression and Benefit of Chemotherapy in Women With Node-Negative, Estrogen Receptor-Positive Breast Cancer J. Clin. Oncol. 2006 0 (2006), p. JCO.2005.04.7985v1
107. Habel LA, Shak S, Jacobs MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. Breast Cancer Res. 2006; 8:R25 (doi:10.1186/bcr1412).
108. Burstein HJ, Paik S, Ravdin PM, Albain KS. Adjuvant chemotherapy for patients with estrogen receptor-postitive breast cancer. 2006 ASCO Educational Sessions. 2006:49-55.
109. Aguado J. Identification of circulating tumour cells in metastatic breast cancer with the CellSearch (TM) system [summary]. Technological Report. Seville, Spain: Agencia de Evaluacion de Tecnologias Sanitarias de Andalucia (AETSA); 2006. Available at: http://www.juntadeandalucia.es/salud/contenidos/aetsa/pdf/Ficha%20CTCs%20ENG.pdf. Accessed October 6, 2006.
110. Hultcrantz R, Olsson R, Danielsson A, et al. A 3-year prospective study on serum tumor markers used for detecting cholangiocarcinoma in patients with primary sclerosing cholangitis. J Hepatol. 1999;30(4):669-673.
111. Chakravatri A, Zehr EM, Zietman AL, et al. Thymosin beta-15 predicts for distant failure in patients with clinically localized prostate cancer-results from a pilot study. Urology. 2000;55(5):635-638.
112. van de Vijver, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002;347(25):1999-2009.
113. Nehls O, Gregor M, Klump B. Serum and bile markers for cholangiocarcinoma. Semin Liver Dis. 2004;24(2):139-154.
114. Levy C, Lymp J, Angulo P, et al. The value of serum CA 19-9 in predicting cholangiocarcinomas in patients with primary sclerosing cholangitis. Dig Dis Sci. 2005;50(9):1734-1740.
115. Hutchinson LM, Chang EL, Becker CM, et al. Development of a sensitive and specific enzyme-linked immunosorbent assay for thymosin beta15, a urinary biomarker of human prostate cancer. Clin Biochem. 2005;38(6):558-571.
116. Locker GY, Hamilton S, Harris J, et al; ASCO. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol. 2006;24(33):5313-5327.
117. Khatcheressian JL, Wolff AC, Smith TJ, et al; American Society of Clinical Oncology. American Society of Clinical Oncology 2006 update of the breast cancer follow-up and management guidelines in the adjuvant setting. J Clin Oncol. 2006;24(31):5091-5097.
118. National Horizon Scanning Centre (NHSC). NMP22 BladderChek proteomic assay for the detection of bladder cancer - horizon scanning technology note. Birmingham, UK: NHSC; 2006.

119. National Horizon Scanning Centre (NHSC). Prostate cancer gene 3 (Progensa PCA3) assay in the diagnosis of prostate cancer: Horizon Scanning Technology Briefing. Birmingham, UK: NHSC; 2006.
120. Parrella A, Mundy L, Merlin T, Hiller J. NMP22 BladderChek(TM) point-of-care diagnostic test for bladder cancer. Horizon Scanning Prioritising Summary - Volume 9. Adelaide, SA: Adelaide Health Technology Assessment (AHTA) on behalf of National Horizon Scanning Unit (HealthPACT and MSAC); 2005.
121. California Technology Assessment Forum (CTAF). Gene expression profiling as a guide for the management of early stage breast cancer. Technology Assessment. San Francisco, CA: CTAF; 2007. Available at: http://ctaf.org/content/general/detail/621. Accessed. April 13, 2007.
122. Myers ER, Havrilesky LJ, Kulasingam SL, et al. Genomic tests for ovarian cancer detection and management. Evidence Report/Technology Assessment 145. Prepared by the Duke Evidence-Based Practice Center for the Agency for Healthcare Research and Quality (AHRQ). AHRQ Publication No. 07-E0001. Rockville, MD: AHRQ; October 2006. Available at: http://www.ahrq.gov/downloads/pub/evidence/pdf/genomicovc/genovc.pdf. Accessed May 27, 2007.
123. Finkelstein SD, Marsh W, Demetris AJ, et al. Microdissection-based allelotyping discriminates de novo tumor from intrahepatic spread in hepatocellular carcinoma. Hepatology. 2003;37(4):871-879.
124. Finkelstein SD, Przygodzki R, Pricolo VE, et al. Prediction of biologic aggressiveness in colorectal cancer by p53/K-ras-2 topographic genotyping. Mol Diagn. 1996;1(1):5-28.
125. Kanbour-shakir A, Kounelis S, Papadaki H, et al. Relationship of p53 genotype to second-look recurrence and survival in ovarian epithelial malignancy. Mol Diagn. 1996;1(2):121-129.
126. Ribeiro U, Safatle-Ribeiro AV, Posner MC, et al. Comparative p53 mutational analysis of multiple primary cancers of the upper aerodigestive tract. Surgery. 1996;120(1):45-53.
127. Przygodzki RM, Koss MN, Moran CA, et al. Pleomorphic (giant and spindle cell) carcinoma is genetically distinct from adenocarcinoma and squamous cell carcinoma by K-ras-2 and p53 analysis. Am J Clin Pathol. 1996;106(4):487-492.
128. Safatle-Ribeiro AV, Ribeiro Junior U, Reynolds JC, et al. Morphologic, histologic, and molecular similarities between adenocarcinomas arising in the gastric stump and the intact stomach. Cancer. 1996;78(11):2288-2299.
129. Przygodzki RM, Finkelstein SD, Keohavong P, et al. Sporadic and thorotrast-induced angiosarcomas of the liver manifest frequent and multiple point mutations in K-ras-2. Lab Invest. 1997;76(1):153-159.
130. Pricolo VE, Finkelstein SD, Bland KI. Topographic genotyping of colorectal carcinoma: From a molecular carcinogenesis model to clinical relevance. Ann Surg Oncol. 1997;4(3):269-278.
131. Holst VA, Finkelstein S, Colby TV, et al. p53 and K-ras mutational genotyping in pulmonary carcinosarcoma, spindle cell carcinoma, and pulmonary blastoma: Implications for histogenesis. Am J Surg Pathol. 1997;21(7):801-811.
132. Jones MW, Kounelis S, Papadaki H, et al. The origin and molecular characterization of adenoid basal carcinoma of the uterine cervix. Int J Gynecol Pathol. 1997;16(4):301-306.
133. Jones MW, Kounelis S, Hsu C, et al. Prognostic value of p53 and K-ras-2 topographic genotyping in endometrial carcinoma: A clinicopathologic and molecular comparison. Int J Gynecol Pathol. 1997;16(4):354-360.
134. Kounelis S, Jones MW, Papadaki H, et al. Carcinosarcomas (malignant mixed mullerian tumors) of the female genital tract: Comparative molecular analysis of epithelial and mesenchymal components. Hum Pathol. 1998;29(1):82-87.
135. Ribeiro U Jr, Finkelstein SD, Safatle-Ribeiro AV, et al. p53 sequence analysis predicts treatment response and outcome of patients with esophageal carcinoma. Cancer. 1998;83(1):7-18.
136. Finkelstein SD, Tiffee JC, Bakker A, et al. Malignant transformation in sinonasal papillomas is closely associated with aberrant p53 expression. Mol Diagn. 1998;3(1):37-

41.
137. Holst VA, Finkelstein S, Yousem SA. Bronchioloalveolar adenocarcinoma of lung: Monoclonal origin for multifocal disease. Am J Surg Pathol. 1998;22(11):1343-1350.
138. Pollack IF, Finkelstein SD, Burnham J, et al.; Children's Cancer Group. Age and TP53 mutation frequency in childhood malignant gliomas: Results in a multi-institutional cohort. Cancer Res. 2001;61(20):7404-7407.
139. Papadaki H, Kounelis S, Kapadia SB, et al. Relationship of p53 gene alterations with tumor progression and recurrence in olfactory neuroblastoma. Am J Surg Pathol. 1996;20(6):715-721.
140. Przygodzki RM, Finkelstein SD, Langer JC, et al. Analysis of p53, K-ras-2, and C-raf-1 in pulmonary neuroendocrine tumors. Correlation with histological subtype and clinical outcome. Am J Pathol. 1996;148(5):1531-1541.
141. Pricolo VE, Finkelstein SD, Wu TT, et al. Prognostic value of TP53 and K-ras-2 mutational analysis in stage III carcinoma of the colon. Am J Surg. 1996;171(1):41-46.
142. Finkelstein SD, Przygodzki R, Pricolo VE, et al. K-ras-2 topographic genotyping of pancreatic adenocarcinoma. Arch Surg. 1994;129(4):367-73.
143. Lin X, Finkelstein SD, Zhu B, Silverman JF. Molecular analysis of multifocal papillary thyroid carcinoma. J Mol Endocrinol. 2008;41(4):195-203.
144. Saad RS, Denning KL, Finkelstein SD, et al. Diagnostic and prognostic utility of molecular markers in synchronous bilateral breast carcinoma. Mod Pathol. 2008;21(10):1200-1207.
145. Krishnamurti U, Sasatomi E, Swalsky PA, et al. Analysis of loss of heterozygosity in atypical and negative bile duct brushing cytology specimens with malignant outcome: Are 'negative' cytologic findings a representation of morphologically subtle molecular alterations? Arch Pathol Lab Med. 2007;131(1):74-80.
146. Khalid A, Nodit L, Zahid M, et al. Endoscopic ultrasound fine needle aspirate DNA analysis to differentiate malignant and benign pancreatic masses. Am J Gastroenterol. 2006;101(11):2493-2500.
147. Maheshwari V, Tsung A, Lin Y, et al. Analysis of loss of heterozygosity for tumor-suppressor genes can accurately classify and predict the clinical behavior of mucinous tumors arising from the appendix. Ann Surg Oncol. 2006;13(12):1610-1616.
148. Lapkus O, Gologan O, Liu Y, et al. Determination of sequential mutation accumulation in pancreas and bile duct brushing cytology. Mod Pathol. 2006;19(7):907-913.
149. Krishnamurti U, Sasatomi E, Swalsky PA, et al. Microdissection-based mutational genotyping of serous borderline tumors of the ovary. Int J Gynecol Pathol. 2005;24(1):56-61.
150. American Cancer Society (ACS). Ovarian cancer has early symptoms. First national consensus on common warning signs. ACS News Center. Atlanta, GA: ACS; June 14, 2007. Available at: http://www.cancer.org/. Accessed July 6, 2007.
151. Berchuck A; Society for Gynecologic Oncologists. Ovarian cancer symptoms consensus statement. Chicago, IL: Society for Gynecologic Oncologists; 2007. Available at: http://www.sgo.org/publications/OvarianCancerSymptoms.pdf. Accessed July 6, 2007.
152. Goff BA, Mandel LS, Melancon CH, Muntz HG. Frequency of symptoms of ovarian cancer in women presenting to primary care clinics. JAMA. 2004;291(22):2705-2712.
153. Daly MB, Ozols RF. Symptoms of ovarian cancer--where to set the bar? JAMA. 2004;291(22):2755-2756.
154. Groskopf J, Aubin SM, Deras IL, et al. APTIMA PCA3 molecular urine test: Development of a method to aid in the diagnosis of prostate cancer. Clin Chem. 2006;52(6):1089-1095.
155. Groskopf J, Nakanishi H, Deras IL, et al. The PCA3 score correlates with tumor volume but not prostate size, and can synergize with other patient information for predicting biopsy outcome. Abstrac 1709. Annual Meeting of the American Urological Association. Anaheim, CA; May 19-24, 2007. Available at: http://www.posters2view.com/aua07/view.php?nu=1709. Accessed August 21, 2007.
156. van Gils MP, Hessels D, van Hooij O, et al The time-resolved fluorescence-based PCA3 test on urinary sediments after digital rectal examination; A Dutch multicenter validation of the diagnostic performance. Clin Cancer Res. 2007;13(3):939-943.
157. Marks LS, Fradet Y, Deras IL, et al. PCA3 molecular urine assay for prostate cancer in men

undergoing repeat biopsy. Urology. 2007;69(3):532-535.
158. Gene-Probe, Inc. Gen-Probe PCA3 assay. Package Insert. San Diego, CA: Gen-Probe; April 2007. Available at: http://www.tdlpathology.com/images/downloads/500614RevB-PCA3%20Assay%20PI.pdf. Accessed August 19, 2007.
159. Haese A, Van Poppel H, Marberger M, et al. The value of the PCA3 assay in guiding decision which men with a negative prostate biopsy need immediate repeat biopsy: Preliminary European data. Abstract. 22nd Annual EAU Congress. Berlin, Germany; March 21-24, 2007. European Association of Urology Web site. Available at: http://www.uroweb.org/publications/eau-abstracts-online/?AID=11696. Accessed August 19, 2007.
160. National Horizon Scanning Center (NHSC). Prostate cancer gene 3 (Progensa PCA3) assay in the diagnosis of prostate cancer. Horizon Scanning Technology Briefing. Birmingham, UK: NHSC; December 2006. Available at: http://pcpoh.bham.ac.uk/publichealth/horizon/PDF_files/2006reports/December06/PCA3%20for%20diagnosis%20of%20prostate%20cancer.pdf. Accessed August 21, 2007.
161. George B, Datar RH, Wu L, et al. p53 gene and protein status: The role of p53 alterations in predicting outcome in patients with bladder cancer. J Clin Oncol. 2007;25(34):5352-5358.
162. Real FX. p53: It has it all, but will it make it to the clinic as a marker in bladder cancer? J Clin Oncol. 2007;25(34):5341-5344.
163. Parekh DJ, Ankerst DP, Troyer D, et al. Biomarkers for prostate cancer detection. J Urol. 2007;178(6):2252-2259.
164. Harris L, Fritsche H, Mennel R, et al; American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. 2007;25(33):5287-5312.
165. Svatek RS, Karam J, Karakiewicz PI, et al. Role of urinary cathepsin B and L in the detection of bladder urothelial cell carcinoma. J Urol. 2008;179(2):478-484; discussion 484.
166. Marchionni L, Wilson RF, Wolff AC, et al. Systematic review: Gene expression profiling assays in early-stage breast cancer. Ann Intern Med. 2008;148(5):358-369.
167. Wolf I, Ben-Baruch N, Shapira-Frommer R, et al. Association between standard clinical and pathologic characteristics and the 21-gene recurrence score in breast cancer patients: A population-based study. Cancer. 2008;112(4):731-736.
168. Australia and New Zealand Horizon Scanning Network (ANZHSN). Genesearch™ breast lymph node (BLN) assay. Horizon Scanning Technology Prioritising Summaries. Canberra, ACT: Australian Government, Department of Health and Ageing; May 2008. Available at: http://www.health.gov.au/. Accessed August 12, 2008.
169. Blumencranz P, Whitworth PW, Deck K, et al. Scientific Impact Recognition Award. Sentinel node staging for breast cancer: intraoperative molecular pathology overcomes conventional histologic sampling errors. Am J Surg. 2007; 194(4):426-432.
170. Blue Cross Blue Shield Association (BCBSA), Technology Evaluation Center (TEC). Use of genesearch breast lymph node assay to detect sentinel node metastases in early stage breast cancer. TEC Assessment Program. Chicago, IL:BCBSA; 2007; 22(8). Available at: http://www.bcbs.com/blueresources/tec/vols/22/22_08.html. Accessed August 12, 2008.
171. U. S. Food and Drug Administration. GeneSearch BLN Testkit. P060017. Rockville, MD: FDA. July 16, 2007. Available at: http://www.fda.gov/cdrh/pdf6/p060017a.pdf. Accessed August 12, 2008.
172. U.S. Food and Drug Administration CDRH Consumer Information. Genesearch BLN Test Kit - P060017. Rockville, MD: FDA. August 3, 2007. Available at: http://www.fda.gov/cdrh/MDA/DOCS/p060017.html. Accessed August 12, 2008.
173. Viale G, Bosari S, Maxxarol G, et al. Intra-operative examination of axillary sentinel lymph nodes in breast carcinoma patients. Cancer 1999; 85(11):2433-2438.
174. Smeets A, Christiaens M. Implications of the sentinel lymph node procedure for local and systemic adjuvant treatment. Current Opinion in Oncology 2005; 17:539-544.
175. Viale G, Dell'Orto P, Biasi MO, et al. Comparative evaluation of an extensive histopathologic examination and a real-time reverse-transcription-polymerase chain reaction assay for mammaglobin and cytokeratin 19 on axillary sentinel lymph nodes of

breast carcinoma patients. Ann Surg. 2008;247(1):136-142.
176. Julian TB, Blumencranz P, Deck K, et al. Novel intraoperative molecular test for sentinel lymph node metastases in patients with early-stage breast cancer. J Clin Oncol. 2008;26(20):3338-3345.
177. Visintin I, Feng Z, Longton G, et al. Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res. 2008;14(4):1065-1072.
178. Society of Gynecologic Oncologists (SGO).. Statement regarding OvaSure. Chicago, IL: SGO; July 2, 2008. Available at: http://blog.targethealth.com/?p=1492. Accessed September 2, 2008.
179. U.S. Food and Drug Administration (FDA), Center for Devices and Radiologic Health (CDRH), Office of In Vitro Diagnostic Device Evaluation and Safety (OVID). Warning Letter to Laboratory Corporation of America. Rockville, MD: FDA; September 29, 2008. Available at: http://www.fda.gov/foi/warning_letters/s6947c.htm. Accessed October 9, 2008.
180. Coradini D, Daidone MG. Biomolecular prognostic factors in breast cancer. Curr Opin Obstet Gynecol. 2004;16(1):49-55.
181. Provista Life Sciences. The BT test and early breast cancer detection [website]. Phoenix, AZ: Provista Life Sciences; 2008. Available at: http://www.provistals.com/BTTestOverview.aspx. Accessed September 8, 2008.
182. Urban N, McIntosh MW, Andersen M, Karlan BY. Ovarian cancer screening. Hematol Oncol Clin North Am. 2003;17(4):989-1005.
183. Lu KH, Patterson AP, Wang L, et al. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res. 2004;10(10):3291-3300.
184. Drapkin R, von Horsten HH, Lin Y, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res. 2005;65(6):2162-2169.
185. Rosenthal AN, Menon U, Jacobs IJ. Screening for ovarian cancer. Clin Obstet Gynecol. 2006;49(3):433-447.
186. Scholler N, Crawford M, Sato A, et al. Bead-based ELISA for validation of ovarian cancer early detection markers. Clin Cancer Res. 2006;12(7 Pt 1):2117-2124.
187. Havrilesky LJ, Whitehead CM, Rubatt JM, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecol Oncol. 2008;110(3):374-382.
188. Lowe KA, Shah C, Wallace E, et al. Effects of personal characteristics on serum CA125, mesothelin, and HE4 levels in healthy postmenopausal women at high-risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2008;17(9):2480-2487.
189. Otchy D, Hyman NH, Simmang C, et al. Practice parameters for colon cancer. Dis Colon Rectum. 2004;47(8):1269-1284.
190. Compton CC. Pathology and prognostic determinants of colorectal cancer. UpToDate [online serial]. Waltham, MA: UpToDate; 2008.
191. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special Report: Pharmacogenomics of cancer-candidate genes.TEC Assessment Program. Chicago, IL: BCBSA; November 2007;22(5). Available at: http://www.bcbs.com/blueresources/tec/vols/22/22_05.pdf. Accessed September 9, 2008.
192. Duffy MJ, van Dalen A, Haglund C, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer. 2007 Jun;43(9):1348-1360.
193. Shankaran V, Wisinski KB, Mulcahy MF, et al. The role of molecular markers in predicting response to therapy in patients with colorectal cancer. Mol Diagn Ther. 2008;12(2):87-98.
194. Welch S, Kocha RB, Rumble K, et al., Gastrointestinal Cancer Disease Site Group. Cancer Care Ontario. The role of bevacizumab (avastin) combined with chemotherapy in the treatment of patients with advanced colorectal cancer: Guideline recommendations. Evidence-based Series #2-25: Section 1. Toronto, ON: Cancer Care Ontario; May 2008.
195. National Institute for Health and Clinical Excellence (NICE). Bevacizumab and cetuximab for the treatment of metastatic colorectal cancer. Technology Appraisal Guidance 118.

London, UK: NICE; January 2007. Available at: http://www.nice.org.uk/guidance/TA118. Accessed July 25, 2007.
196. Secord AA, Darcy KM, Hutson A, et al; Gynecologic Oncology Group study. Co-expression of angiogenic markers and associations with prognosis in advanced epithelial ovarian cancer: a Gynecologic Oncology Group study. Gynecol Oncol. 2007;106(1):221-232.
197. Hellstrom I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res. 2003;63:3695.
198. Hellstrom I, Hellstrom KE. SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA125 and/or each other. Adv Exp Med Biol. 2008;622:15-21
199. Moore RG, Brown AK, Miller MC, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol. 2008;108(2):402-408.
200. Bast RC Jr, Brewer M, Zou C, et al. Prevention and early detection of ovarian cancer: Mission impossible? Recent Results Cancer Res. 2007;174:91-100.
201. Scholler N, Crawford M, Sato A, et al. Bead-based ELISA for validation of ovarian cancer early detection markers. Clin Cancer Res. 2006;12(7 Pt 1):2117-2124.
202. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26:1324-1337.
203. Salgia R. Molecular markers in non-small cell lung cancer. Waltham, MA; UpToDate [online serial]; 2008.
204. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. Blood. 2008;111(7):3322-3330.
205. Bos JL. Ras oncogenes in human cancer: A review. Cancer Res. 1989;49(17):4682-4689.
206. Lièvre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66(8):3992-3995.
207. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. Br J Cancer. 2007;96(8):1166-1169.
208. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol. 2008;19(3):508-515.
209. Lièvre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol. 2008;26(3):374-379.
210. Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol. 2005;6(5):279-286.
211. Gonçalves A, Esteyries S, Taylor-Smedra B, et al. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. BMC Cancer. 2008;8(169). Available at: http://www.biomedcentral.com/1471-2407/8/169. Accessed October 16, 2008.
212. Van Cutsem E, Lang I., D'haens G., et al. KRAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treated with FOLFIRI with or without cetuximab: The CRYSTAL experience. J Clin Oncol. 2008;26: Abstr. 2. Available at: http://www.asco.org/ASCO/Abstracts+%26+Virtual+Meeting/Abstracts?&vmview=abst_detail_view&confID=55&abstractID=34491. Accessed October 16, 2008.
213. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: A systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol. 2008;9(10):962-972.
214. Bokemeyer C, Bondarenko I, Hartmann J, et al. KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: The OPUS experience. J Clin Oncol. 2008; 26: (May 20 Suppl; Abstr. 4000). Available at:

http://www.asco.org/ASCO/Abstracts+%26+Virtual+Meeting/Abstracts?&vmview=abst_detail_view&confID=55&abstractID=35260. Accessed October 16, 2008.
215. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(10):1626-1634.
216. Blue Cross Blue Shield Association (BCBSA), Technology Evaluation Center (TEC). KRAS mutations and epidermal growth factor receptor inhibitor therapy in metastatic colorectal cancer. TEC Assessment Program. Chicago, IL: BCBSA; January 2009;25(6).
217. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special report: Recent developments in prostate cancer genetics and genetic testing. TEC Assessments in Press. Chicago, IL: BCBSA; September 2008.
218. Nielsen KV, Ejlertsen B, Møller S, et al. The value of TOP2A gene copy number variation as a biomarker in breast cancer: Update of DBCG trial 89D. Acta Oncol. 2008;47(4):725-734.
219. Järvinen TA, Liu ET. Simultaneous amplification of HER-2 (ERBB2) and topoisomerase IIalpha (TOP2A) genes--molecular basis for combination chemotherapy in cancer. Curr Cancer Drug Targets. 2006;6(7):579-602.
220. Pritchard KI, Messersmith H, Elavathil L, et al. HER-2 and topoisomerase II as predictors of response to chemotherapy. J Clin Oncol. 2008;26(5):736-744.
221. Järvinen TA, Liu ET. Simultaneous amplification of HER-2 (ERBB2) and topoisomerase IIalpha (TOP2A) genes--molecular basis for combination chemotherapy in cancer. Curr Cancer Drug Targets. 2006;6(7):579-602.
222. Everhard S, Kaloshi G, Crinière E, et al. MGMT methylation: A marker of response to temozolomide in low-grade gliomas. Ann Neurol. 2006;60(6):740-743.
223. Palanichamy K, Erkkinen M, Chakravarti A. Predictive and prognostic markers in human glioblastomas. Curr Treat Options Oncol. 2006;7(6):490-504.
224. van den Bent MJ, Kros JM. Predictive and prognostic markers in neuro-oncology. J Neuropathol Exp Neurol. 2007;66(12):1074-1081.
225. Stupp R, Hegi ME, Gilbert MR, et al. Chemoradiotherapy in malignant glioma: standard of care and future directions. J Clin Oncol. 2007;25(26):4127-4136.
226. Murat A, Migliavacca E, Gorlia T, et al. Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. J Clin Oncol. 2008;26(18):3015-3024.
227. Secord AA, Darcy KM, Hutson A, et al. Co-expression of angiogenic markers and associations with prognosis in advanced epithelial ovarian cancer: A Gynecologic Oncology Group study. Gynecol Oncol. 2007;106(1):221-232.
228. Ghoneim C, Soula-Rothhut M, Rothhut B. Thrombospondin-1 in differentiated thyroid cancer: Dr. Jekyll and Mr. Hyde. Connect Tissue Res. 2008;49(3):257-260.
229. Brostjan C, Gebhardt K, Gruenberger B, et al. Neoadjuvant treatment of colorectal cancer with bevacizumab: the perioperative angiogenic balance is sensitive to systemic thrombospondin-1 levels. Clin Cancer Res. 2008;14(7):2065-2074.
230. Garcia AA, Hirte H, Fleming G, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: A trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol. 2008;26(1):76-82.
231. List AF, Spier CM. Multidrug resistance in acute leukemia: A conserved physiologic function. Leuk Lymphoma. 1992;8(1-2):9-14.
232. Yuen AR, Sikic BI. Multidrug resistance in lymphomas. J Clin Oncol. 1994;12(11):2453-2459.
233. Solary E, Drenou B, Campos L, et al; Groupe Ouest Est Leucémies Aiguës Myéloblastiques. Quinine as a multidrug resistance inhibitor: A phase 3 multicentric randomized study in adult de novo acute myelogenous leukemia. Blood. 2003;15;102(4):1202-1210.
234. Gruber A, Björkholm M, Brinch L, et al. A phase I/II study of the MDR modulator Valspodar (PSC 833) combined with daunorubicin and cytarabine in patients with relapsed and primary refractory acute myeloid leukemia. Leuk Res. 2003;27(4):323-328.

235. Takara K, Sakaeda T, Okumura K. An update on overcoming MDR1-mediated multidrug resistance in cancer chemotherapy. Curr Pharm Des. 2006;12(3):273-286.
236. Miyake M, Nakano K, Ieki Y, et al. Motility related protein 1 (MRP-1/CD9) expression: inverse correlation with metastases in breast cancer. Cancer Res. 1995;55(18):4127-4131.
237. Erovic BM, Pammer J, Hollemann D, et al. Motility-related protein-1/CD9 expression in head and neck squamous cell carcinoma Head Neck. 2003;25(10):848-857.
238. Mimori K, Kataoka A, Yoshinaga K, et al. Identification of molecular markers for metastasis-related genes in primary breast cancer cells. Clin Exp Metastasis. 2005;22(1):59-67.
239. Mhawech P, Dulguerov P, Tschanz E, et al. Motility-related protein-1 (MRP-1/CD9) expression can predict disease-free survival in patients with squamous cell carcinoma of the head and neck. Br J Cancer. 2004;90(2):471-475.
240. Mhawech P, Herrmann F, Coassin M, et al. Motility-related protein 1 (MRP-1/CD9) expression in urothelial bladder carcinoma and its relation to tumor recurrence and progression. Cancer. 2003;98(8):1649-1657.
241. National Cancer Institute. Carcinoma of unknown primary treatment (PDQ®). Bethesda, MD: NCI; 2008. Available at: http://www.cancer.gov/cancertopics/pdq/treatment/unknownprimary. Accessed October 13, 2008.
242. U.S. Food and Drug Administration (FDA). FDA clears test that helps identify type of cancer in tumor sample. FDA News. Rockville, MD: FDA; July 31, 2008. Available at: http://www.fda.gov/bbs/topics/NEWS/2008/NEW01870.html. Accessed October 13, 2008.
243. Dumur CI, Lyons-Weiler M, Sciulli C, et al. Interlaboratory performance of a microarray-based gene expression test to determine tissue of origin in poorly differentiated and undifferentiated cancers. J Mol Diagn. 2008;10(1):67-77.
244. Pathwork Diagnostics [website]. Announcing the pathwork tissue of origin test [website]; Sunnyvale, CA. Available at: http://www.pathworkdx.com/index.html. Accessed October 13, 2008.
245. Monzon FA, Dumur CI, Lyons-Weiler M, et al. Validation of a gene expression-based tissue of origin test applied to poorly differentiated and undifferentiated cancers. Abstract presented at the 13th Annual Meeting of the Association for Molecular Pathology, Los Angeles, CA, November 7-10, 2007.
246. Flynn K. Bladder cancer surveillance. Brief Overview. Final Report. Boston, MA: Veterans Health Administration, Office of Patient Care Services, Technology Assessment Program; November 2007.
247. Nauseef WM, Olsson I, Arnljots K. Biosynthesis and processing of myeloperoxidase--a marker for myeloid cell differentiation. Eur J Haematol. 1988;40(2):97-110.
248. Storr J, Dolan G, Coustan-Smith E, et al. Value of monoclonal anti-myeloperoxidase (MPO7) for diagnosing acute leukaemia. J Clin Pathol. 1990;43(10):847-849.
249. Dunphy CH, Polski JM, Evans HL, Gardner LJ. Evaluation of bone marrow specimens with acute myelogenous leukemia for CD34,CD15, CD117, and myeloperoxidase. Arch Pathol Lab Med. 2001;125(8):1063-1069.
250. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292-2302.
251. Matsuo T, Kuriyama K, Miyazaki Y, et al. The percentage of myeloperoxidase-positive blast cells is a strong independent prognostic factor in acute myeloid leukemia, even in the patients with normal karyotype. Leukemia. 2003;17:1538-1543.
252. British Committee for Standards in Haematology, Milligan DW, Grimwade D, Cullis JO, et al. Guidelines on the management of acute myeloid leukaemia in adults. Br J Haematol. 2006;135(4):450-474.
253. Storr J, Dolan G, Coustan-Smith E, et al. Value of monoclonal anti-myeloperoxidase (MPO7) for diagnosing acute leukaemia. J Clin Pathol. 1990;43(10):847-849.
254. British Committee for Standards in Haematology, Milligan DW, Grimwade D, Cullis JO, et al. Guidelines on the management of acute myeloid leukaemia in adults. Br J Haematol. 2006;135(4):450-474.
255. National Comprehensive Cancer Network (NCCN). Acute myeloid leukemia. NCCN

Clinical Practice Guidelines in Oncology v.1.2009. Fort Washington, PA: NCCN; September 2008. Available at: http://www.nccn.org/professionals/physician_gls/PDF/aml.pdf. Accessed January 26, 2009.
256. Liebman HA, Furie BC, Tong MJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. NEJM. 1984;310(22):1427-1431.
257. Fujiyama S, Izuno K, Gohshi K, et al. Clinical usefulness of des-gamma-carboxy prothrombin assay in early diagnosis of hepatocellular carcinoma. Dig Dis Sci. 1991;36(12):1787-1792.
258. Weitz IC; Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: A critical review. Hepatology. 1993;18(4):990-997.
259. Nomura F, Ishijima M, Horikoshi A, et al. Determination of serum des-gamma-carboxy prothrombin levels in patients with small-sized hepatocellular carcinoma: Comparison of the conventional enzyme immunoassay and two modified methods. Am J Gastroenterol. 1996;91(7):1380-1383.
260. Aoyagi Y, Oguro M, Yanagi M, et al. Clinical significance of simultaneous determinations of alpha-fetoprotein and des-gamma-carboxy prothrombin in monitoring recurrence in patients with hepatocellular carcinoma. Cancer. 1996;77(9):1781-1786.
261. Hamamura K, Shiratori Y, Shiina S, et al. Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. Cancer. 2000;88(7):1557-1564.
262. Ikoma J, Kaito M, Ishihara T, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: A prospective study. Hepatogastroenterology. 2002;49(43):235-238.
263. Nagaoka S, Yatsuhashi H, Hamada H, et al. The des-gamma-carboxy prothrombin index is a new prognostic indicator for hepatocellular carcinoma. Cancer. 2003;98(12):2671-2677.
264. Yuan LW, Tang W, Kokudo N, et al. Measurement of des-gamma-carboxy prothrombin levels in cancer and non-cancer tissue in patients with hepatocellular carcinoma. Oncol Rep. 2004;12(2):269-273.
265. Yuen MF, Lai CL. Serological markers of liver cancer. Best Pract Res Clin Gastroenterol. 2005;19(1):91-99.
266. Nakamura S, Nouso K, Sakaguchi K, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. Am J Gastroenterol. 2006;101(9):2038-2043.
267. Toyoda H, Kumada T, Kiriyama S, et al. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2006;4(1):111-117.
268. Shirabe K, Itoh S, Yoshizumi T, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. J Surg Oncol. 2007;95(3):235-340.
269. Schwartz JM, Carithers RL. Clinical features and diagnosis of primary hepatocellular carcinoma. UpToDate [online serial]. Waltham, MA: UpToDate; 2008.
270. Sherman M. Surveillance for hepatocellular carcinoma in adults with chronic liver disease. UpToDate [online serial]. Waltham, MA: UpToDate; 2008.
271. Taylor CR. Cirrhosis. eMedicine Medicine Topic 175. Omaha, NE: eMedicine.com. January 7, 2009. Available at: http://emedicine.medscape.com/article/366426-overview. Accessed January 13, 2009.
272. National Comprehensive Cancer Network (NCCN). Hepatobiliary cancers. NCCN Clinical Practice Guidelines in Oncology v.1.2010. Fort Washington, PA: NCCN; 2010.
273. Lüftner D, Possinger K. Nuclear matrix proteins as biomarkers for breast cancer. Expert Rev Mol Diagn. 2002;2(1):23-31.
274. Wright T, McGechan A. Breast cancer: New technologies for risk assessment and diagnosis. Mol Diagn. 2003;7(1):49-55.
275. Matritech, Inc. [website]. Newton, MA; Matritech; 2009. Available at: http://www.matritech.com/. Accessed on January 27, 2009.
276. National Comprehensive Cancer Network (NCCN). Occult primary. NCCN Clinical

Practice Guidelines in Oncology v.1.2009. Fort Washington, PA: NCCN; July 2008.
277. Singletary SE, Allred C, Ashley P, et al. Revision of the American Joint Committee on Cancer staging system for breast cancer. J Clin Oncol. 2002;20(17):3628-3636.
278. National Cancer Institute. Hormone therapy with or without combination chemotherapy in treating women who have undergone surgery for node-negative breast cancer (the TAILORx Trial). ClinicalTrials.gov ID. NCT00310180. Bethesda, MD: National Library of Medicine; updated August 11, 2009.
279. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008;359(17):1757-1765.
280. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: Testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009;27(12):2091-2096.
281. Marchionni L, Wilson RF, Marinopoulos SS, et al. Impact of gene expression profiling on breast cancer outcomes. Evidence Report/Technology Assessment No. 160. Prepared by the Johns Hopkins University Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ). AHRQ Publication No. 08-E002. Rockville, MD: AHRQ; January 2008.
282. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: Can tumor gene expression profiling improve outcomes in patients with breast cancer? Genet Med. 2009;11(1):66-73.
283. de Boer M, van Deurzen CH, van Dijck JA, et al. Micrometastases or isolated tumor cells and the outcome of breast cancer. N Engl J Med. 2009;361(7):653-663.
284. Goodman S, Dickerson K, Wilson R. Gene expression profile tests for early-stage breast cancer. Effectiveness Guidance Document: Methodological Guidance for the Design of Comparative Effectiveness Studies. Version 1.0. Baltimore, MD: Center for Medical Technology Policy (CMTP); June 2009.
285. Kawai M, Uchiyama K, Tani M, et al. Clinicopathological features of malignant intraductal papillary mucinous tumors of the pancreas: The differential diagnosis from benign entities. Arch Surg. 2004;139(2):188-192.
286. Donovan MJ, Hamann S, Clayton M, et al. Systems pathology approach for the prediction of prostate cancer progression after radical prostatectomy. J Clin Oncol. 2008;26(24):3923-3929.
287. Klein EA, Stephenson AJ, Raghavan D, Dreicer R. Systems pathology and predicting outcome after radical prostatectomy. J Clin Oncol. 2008;26(24):3916-3917.
288. Shen J, Brugge WR, Dimaio CJ, Pitman MB. Molecular analysis of pancreatic cyst fluid: A comparative analysis with current practice of diagnosis. Cancer Cytopathol. 2009;117(3):217-227.
289. Leung KK, Ross WA, Evans D, et al. Pancreatic cystic neoplasm: The role of cyst morphology, cyst fluid analysis, and expectant management. Ann Surg Oncol. 2009;16(10):2818-2824.
290. Sutcliffe P, Hummel S, Simpson E, et al. Use of classical and novel biomarkers as prognostic risk factors for localised prostate cancer: A systematic review. Health Technol Assess. 2009;13(5):iii, xi-xiii 1-219.
291. Gelmann EP, Henshall SM. Clinically relevant prognostic markers for prostate cancer: The search goes on. Ann Intern Med. 2009;150(9):647-649.
292. Tosoian JJ, Loeb S, Kettermann A, et al. Accuracy of PCA3 measurement in predicting short-term biopsy progression in an active surveillance program. J Urol. 2010;183(2):534-538.
293. Noon AP, VlatkoviÄ‡ N, PolaÅ„ski R, et al. p53 and MDM2 in renal cell carcinoma: Biomarkers for disease progression and future therapeutic targets? Cancer. 2010;116(4):780-790.
294. Knauer M, Mook S, Rutgers EJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. Breast Cancer Res Treat. 2010;120(3):655-661.
295. Fung ET. A recipe for proteomics diagnostic test development: the OVA1 test,

from biomarker discovery to FDA clearance. Clin Chem. 2010;56(2):327-329.
296. Trikalinos TA, Terasawa T, Raman G, et al. A systematic review of loss-of-heterozygosity based topographic genotyping with PathfinderTG. Technology Assessment Report. Project ID: GEND0308. Prepared by the Tufts Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ) under Contract No. HHSA 290 2007 10055 I. Rockville, MD: AHRQ; March 1, 2010.
297. National Institute for Health and Clinical Excellence (NICE). Diagnosis and management of metastatic malignant disease of unknown primary origin. Clinical Guideline. Draft for Consultation. London, UK: NICE; December 2009.
298. National Comprehensive Cancer Network (NCCN). Occult primary (cancer of unknown primary [CUP]). NCCN Clinical Practice Guidelines in Oncology v.1.2010. Fort Washington, PA: NCCN; 2010.
299. Ring BZ, Seitz RS, Beck R, et al. Novel prognostic immunohistochemical biomarker panel for estrogen receptor-positive breast cancer. J Clin Oncol. 2006; 24(19):3039-3047.
300. Ross DT, Kim CY, Tang G, et al. Chemosensitivity and stratification by a five monoclonal antibody immunohistochemistry test in the NSABP B14 and B20 trials. Clin Cancer Res. 2008;14(20):6602-6609.
301. Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(19):3213-3221.
302. De Giorgi U, Valero V, Rohren E, et al. Circulating tumor cells and [18F]fluorodeoxyglucose positron emission tomography/computed tomography for outcome prediction in metastatic breast cancer. J Clin Oncol. 2009;27(20):3303-3311.
303. Mejia A, Schulz S, Hyslop T, et al. GUCY2C reverse transcriptase PCR to stage pN0 colorectal cancer patients. Expert Rev Mol Diagn. 2009;9(8):777-785.
304. Pénzváltó Z, Mihály Z, Gyorffy B. Gene expression based multigene prognostic and predictive tests in breast cancer. Magy Onkol. 2009;53(4):351-359.
305. Bartlett JM, Thomas J, Ross DT, et al. Mammostrat as a tool to stratify breast cancer patients at risk of recurrence during endocrine therapy. Breast Cancer Res. 2010;12(4):R47.
306. Boyle P, Chapman CJ, Holdenrieder S, et al. Clinical validation of an autoantibody test for lung cancer. Ann Oncol. 2011;22(2):383-389.
307. Murray A, Chapman CJ, Healey G, et al. Technical validation of an autoantibody test for lung cancer. Ann Oncol. 2010;21(8):1687-1693.
308. U.S. Preventive Services Task Force. Lung cancer screening: Recommendation statement. Ann Intern Med. 2004;140(9):738-739.
309. Humphrey LL, Teutsch S, Johnson M; U.S. Preventive Services Task Force. Lung cancer screening with sputum cytologic examination, chest radiography, and computed tomography: An update for the U.S. Preventive Services Task Force. Ann Intern Med. 2004;140(9):740-753.
310. Gradishar WJ. Male breast cancer. UpToDate [online serial]. Waltham, MA: UpToDate; 2010.
311. Shak S. Palmer G, Baehner FL, et al; Genomic Health, Redwood City, CA; Indiana University School of Medicine, Indianapolis, IN. Molecular characterization of male breast cancer by standardized quantitative RT-PCR analysis: First large genomic study of 347 male breast cancers compared to 82,434 female breast cancers. J Clin Oncol. 2009;27:15s (suppl; abstr 549).
312. Monzon FA, Lyons-Weiler M, Buturovic LJ, et al. Multicenter validation of a 1,550-gene expression profile for identification of tumor tissue of origin. J Clin Oncol. 2009;27(15):2503-2508.
313. Yurkovetsky Z, Skates S, Lomakin A, et al. Development of a multimarker assay for early detection of ovarian cancer. J Clin Oncol. 2010;28(13):2159-2166.
314. Muller CY. Doctor, should I get this new ovarian cancer test-OVA1? Obstet Gynecol. 2010;116(2 Pt 1):246-247.
315. Monzon FA, Koen TJ. Diagnosis of metastatic neoplasms: molecular approaches for identification of tissue of origin. Arch Pathol Lab Med. 2010;134(2):216-224.

316. Anderson GG, Weiss LM. Determining tissue of origin for metastatic cancers: Meta-analysis and literature review of immunohistochemistry performance. Appl Immunohistochem Mol Morphol. 2010;18(1):3-8.
317. Messick CA, Sanchez J, Dejulius KL, et al. CEACAM-7: A predictive marker for rectal cancer recurrence. Surgery. 2010;147(5):713-719.
318. Castro MA, Dal-Pizzol F, Zdanov S, et al. CFL1 expression levels as a prognostic and drug resistance marker in nonsmall cell lung cancer. Cancer. 2010;116(15):3645-3655.
319. Shanmugam C, Jhala NC, Katkoori VR, et al. Prognostic value of mucin 4 expression in colorectal adenocarcinomas. Cancer. 2010;116(15):3577-3586.
320. Ratner E, Lu L, Boeke M, et al. A KRAS-variant in ovarian cancer acts as a genetic marker of cancer risk. Cancer Res. 2010;70(16):6509-6515.
321. Hollestelle A, Pelletier C, Hooning M, et al. Prevalence of the variant allele rs61764370 T>G in the 3'UTR of KRAS among Dutch BRCA1, BRCA2 and non-BRCA1/BRCA2 breast cancer families. Breast Cancer Res Treat. 2011;128(1):79-84.
322. Cocco E, Bellone S, El-Sahwi K, et al. Serum amyloid A: A novel biomarker for endometrial cancer. Cancer. 2010;116(4):843-851.
323. Park CM, Lee WY, Chun HK, et al. Relationship of polymorphism of the tandem repeat sequence in the thymidylate synthase gene and the survival of stage III colorectal cancer patients receiving adjuvant 5-flurouracil-based chemotherapy. J Surg Oncol. 2010;101(1):22-27.
324. Sumi T, Katsumata K, Tsuchida A, et al. Correlations of clinicopathological factors with protein expression levels of thymidylate synthase, dihydropyrimidine dehydrogenase and orotate phosphoribosyltransferase in colorectal cancer. Chemotherapy. 2010;56(2):120-126.
325. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Gene expression profiling for women with lymph-node-positive breast cancer to select adjuvant chemotherapy treatment. TEC Assessment Program. Chicago, IL: BCBSA; November 2010;25(1).
326. Albain KS, Barlow WE, Shak S, et al; Breast Cancer Intergroup of North America. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: A retrospective analysis of a randomised trial. Lancet Oncol. 2010;11(1):55-65.
327. No authors listed. Committee opinion no. 477: The role of the obstetrician-gynecologist in the early detection of epithelial ovarian cancer. Obstet Gynecol. 2011;117(3):742-746.
328. Oratz R, Kim B, Chao C, et al. Physician survey of the effect of the 21-gene recurrence score assay results on treatment recommendations for patients with lymph node-positive, estrogen receptor-positive breast cancer. J Oncol Practice. 2011;7(2):94-99.
329. Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(19):3213-3221.
330. De Giorgi U, Valero V, Rohren E, et al. Circulating tumor cells and [18F]fluorodeoxyglucose positron emission tomography/computed tomography for outcome prediction in metastatic breast cancer. J Clin Oncol. 2009;27(20):3303-3311.
331. Gunven P, Randén M, Elmberger G, et al. Gene expression profiling guiding diagnosis and therapy of rare mammary-like anogenital gland carcinomas. Med Oncol. 2012;29(1):127-132.
332. Gevensleben H, Gohring UJ, Buttner R, et al. Comparison of MammaPrint and TargetPrint results with clinical parameters in German patients with early stage breast cancer. Int J Mol Med. 2010;26(6):837-843.
333. Roepman P, Horlings HM, Krijgsman O, et al. Microarray-based determination of estrogen receptor, progesterone receptor, and HER2 receptor status in breast cancer. Clin Cancer Res. 2009;15(22):7003-7011.
334. Von Hoff DD, Stephenson JJ Jr, Rosen P, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. J Clin Oncol. 2010;28(33):4877-4883.
335. Doroshow JH. Selecting systemic cancer therapy one patient at a time: Is there a role for

molecular profiling of individual patients with advanced solid tumors? J Clin Oncol. 2010;28(33):4869-4876.
336. Dutch College of Health Insurances (CvZ). Genexpressietest Mammaprint bij de behandeling van het mammacarcinoom. Amsterdam, The Netherlands: CvZ; October 29, 2010.
337. Smartt P. A comparison of gene expression profiling tests for breast cancer. A systematic review update. HSAC Report. Christchurch, New Zealand; Health Services Assessment Collaboration (HSAC); November 2009;3 (16).
338. Tice JA. The 70-gene signature (MammaPrint) as a guide for the management of early stage breast cancer. Technology Assessment. San Francisco, CA: California Technology Assessment Forum (CTAF); June 2, 2010.
339. Sarbia M, Borchard F, Hengels KJ. Histogenetical investigations on adenocarcinomas of the esophagogastric junction. An immunohistochemical study. Pathol Res Pract. 1993;189(5):530-535.
340. Deeb G, Wang J, Ramnath N, et al. Altered E-cadherin and epidermal growth factor receptor expressions are associated with patient survival in lung cancer: A study utilizing high-density tissue microarray and immunohistochemistry. Mod Pathol. 2004;17(4):430-439.
341. Murray NP, Calaf GM, Badinez L, et al. P504S expressing circulating prostate cells as a marker for prostate cancer. Oncol Rep. 2010;24(3):687-692.
342. Aureon Laboratories, Inc. Aureon Laboratories announces name change for existing post-surgical prostate cancer recurrence test commercially available since February 2006, Post-Op Px(TM) is the first diagnostic test to predict prostate cancer recurrence. March 5, 2010, GlobeNewswire. Nasdaz Omx. Available at: http://www.globenewswire.com/newsroom/news.html?d=185946. Accessed December 9, 2011.
343. Ellis PM, Blais N, Soulieres D, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. J Thorac Oncol. 2011;6(8):1379-1391.
344. Gray RG, Quirke P, Handley K, et al. Validation study of a quantitative multigene reverse transcriptase-polynmerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. J Clin Oncol. 2011;29:1-13.
345. Juntermanns B, Kaiser GM, Reis H, et al. Klatskin-mimicking lesions: Still a diagnostical and therapeutical dilemma? Hepatogastroenterology. 2011;58(106):265-269.
346. Kumaresan K, Kakkar N, Verma A, et al. Diagnostic utility of α-methylacyl CoA racemase (P504S) & HMWCK in morphologically difficult prostate cancer. Diagn Pathol. 2010;22(5):83.
347. National Comprehensive Cancer Network (NCCN). Esophageal and Esophagogastric Junction Cancers. NCCN Clinical Practice Guidelines in Oncology v.2.2011. Fort Washington, PA: NCCN; May, 2011. Available at: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site. Accessed: December 2, 2011.
348. Sinakos E, Saenger AK, Keach J, et al. Many patients with primary sclerosing cholangitis and increased serum levels of carbohydrate antigen 19-9 do not have cholangiocarcinoma. Clin Gastroenterol Hepatol. 2011;9(5):434-439.
349. Yoshida A, Tsuta K, Nitta H, et al. Bright-field dual-color chromogenic in situ hybridization for diagnosing echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase-positive lung adenocarcinomas. Am J Surg Pathol. 2011;35(8):1226-1234.
350. Ueland, FR, Desimone, CP, Seamon LG, et al. Effectiveness of a multivariate index assay in the preoperative assessment of ovarian tumors. Obstet Gynecol. 2011;117(6):1289-1297.
351. Ware, MR, Smith, A, DeSimone CP, et al. Performance of the American College of Obstetricians and Gynecologists' ovarian tumor referral guidelines with a multivariate index assay. Obstet Gynecol. 2011;117(6):1298-1306.
352. Febbo, PG, Ladanyi, M, Aldape, KD, et al. NCCN Task Force Report: Evaluating the Clinical Utility of Tumor Markers in Oncology. J Natl Compre Canc Netw. 2011;9:S1-

S31.
353. National Comprehensive Cancer Network (NCCN). Breast cancer. NCCN Clinical Practice Guidelines in Oncology. Version 2.2011. Fort Washington, PA: NCCN; 2011.
354. Adelstein BA, Dobbins TA, Harris CA, et al. A systematic review and meta-analysis of KRAS status as the determinant of response to anti-EGFR antibodies and the impact of partner chemotherapy in metastatic colorectal cancer. Eur J Cancer. 2011;47(9):1343-1354.
355. Ellis PM, Blais N, Soulieres D, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. J Thorac Oncol. 2011;6(8):1379-1391.
356. Nair VS, Maeda LS, Ioannidis JP. Clinical outcome prediction by microRNAs in human cancer: A systematic review. J Natl Cancer Inst. 2012;104(7):528-540.
357. Raman G, Wallace B, Patel K, et al. Update on horizon scans of genetic tests currently available for clinical use in cancers. Technology Assessment Report. Final Report. Prepared by the Tufts Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ) under contract no. HHSA 290-2007-10055-I. Project ID: GEND0508. Rockville, MD: AHRQ; April 15, 2011.
358. Sanz-Pamplona R, Berenguer A, Cordero D, et al. Clinical value of prognosis gene expression signatures in colorectal cancer: A systematic review. PLoS One. 2012;7(11):e48877.
359. Siddiqui AA, Kowalski TE, Kedika R, et al. EUS-guided pancreatic fluid aspiration for DNA analysis of KRAS and GNAS mutations for the evaluation of pancreatic cystic neoplasia: A pilot study. Gastrointest Endosc. 2013;77(4):669-670.
360. Finkelstein SD, Bibbo M, Loren DE, et al. Molecular analysis of centrifugation supernatant fluid from pancreaticobiliary duct samples can improve cancer detection. Acta Cytol. 2012;56(4):439-447.
361. Lin X, Zhu B, Finkelstein SD, et al. Significance of loss of heterozygosity in predicting axillary lymph node metastasis of invasive ductal carcinoma of the breast. Appl Immunohistochem Mol Morphol. 2012;20(2):116-123.
362. Ellsworth EM, Palma JF, Spence WC, et al. Mutational profiling of sporadic versus toxin-associated brain cancer formation: Initial findings using loss of heterozygosity profiling. Int J Hyg Environ Health. 2012;215(3):427-433.
363. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients With carcinoma of unknown primary site: A prospective trial of the Sarah Cannon Research Institute. J Clin Oncol. 2013;31(2):217-223.
364. Drukker CA, Bueno-de-Mesquita JM, Retèl VP, et al. A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. Int J Cancer. 2013 Jan 31. [Epub ahead of print]
365. Bradley LA, Palomaki G, Gutman S, et al. PCA3 testing for the diagnosis and management of prostate cancer. Comparative Effectiveness Review No. 98. Prepared by the Blue Cross and Blue Shield Technology Evaluation Center Evidence-based Practice Center under Contract No. 290-2007-10058-I). AHRQ Publication No. 13-EHC030-EF. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); April 2013.
366. Little J, Wilson B, Carter R, et al. Multigene panels in prostate cancer risk assessment. Evidence Report/Technology Assessment No. 209. Prepared by the McMaster University Evidence-based Practice Center (EPC) under contract to the Agency for Healthcare Research and Quality (AHRQ), Contract No. 290-2007-10060-1. AHRQ Publication No. 12-E020-EF. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); 2012.
367. Alberts SR, Yu T, Behrens RJ, et al. Real-world comparative economics of a 12-gene assay for prognosis in stage II colon cancer [abstract]. J Clin Oncol. 2012;30(suppl 34):Abstract 391.
368. Srivastava G, Renfro LA, Behrens RJ, et al. Prospective evaluation of a 12-gene assay on treatment recommendations in patients with stage II colon cancer [abstract]. J Clin Oncol. 2012;30(suppl 34):Abstract 453.

369. Venook AP, Niedzwiecki D, Lopatin M, et al. Biologic determinants of tumor recurrence in stage II colon cancer: Validation study of the 12-gene recurrence score in cancer and leukemia group B (CALGB) 9581. J Clin Oncol. 2013 Mar 25. [Epub ahead of print]
370. National Horizon Scanning Centre (NHSC). UPDATED: OVA1™ test for the assessment of suspected ovarian cancer. Horizon Scanning Review. Birmingham, UK: National Horizon Scanning Centre (NHSC); 2012.
371. National Horizon Scanning Centre (NHSC). UPDATED: RealTime mS9 Colorectal Cancer (CRC) Assay for the early detection of colorectal cancer. Horizon Scanning Review. Birmingham, UK: National Horizon Scanning Centre (NHSC); 2012.
372. National Collaborating Centre for Cancer. Diagnosis and management of metastatic malignant disease of unknown primary origin. Developed for NICE. July 2010. Available at: http://www.nice.org.uk/nicemedia/live/13044/49864/49864.pdf. Accessed March 29, 2013.
373. Alberta Provincial Thoracic Tumour Team. Non-small cell lung cancer stage IV. Clinical Practice Guideline No. LU-004. Edmonton, AB: Alberta Health Services, Cancer Care; June 2011.
374. Gao G, Ren S, Li A, et al. Epidermal growth factor receptor-tyrosine kinase inhibitor therapy is effective as first-line treatment of advanced non-small-cell lung cancer with mutated EGFR: A meta-analysis from six phase III randomized controlled trials. Int J Cancer. 2012;131(5):E822-E829.
375. Krijgsman O, Roepman P, Zwart W, et al. A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response. Breast Cancer Res Treat. 2012;133(1):37-47.
376. Trock BJ, Brotzman MJ, Mangold LA, et al. Evaluation of GSTP1 and APC methylation as indicators for repeat biopsy in a high-risk cohort of men with negative initial prostate biopsies. BJU Int. 2012;110(1):56-62.
377. BlueCross BluShield Association (BCBSA). Technology Evaluation Center (TEC). Multi-analyte testing for the evaluation of adnexal masses. TEC Assessment Program. Chicago, IL: BCBSA; 2013. Available at: http://www.bcbs.com/blueresources/tec/press/multi-analyte-testing-for-the.html. Accessed March 29, 2013.
378. Yu D, Shi J, Sun T, et al. Pharmacogenetic role of ERCC1 genetic variants in treatment response of platinum-based chemotherapy among advanced non-small cell lung cancer patients. Tumour Biol. 2012;33(3):877-884.
379. Wang Z, Chen JQ, Liu JL, et al. Polymorphisms in ERCC1, GSTs, TS and MTHFR predict clinical outcomes of gastric cancer patients treated with platinum/5-Fu-based chemotherapy: A systematic review. BMC Gastroenterol. 2012;12:137.
380. Gong W, Zhang X, Wu J, et al. RRM1 expression and clinical outcome of gemcitabine-containing chemotherapy for advanced non-small-cell lung cancer: A meta-analysis. Lung Cancer. 2012;75(3):374-380.
381. Friboulet L, Olaussen KA, Pignon JP, et al. ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. N Engl J Med. 2013;368(12):1101-1110.
382. Besse B, Olaussen KA, Soria JC. ERCC1 and RRM1: Ready for prime time? J Clin Oncol. 2013;31(8):1050-1060.
383. Fletcher SW. Screening for breast cancer. Last reviewed February 2013. UpToDate Inc. Waltham, MA.
384. Collins LC, Laronga C, Wong JS. Ductal carcinoma in situ: Treatment and prognosis. Last reviewed February 2013. UpToDate Inc. Waltham, MA.
385. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology. Breast cancer (Version 2.2013). NCCN: Fort Washington: PA.
386. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology. Multiple myeloma (Version 2.2013). NCCN: Fort Washington: PA.
387. Oxnard GR, Binder A, Jänne PA. New targetable oncogenes in non-small-cell lung cancer. J Clin Oncol. 2013;31(8):1097-1104.
388. Shin SJ, Hwang JW, Ahn JB, et al. Circulating vascular endothelial growth factor receptor 2/pAkt-positive cells as a functional pharmacodynamic marker in metastatic colorectal cancers treated with antiangiogenic agent. Invest New Drugs. 2013;31(1):1-13.

389. Yu Z, Li Z, Cai B, et al. Association between the GSTP1 Ile105Val polymorphism and prostate cancer risk: A systematic review and meta-analysis. Tumour Biol. 2013 34(3):1855-1863.
390. Janku F, Wheler JJ, Hong DS, Kurzrock R. Bevacizumab-based treatment in colorectal cancer with a NRAS Q61K mutation. Target Oncol. 2013 Feb 12. [Epub ahead of print]
391. Khatcheressian JL, Hurley P, Bantug E, et al.; American Society of Clinical Oncology. Breast cancer follow-up and management after primary treatment: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013;31(7):961-965.
392. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special report: Multiple molecular testing of cancers to identify targeted therapies. TEC Assessments in Press. Chicago, IL: BCBSA; February 2013.
393. Meleth S, Whitehead N, Swinson T, Lux L. Technology assessment on genetic testing or molecular pathology testing of cancers with unknown primary site to determine origin. Technology Assessment Report. Final. Prepared by RTI International-University of North Carolina at Chapel Hill Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ) under contract no. HHSA290200710057I #8. Project ID: CANU5011. Rockville, MD: AHRQ; February 20, 2013.
394. Deverka P, Messner D, Dutta T. Evaluation of clinical validity and clinical utility of actionable molecular diagnostic tests in adult oncology. Effectiveness Guidance Document. Baltimore, MD: Center for Medical Technology Policy (CMTP); 2013.
395. Andreopoulou E, Yee H, Warycha MA, et al. Mucinous cancer of the appendix: challenges in diagnosis and treatment. J Chemother. 2007;19(4):451-454.
396. Carmignani CP, Sugarbaker PH. Synchronous extraperitoneal and intraperitoneal dissemination of appendix cancer. Eur J Surg Oncol. 2004a;30(8):864-868.
397. Carmignani CP, Hampton R, Sugarbaker CE, et al. Utility of CEA and CA 19-9 tumor markers in diagnosis and prognostic assessment of mucinous epithelial cancers of the appendix. J Surg Oncol. 2004b;87(4):162-166.
398. Rasamny JJ, Allak A, Krook KA, et al. Cyclin D1 and FADD as biomarkers in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg. 2012;146(6):923-31.
399. O'Sullivan P, Sharples K, Dalphin M, et al. A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. J Urol. 2012;188(3):741-747.
400. National Institute for Health and Care Excellence. DG10 gene expression profiling and expanded immunohistochemistry tests for guiding adjuvant chemotherapy decisions in early breast cancer management: MammaPrint, Oncotype DX, IHC4 and Mammostrat: Guidance. NICE: London, UK. September 2013. Available at: http://guidance.nice.org.uk/DG10/Guidance/pdf/English. Accessed January 7, 2014.
401. Lotan Y, Choueiri TK. Clinical presentation, diagnosis, and staging of bladder cancer. Last reviewed December 2013. UpToDate Inc., Waltham, MA.
402. National Comprehensive Cancer Network. Clinical practice guideline: Bladder cancer. Version 1.2014. NCCN: Fort Washington, PA.
403. Delahaye LJM, Wehkamp D, Floore AN, et al. Performance characteristics of the MammaPrint breast cancer diagnostic gene signature. Personalized Medicine. 2013;10(8):801.
404. Sapino A, Roepman P, Linn SC, et al. MammaPrint molecular diagnostics on formalin-fixed, paraffin-embedded tissue. J Mol Diagn. 2014;16(2):190-197.
405. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Actions Taken by the BlueCross BlueShield Association Medical Advisory Panel (MAP). Chicago, IL: BCBSA; February 19, 2014.
406. Cheng S, Qu K, Abdool A, et al. A molecular diagnostic panel for thyroid cancer disease management. J Clin Oncol 30, 2012 (suppl; abstr 5510). Available at: http://meetinglibrary.asco.org/content/94089-114. Accessed April 1, 2014.
407. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Gene expression analysis for prostate cancer management. TEC Assessment Program. Chicago, IL: BCBSA; April 2014;28(11).

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Review

Biomarkers and diagnostics in heart failure ☆

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ABSTRACT

Heart failure (HF) biomarkers have dramatically impacted the way HF patients are evaluated and managed. B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are the gold standard biomarkers in determining the diagnosis and prognosis of HF, and studies on natriuretic peptide-guided HF management look promising. An array of additional biomarkers has emerged, each reflecting different pathophysiological processes in the development and progression of HF: myocardial insult, inflammation and remodeling. Novel biomarkers, such as mid-regional pro atrial natriuretic peptide (MR-proANP), mid-regional pro adrenomedullin (MR-proADM), highly sensitive troponins, soluble ST2 (sST2), growth differentiation factor (GDF)-15 and Galectin-3, show potential in determining prognosis beyond the established natriuretic peptides, but their role in the clinical care of the patient is still partially defined and more studies are needed. This article is part of a Special Issue entitled: Heart failure pathogenesis and emerging diagnostic and therapeutic interventions.

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1. Introduction

A wide range of cardiovascular disorders that result in the impairment of the heart's ability to fill or to pump out blood may eventually lead to the clinical syndrome of heart failure (HF). In recognition of the multiplicity of causes of the diagnosis, it is not surprising that HF is a common affliction. Currently an estimated 5.8 million people in the United States [1] and 23 million people worldwide [2] are living with HF. As the population ages and treatments for cardiovascular diseases are improving mortality in affected patients, the number of HF patients is expected to grow.

Patients with HF often present with signs and symptoms that are often nonspecific and with a wide differential diagnosis, making diagnosis by clinical presentation alone challenging. Some of the signs and symptoms, such as dyspnea, orthopnea and paroxysmal nocturnal dyspnea, are due to congestion while some are due to lack of adequate cardiac output, including fatigue, weakness and exercise intolerance. This heterogeneity of presentation often results in delays in definitive diagnosis and treatment, and such delays are linked with poor prognosis [3]. Thus, together with HF's well-described risk for death and hospitalization, the growing incidence and prevalence make it a priority.

The evaluation of a patient suspected of HF was traditionally based on clinical assessment with history, physical examination and chest x-ray. However, in isolation, the performance of these methods of diagnosis can be limited in accurately diagnosing HF. As a matter of fact, isolated symptoms and signs correlate poorly with objective methods of cardiac dysfunction [4–6]. One study looked at the relationship between three clinical criteria that combined findings from history, physical examination and chest x-ray for the diagnosis of HF: the Framingham, the Duke and the Boston criteria [7]. All of the clinical criteria were limited in sensitivity (50–73%) and/or specificity (54–78%). Beyond clinical variables, noninvasive imaging studies (such as echocardiography and radionuclide angiography) may be useful to identify or exclude HF. These modalities can determine ventricular ejection fraction and diastolic dysfunction, as well as estimate chamber pressures. Echocardiograms can also provide clues to the underlying etiology of HF and are an essential part of assessment once the diagnosis is secured. However, many patients with abnormal ventricular systolic function on imaging studies are asymptomatic and do not necessarily have the clinical syndrome of HF [8,9]. For example, in one study of community-dwelling urban patients, 48% of patients with left ventricular ejection fraction ≤30% were asymptomatic [8]. Echocardiograms are also costly and time consuming

Abbreviations: ADHERE, Acute Decompensated Heart Failure National Registry; ADM, Adrenomedullin; ANP, Atrial natriuretic peptide; AUC, Area under the curve; BACH, Biomarkers in Acute Heart Failure; BNP, B-type natriuretic peptide; CI, Confidence interval; CORONA, Controlled Rosuvastatin Multinational Trial in Heart Failure; GDF-15, Growth differentiation factor-15; GISSI-HF, Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca–Heart Failure; HF, Heart failure; HFpEF, HF with preserved left ventricular ejection fraction; HR, Hazard ratio; hsTn, Highly sensitive troponin; hsTnI, Highly sensitive troponin I; hsTnT, Highly sensitive troponin T; ICON, International Collaborative of NT-proBNP; LVSD, Left ventricular systolic dysfunction; MI, Myocardial infarction; MR-proADM, Mid-regional pro adrenomedullin; MR-proANP, Mid-regional pro atrial natriuretic peptide; NHANES, National Health and Nutrition Examination Survey; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; PRIDE, ProBNP Investigation of Dyspnea in the Emergency Department; sST2, Soluble ST2; ST2L, ST2 ligand; Val-HeFT, Valsartan Heart Failure Trial

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Review

A Novel Cardiac Bio-Marker: ST2: A Review

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Abstract: Cardiovascular diseases (CVD) are the major cause of death worldwide. The identification of markers able to detect the early stages of such diseases and/or their progression is fundamental in order to adopt the best actions in order to reduce the worsening of clinical condition. Brain natriuretic peptide (BNP) and NT-proBNP are the best known markers of heart failure (HF), while troponins ameliorated the diagnosis of acute and chronic coronary artery diseases. Nevertheless, many limitations reduce their accuracy. Physicians have tried to develop further detectable molecules in order to improve the detection of the early moments of CVD and prevent their development. Soluble ST2 (suppression of tumorigenicity 2) is a blood protein confirmed to act as a decoy receptor for interleukin-33. It seems to be markedly induced in mechanically overloaded cardiac myocytes. Thus, HF onset or worsening of a previous chronic HF status, myocardial infarct able to induce scars that make the myocardium unable to stretch well, *etc*, are all conditions that could be detected by measuring blood levels of soluble ST2. The aim of this review is to explore the possible role of ST2 derived-protein as an early marker of cardiovascular diseases, above all in heart failure and ischemic heart diseases.

Keywords: ST2; heart failure; coronary artery diseases; early markers

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1. Introduction

Cardiovascular diseases (CVD) are the main leading cause of death worldwide [1]. Their incidence is still increasing, even in developing countries [2] and it greatly diminishes individuals' quality of life and survival rates.

Primary and secondary preventive actions are required in order to arrest the negative development of CVD. Thus, physicians are involved in searching for tools able to detect the early moments of CVD development. Early markers of CVD will allow immediate interventions in order to prevent the beginning of the diseases or, at least, minimize their negative consequences. Carotid intima-media thickness and brachial artery flow-mediated dilatation are instrumental, well-established early markers of atherosclerosis and chronic heart failure [3,4]. Biochemical markers work side by side with the latter ultrasonographic tools in order to detect early alterations of cardiovascular system. Ciccone *et al.* [5] recently pointed out the role of osteoprotegerin in acute/chronic coronary artery diseases (CAD). The natriuretic peptides (brain natriuretic peptide [BNP] and NT-proBNP) are the best known markers of heart failure (HF) condition [6]. Nevertheless, due to their limitations, research has been undertaken in order to implement and to find new, more accurate and complete markers of both CAD and HF conditions. Further research focused on cardiac remodelling has focused on biomechanical pathways activated during cardiac remodelling after the onset of cardiovascular diseases that could enhance production of compounds released in the bloodstream. Their early detection could detect the early moments of cardiac impairment in the case of CAD and HF. Genomic technology has discovered new genes and pathways involved in this process. A new gene, called ST2 (suppression of tumorigenicity 2) and its related protein was markedly induced in mechanically overloaded cardiac myocytes. This suggests that the protein can be overexpressed in remaining viable myocardium that has undergone great stress [7]. The aim of this review is to explore the possible role of ST2 derived-protein as an early marker of cardiovascular diseases, above all in heart failure and ischemic heart diseases.

2. ST2 Protein: Biological Structure and Function

Discovered in 1989, the gene named ST2 (also known as T1, IL1RL1, or Fit1) is placed on chromosome 2q12 as part of the wider interleukin 1 (IL-1) gene cluster [8]. Four isoforms are the transcriptional product of the gene and two of them are the most important: IL1RL1-b or ST2L, which is a membrane receptor member of the interleukin-1 receptor family, and IL1RL1-a or sST2a, which is a truncated soluble receptor that can be detected in serum. Alternative promoter splicing and 3' processing of the same mRNA seems to be responsible for the production of sST2 and ST2L [8]. ST2 gene shows two promoters: a proximal and a distal one, which are able to influence the mechanism of transcriptional regulation of the gene [9]. Each promoter could influence the expression of sST2 and ST2L mRNA: The alternative splicing involving the 3' end of the gene is responsible for such a difference. Baba *et al.* [9] found a different function of two GATA motifs (1 and 2), positioned upstream of the site where transcription of ST2 gene begins, which were able to influence the mRNA production and the final expression of the gene in human mast cells and in basophilic human cell line. In particular, GATA2 seemed to exert the major control in ST2 mRNA expression in both cells lines. Thus, the different expression and function of GATA motifs could play a role in the final expression

and function of ST2 in immune human cells and, probably, in other cells constitutively expressing the final products of the ST2 gene transcription. Nevertheless, poor data still persists about the inner control of sST2 and ST2L expressions. Caporali *et al.* [10] found that the neurotrophin p75 receptor, a member of tumor necrosis factor (TNF)-alpha receptors family involved in post-ischemic neovascularisation in diabetes mellitus induced critical limb ischemia, is able to enhance the sST2 transcription and this action did not allow a full revascularization attempt by endothelial cells.ST2L is composed by 3 extracellular immunoglobulin G domains, a single transmembrane domain, and an intracellular domain [7,8,11]. The sST2 lacks the transmembrane and intracellular domains and it travels freely through the bloodstream.

Interleukin-33 (IL-33) has been identified as a functional ligand of ST2L [7,11]. It binds ST2L on inflammatory cells membranes: this activates mitogen-activated protein kinase (MAPK)-kinases and several biochemical pathways. The end of these reactions is the activation of the inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) complex, which makes NF-kB active and able to exert its pro-inflammatory actions [8]. sST2 seems to act as a decoy-receptor for IL-33: it binds IL-33 thus subtracting such a molecule from the interaction with ST2L. sST2 interaction with IL-33 could reduce the production and activation of NF-kB, thus it would reduce inflammatory response. IL-33 has been supposed to regulate by itself the ST2L and sST2 mRNA transcription [12]. In their experimental research on highly metastatic human pancreatic adenocarcinoma cell line Colo357, Schmieder *et al.* [12] observed the influence of IL-33 and (interleukin-1) IL-1 concentrations on both ST2L and sST2 mRNA expression. The particular behavior of both cytokines lied on their skill in increasing the mRNA expression of ST2L while reducing that of sST2. When considering low doses of IL-33 mRNA expression, the presence of IL-1 was sufficient to up-regulate ST2L mRNA transcription, as well as INF-γ concentrations which rose the expression of both mRNA and ST2L protein [12]. These aspects introduce the immunomodulatory role of ST2 [13] which is more complex than previously expressed. As Sweet *et al.* [13] demonstrated, sST2 is able to directly bind and interact with macrophages when bacterial lipopolysaccharide (LPS) flows through bloodstream [13]. The interaction seems to induce an inhibition of cytokines production such as interleukin-6 (IL-6), interleukin-12 (IL-12) and TNF-alpha thus modulating inflammatory response [13]. Furthermore, ST2L seems to be able to activate T helper type 2 (Th2) lymphocytes and to induce the production of several cytokines from these cells such as IL-4, IL-5, and IL-13 which enhance Th2 response to inflammatory events [7,14].

It is interesting to note that there is a reciprocal influence between immune system cells, their production of cytokines and the final expression of ST2. IL-6 production from antigen-presenting cell is able to increase ST2 expression during Th2 differentiation [15]. Minor effects are detected for other cytokines such as IL-1, TNF-α and IL-5. Therefore, while ST2 is able to activate naive T cell to type 2 conformation, immune system is parallel able to enhance ST2 expression [15]. Nevertheless, the inner mechanisms able to explain such interactions are not fully elucidated.

3. The Heart and the ST2: A Tight Relationship

ST2 is involved in cardiac function and dysfunction. ST2 action in cardiac muscle is complex and not fully understood and it is tightly related to IL-33 mode of action. IL-33 could be localized simultaneously onto nuclear euchromatin and membrane-bound cytoplasmic vesicles. Kakkar *et al.* [16]

demonstrated that the mechanical stretch of living cells could enhance the release of IL-33 from the cytoplasmatic vesicles. Such an exocytosis was not related to a possible stretch-induced necrosis: the cells viability was demonstrated after the periods of biaxial stretch [16]. Thus, the cellular stretch is able to increase the release of IL-33. Cardiomyocytes and cardiac fibroblasts show a parallel, increased release of both ST2L and sST2 when undergone biomechanical strain [14,17].

Such a release is an important step in the overall mechanism able to induce cardiac remodelling due to volume and/or pressure overload. IL-33 could exert an antihypertrophic effect by blocking the actions of angiotesin II or phenylephrine on myocardium. Angiotesin II or phenylephrine favour cardiac hypertrophy by inducing NF-kB action: IL-33, by interacting with ST2L, is able to attenuate NF-kB promoter activity and the phosphorylation of IKK [17]. Furthermore, it seems that the action of IL-33 via ST2L is to prevent MAPK pathway activation induced by angiotesin II or phenylephrine [17]. Histological analysis even demonstrated that ST2 presence reduced cardiac fibrosis and cardiomyocyte cross-sectional area as compared to ST2 deficient mice [17].

sST2, by acting as a decoy receptor, reduces the positive actions of IL-33 [17]. By binding IL33, it reduces the availability of the IL-33 for interaction with sarcolemmal receptor. Thus, an increase in sST2 could reduce the cardioprotective action of IL-33 on cardiac cells and could induce a negative prognostic effect on the overall cardiovascular risk profile [17]. As demonstrated by Weinberg *et al.* [14], the increased plasma levels of sST2 soon after a myocardial infarction event could be considered as a negative prognostic factor according to previous sentences.

Sanada *et al.*'s work [17] offers a more interesting point of view about the biological properties and uses of IL-33/ST2 in clinical practice. In particular, beyond its biomarker value, ST2 could be considered as a real therapeutic target. In order to demonstrate the biochemical properties of IL-33/ST2 interaction, Sanada *et al.* adopted IL-33 administration in their *in vivo*/*in vitro* experiments. It seemed that IL-33 administration was able to decrease p38 MAPK and Jun N-terminal kinase phosphorylation in MAPK pathway activation induced by angiotensin II [17]. Angiotensin II is able to exert cardiac damages by creating several reactive oxygen species (ROS): IL-33 external administration was able to reduce the ROS generation induced by angiotensin II in experimental models. Once more, an intriguing result was that the purified IL-33 2 μg/d i.p. for 4 weeks use in mice models wild type for ST2 receptor function and presence, was able to reduce the fibrotic and hypertrophic burden of cardiac cardiomyocytes and structures, as compared to ST2 −/− mice [17]. Although Sanada *et al.* concluded such part of their experiment only outlining the importance of deletion of ST2 in the general outcome of cardiac structure undergone angiotensin II action, one more idea could be represented by the possible role of ST2 as therapeutic target: IL-33 external administration in patients suffering from cardiac diseases could enhance ST2L activation and its own biochemical intracellular pathway in order to prevent or at least reduce the worsening of the clinical and structural condition of the heart. The same mice of Sanada *et al.* even demonstrated echocardiographic improvement in hypertrophy and systolic function of their heart after ST2 activation by IL-33 administration. These findings are still experimental and coming from animal models; nevertheless, the idea to develop drugs able to enhance ST2 activation in cardiac human cells could be considered as an interesting development for the next clinical future.

Nevertheless, such enthusiastic results should be tempered by novel insights about ST2 [18]. Ho *et al.* [18] pointed out the genetic landscape controlling ST2L and sST2 expression. There is a

strong genetic variability among individuals about sST2 blood concentrations in basal conditions and it could account for up to 40% in inter-individual variability. The reason could be attributed to single nucleotide polymorphisms that could account for the natural variation in ST2 expression. Some variants of IL1RL1 gene (G allele of rs1558648; T allele of rs13019803) were associated with lower sST2 levels and higher mortality rate [18]. Furthermore, missense mutations in the sequence of the IL1RL1 gene regulating intracellular domain were related to peculiar behavior: these variants were able to alter the biochemical pathway downstream ST2L and this induced an up-regulation in sST2 mRNA transcription and conversion in protein. This condition was even related to higher IL-33 production [18]. Thus, the genetic variability should be taken into account when considering the role of sST2 as biomarker and/or prognostic factor; in second line, genetics should be even considered for further trial considering therapeutic actions of the IL-33/ST2L on human organs.

Thus, despite the intriguing results coming from the biological and biochemical analyses conducted, many aspects of IL-33/ST2 interaction remained unclear and deserve much more attentions and further studies. This, far from reducing the role of ST2 in clinical settings, enforces the need of more objectives evaluations in order to obtain an objective picture of the ST2 use.

4. sST2 Assessment: A Novel Biomarker in Cardiovascular Diseases Detection

The increased knowledge about sST2 and ST2L actions on cardiovascular system led physicians to consider the assessment of plasma levels of sST2 as a novel marker of cardiovascular events and clinical conditions, above all related to heart failure and ischemic heart diseases [19,20].

The identification of novel biomarkers able to early detect worsening signs of such clinical conditions is a fundamental step in the evolution of clinical diagnosis in order to adopt measures able to stop or at least reduce their evolution and negative outcomes. Furthermore, they could be used to detect early the benefits from therapies and the prognosis of the disease [11,21].

Nevertheless, the identification of novel molecules able to make possible a clearer and earlier overview on diseases should face the presence and the authority of previous, well-established biomarkers [20,22,23]. Troponins, natriuretic peptides, *etc*., are well-established cardiovascular diseases biomarkers already entered the international guidelines for detection of heart pathologies [24,25].

Furthermore, the optimal biomarker should be independent from other factors. The blood measurements of sST2 should not be associated to other conditions than myocardial diseases, nor linked to confounding factors that could influence its blood concentrations. This should be the "optimal condition" for a molecule in order to become a reliable biomarker. Against such a preface, one could point out the limitations of the previous "well-established" biomarkers and their dependence from clinical conditions other than the pre-established targets and, despite such limitations, they continued to be considered as important biomarkers all over the world. Why we could consider sST2 as a reliable biomarker for cardiac diseases despite its limitations will be further discussed below.

5. sST2 Assessment in Patients with Acute Dyspnea

sST2 has been considered as a possible biomarker in dyspnoeic patients with and without acute destabilized HF referred to the emergency department [7,11,26]. Dyspnoea is the subjective symptom of breathlessness and it often characterizes different pathologies: heart failure, chronic obstructive

pulmonary disease/bronchitis/asthma, pneumonia, malignancy, pulmonary embolism, *etc*. [27]. The possibility to adopt a marker able to ameliorate diagnosis and, perhaps, differential diagnosis in dyspnoeic patients would improve their treatment and management [28–30]. The Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department (PRIDE) project was a prospective, blinded study, aimed at evaluating sST2, amino-terminal pro-brain natriuretic peptide (NT-proBNP) and C-reactive protein (CRP) concentrations in 599 dyspnoeic subjects with and without acute destabilized HF admitted to the emergency department of the Massachussets General Hospital [26]. Patients supposed to show symptoms related to acute HF demonstrated sST2 serum concentrations higher than those whose symptoms seemed no-HF related (0.50 *vs.* 0.15 ng/mL; $p < 0.001$). Nevertheless, the relationship between sST2 and NT-proBNP concentrations revealed interesting results: receiver-operating characteristics (ROC) curves showed an area under the curve (AUC) of 0.74 (95% CI 0.70 to 0.78; $p < 0.001$) for sST2 when considered as a marker of acute HF [26]. The datum is inferior than that related to NT-proBNP for the same trial, *i.e.*, 0.94 ($p < 0.0001$) [31], thus the NT-proBNP confirmed its leadership as biomarker in acute HF rather than sST2. Nevertheless, sST2 seemed to have prognostic features: the combination of elevated NT-proBNP and low sST2 was observed in patients suffering from dyspnoea not related to acute HF onset [26]. Furthermore, a subgroup of patients with acute HF related symptoms showed an AUC of 0.80 (95% CI 0.75 to 0.84; $p < 0.001$) at ROC analyses regarding the relationship between sST2 and 1 year mortality [26], which was higher than the AUC value related to NT-proBNP for the same population (0.76, $p < 0.001$) [32].

Results from Socrates *et al.*'s research [29] pointed out no statistical significant difference between ROC analyses AUC regarding sST2, BNP and NT-proBNP in predicting 30 days, 90 days and 1 year mortality (30 days AUCs: sST2 = 0.76 *vs.* BNP = 0.63, $p = 0.14$; *vs.* NT-proBNP = 0.71, $p = 0.79$; 90 days AUCs: sST2 = 0.74 *vs.* BNP = 0.71, $p = 0.5$; *vs.* NT-proBNP = 0.75, $p = 0.72$. 1 year AUCs: sST2 = 0.72 *vs.* BNP = 0.71, $p = 0.76$; *vs.* NT-proBNP = 0.73, $p = 0.68$). Nevertheless, Kaplan-Meier curves showed further interesting information: the combination of sST2 to BNP and NT-proBNP measurements is able to increase the accuracy in detecting high risk dyspnoeic patients for acute HF onset. Socrates *et al.* outlined that the subgroup of patients with high sST2 levels and low levels of natriuretic peptides (both BNP/NT-proBNP) had increased mortality risk [29]. According to international guidelines [24], which do not advise sST2 evaluation in general assessment of heart failure patients, this subgroup would not receive a correct assignment. Dieplinger *et al.* [33] confirmed the same results in their recent evaluation of 251 dyspnoeic patients: sST2 is a good biomarker able to predict mortality at 1 years, above all if combined with detection of other plasma molecules (midregional-proadrenomedullin and chromogranin A).

According to Dieplinger *et al.*'s study, sST2 evaluation was not able to discriminate between patients suffering from dyspnoea due to HF or inflammatory pulmonary diseases, thus limiting its usefulness in differential diagnosis [33]. A subgroup analysis from the PRIDE [34] better evaluated such discriminatory point. The authors considered 139 patients (66% were further diagnosed with HF) who underwent complete echocardiographic evaluation at admission at the emergency department. sST2 demonstrated its tight relationship with myocardial function: both in HF and non-HF patients, sST2 was independently related to left (LV) and right (RV) ventricular function and morphological parameters at multivariate analysis (*i.e.*, RV systolic pressure: $t = 2.29$, $p = 0.002$; LV ejection fraction: $t = -2.15$, $p = 0.05$; LV end systolic dimension: $t = 2.57$, $p = 0.01$; LV end diastolic dimension:

t = 2.98, $p = 0.005$; transmitral E to tissue Doppler Ea ratio: t = −2.13, $p = 0.03$; tissue Doppler A wave peak velocity: t = 2.11, $p = 0.05$). Shah *et al.* further demonstrated the deep impact of sST2 as a prognostic biomarker when considering a 4-year mortality. The relationship was independent from others variables: addition of log-transformed sST2 concentration as a continuous variable to other markers of clinical risk really improved the C-statistic from 0.76 to 0.80 in a statistically significant way [34].

6. Role of sST2 Measurements in Acute De-Compensated HF

Acute decompensated HF is the ideal clinical condition to evaluate the real burden of sST2 as biomarker and its power as prognostic factor. Boisot *et al.* enrolled 150 patients suffering from acute decompensated HF in order to evaluate the predictive 90-day mortality power of sST2 percent change as compared to well-established heart failure biomarkers such as BNP or NT-proBNP [35]. ROC analysis demonstrated the comparable predictive value of sST2 and NT-proBNP changes in predicting 90-day mortality (sST2 AUC = 0.78 CI = 0.69 to 0.88 *vs.* NT-proBNP AUC = 0.78 CI = 0.67 to 0.90) rather than BNP (AUC = 0.67 CI = 0.56 to 0.79). The optimization of the statistical analysis led physicians to consider a reduction of more than 15.5% as the best cut-off value able to identify acute decompensated HF patients at high cardiovascular risk [35]. Some authors demonstrated that a sST2 ratio (*i.e.*, the ratio between sST2 two week values and sST2 baseline values) >0.75 would be able to really predict cardiac event rates independently from others confounding factors [36]. The predictive value of sST2 still persists at 1-year follow-up: the higher the sST2 plasma levels, the higher the mortality rates at 1-year, independently of possible confounders, remaining associated to that of BNP (rs = 0.317; 95% CI, 0.157 to 0.460; $p < 0.001$) [37]. A study from Rehman *et al.* [38] revealed that CRP (T = 6.79; $p < 0.001$), body temperature (T = 5.66; $p < 0.001$), heart rate (T = 4.44; $p < 0.001$), BNP (T = 3.0; $p = 0.003$), blood urea nitrogen (T = 2.76; $p = 0.006$), smoking history (T = 2.32; $p = 0.021$), and systolic blood pressure (T = 2.01; $p = 0.045$) are all predictors of sST2 blood concentrations in acute decompensated HF [38]. Surprisingly and in agreement with the work from Manzano-Fernández *et al.* [19], left ventricular ejection fraction is not involved in prediction of sST2 blood concentrations in acute decompensated HF patients. Furthermore, Rehman *et al.* found a 72% sensitivity (95% CI: 62% to 81%), 56% specificity (95% CI: 49% to 62%), a positive predictive value of 39%, and a negative predictive value of 84% for sST2 as a predictor of mortality [38]. The combination of sST2, NT-proBNP, BNP, renal function and C-reactive protein values was able to increase the AUC for 1-year mortality prediction till 0.80 in a statistically significant manner [38]. Such results were confirmed by further studies about this matter [22] which made reliable sST2 as a real marker of acute HF and/or a good mortality predictor in the same de-compensated patients [39].

7. Role of sST2 Plasma Levels in Chronic HF

One of the first attempts to define the role of sST2 in chronic HF was realized in the neurohormone-sub-study of the Prospective Randomized Amlodipine Survival Evaluation 2 (PRAISE-2) study [40], *i.e.*, a multicenter, randomized, double-blinded, placebo-controlled study evaluating the effects of amlodipine 10 mg/day on survival of patients suffering from congestive HF due to non-ischemic conditions. sST2 was significantly higher in patients with severe HF (0.24 ng/mL, CI: 0.16–0.70) than

controls (0.14 ng/mL, CI: 0.13–0.17; $p < 0.0001$). Scatter plots analysis suggested that sST2 values were related to BNP (r = 0.36, $p < 0.0001$), proatrial natriuretic peptide (ProANP) (r = 0.36, $p < 0.0001$), and norepinephrine (r = 0.39, $p < 0.0001$) concentrations. Nevertheless, sST2 was not able to predict mortality by itself in such a category of patients: multivariate analysis confirmed that the sST2 ratio (defined as the change in sST2 from baseline to 2 weeks treatment) was an independent predictor of mortality and/or transplantation ($p = 0.048$), above all if associated with BNP ($p < 0.0001$) and Pro-ANP ($p < 0.0001$) evaluation [40]. More interesting results came from Penn Heart Failure Study (PHFS), a multicenter, prospective cohort study involving chronic heart failure patients suffering from different range of disease severity [41]. Far from the severe HF patients of PRAISE-2, those from PHFS demonstrated that sST2 evaluation had a predictive 1-year mortality/transplantation rate similar at NT-proBNP one: the ROC curves showed comparable predictive values between the two biomarkers (sST2 AUC: 0.75, 95% CI, 0.69 to 0.79 *vs.* NT-proBNP AUC: 0.77, 95% CI, 0.72 to 0.81; $p = 0.24$) [41]. Furthermore, by combining sST2 and NT-proBNP evaluations, the AUC reached a 0.80 (95% CI, 0.76 to 0.84) value. Ky *et al.* [20] included the sST2 evaluation in a multi-markers score to be adopted in chronic HF patients in order to depict the mortality risk of such a category of patients. The new multi-markers model for chronic HF patients was superior than the Seattle Heart Failure Model (SHFM): the multi-markers score involving sST2 evaluation in combination with high-sensitivity CRP (hsCRP), myeloperoxidase (MPO), BNP, soluble fms-like tyrosine kinase receptor-1 (sFlt-1), troponin I (TnI), creatinine, and uric acid reached an AUC higher than that of SHFM (AUC multi-markers score [0.798, 95% CI 0.763–0.833 *vs.* AUC SHFM: 0.756, 95% CI 0.717–0.795)] at 1 year. But the combination of SHFM to the multi-markers allowed an improvement in 1-year mortality prediction up to an overall AUC of 0.803, 95% CI 0.769–0.837, $p = 0.003$.

In agreement with acute de-compensated data [19,38], Bartunek *et al.* [42] demonstrated the independence of sST2 blood levels from left ventricular systolic function. Nevertheless, chronic HF patients, above all if suffering with LV hypertrophy and/or aortic stenosis, revealed that sST2 levels were related to the diastolic overload. This reveals novel insights in the evaluation of sST2 role in myocardial function and the great importance that such a marker could play in the overall assessment of chronic HF patients [43,44]. Despite such evidences, an analysis involving 2,460 Framingham Heart Study participants (mean age 58 years) outlined that left ventricular hypertrophy and left ventricular systolic dysfunction were not related to sST2 concentrations [45]. In this recent research involving a wide sample size, sST2 failed in its predictive value, above all according to those cardiac characteristics (left ventricular hypertrophy and systolic dysfunction) that should theoretically be the main determinants of sST2 plasma levels.

8. sST2, IL-33 and ST2L Roles in Ischemic Heart Disease

The role of IL-33/ST2 signaling pathway in ischemic heart disease has been highlighted. *In vitro* and *in vivo* studies demonstrated that IL-33 plays a regulatory role in cardiac dysfunction after myocardial infarction [46]. In cultured rat cardiomyocytes, IL-33 treatment was dose-dependently able to inhibit hypoxia-induced apoptosis by increasing the expression of inhibitor of apoptosis proteins and by decreasing the activation of caspase-3, *i.e.*, an enzyme able to enhance apoptotic process [46]. sST2 attenuated such an effect by acting as a decoy receptor. Subcutaneous injection of IL-33 reduced

infarct volume, fibrosis, and apoptosis of myocardium after infarction due to coronary ligation, while the presence of sST2 was able to counteract the positive effects of IL-33 in a dose-dependent manner [46]. IL-33/ST2 signaling pathway seems to strengthen the stability of plaques. It is well-known that interferon (IFN)-γ from Th1 lymphocytes could enhance matrix metalloproteinases (MMPs) production from macrophages [47,48]. MMPs are enzymes able to damage the fibrous cap of an atherosclerotic plaque, making it unstable [49,50]. Serum levels of MMP-9 are effectively elevated in the acute phase of myocardial infarction [51,52], showing the maximum levels of concentrations in the culprit coronary artery rather than systemic circulation [53]. IL-33 decreases serum levels of IFN-γ, thus preventing MMPs activation, extracellular matrix destruction and plaque rupture [13]. Guzel *et al.* [54] found IL-33 serum levels significantly lower in non-ST segment elevation myocardial infarction (NSTEMI) patients than controls ($p < 0.05$) and negatively correlated with MMP-9 ($r = -0.461$, $p < 0.05$) levels [54]. Considering an increase in sST2 [14] and a parallel decrease in IL-33 [54] levels during myocardial infarction, one can image the increased risk of patients for coronaries damages.

Shimpo *et al.* developed the predictive value of sST2 in patients suffering from acute myocardial infarction presenting in form of ST-segment elevation myocardial infarction (STEMI) [55]. Patients with higher levels of serum sST2 at STEMI presentation had a statistically significant higher risk of death or of developing new congestive heart failure at 30 days ($p = 0.0009$) [55]. Such a relationship still persisted after adjustment for confounding factors (OR: 1.77, 95% CI 1.01 to 3.12; $p = 0.047$), although the adding of BNP and cTnI made the significance loss [55]. This enforces the important role of BNP and cTnI in STEMI patients rather than sST2 blood concentrations. Considering a subgroup of the CLopidogrel as Adjunctive ReperfusIon TherapY–Thrombolysis in Myocardial Infarction 28 (CLARITY-TIMI 28) trial involving STEMI patients, Sabatine *et al.* [23] demonstrated the independent predictive value of both sST2 and NT-proBNP in a multi-markers model, with an OR of 3.57 (95% CI 1.87 to 6.81) for sST2 and 2.41 (95% CI 1.17 to 4.95) for NT-proBNP. Furthermore, the authors demonstrated that, by introducing both sST2 and NT-proBNP to the TIMI Risk Score, the c statistic of this score improved from 0.73 (95% CI, 0.68 to 0.78) to 0.78 (95% CI, 0.74 to 0.83; $p = 0.0025$) [23].

Non-ST segment elevation acute coronary syndrome (NSTE-ACS) patients did not differ from their STE-ACS counterpart [56]. sST2 plasma levels deeply increased at early stages of the event, with a peak value reached at 6–11 h. According to outcome prediction, the sST2 c-statistics for 1-year mortality was 0.67 (95% CI 0.54–0.79), which was not different from that of cTnT (0.64 [95% CI 0.53–0.76], $p = 0.66$), although lower than that of NT-proBNP one (0.78 [95% CI 0.66–0.89], $p = 0.03$). The authors further demonstrated that 1-SD increase in ln-transformed ST2 was associated with an OR 2.3 [95% CI 1.1–4.6, $p = 0.03$] in a risk model adopted within their population by considering all the clinical variable of the sample [56]. These results were similar to those from Kohli *et al.* [57] who demonstrated that high sST2 levels (*i.e.*, >35 μg/L) in NSTE-ACS patients predicted a >3-fold higher risk of cardiovascular death and heart failure at 30 days and 1 year, even after adjustment for major clinical risk factors and biomarkers (cTnI and BNP) [57]. Nevertheless, adding sST2 evaluation to GRACE score and NT-proBNP values in NSTE-ACS patients, seemed not to be long-term predictive, thus did not improve cardiovascular risk stratification [58].

Furthermore, the value of sST2 as predictor of functional recovery and remodeling of LV in the post-infarct phase was also evaluated. Weir *et al.* [59] effectively noted that sST2 serum

concentrations were positively correlated with endocardial, epicardial extent and microvascular obstruction of infarct in 100 acute myocardial infarct patients. sST2 values significantly decreased from baseline to 24 weeks, predicting the degree in adverse LV functional recovery and remodeling: a slow change in sST2 concentrations was related to worse LVEF, LV end-diastolic volume index and infarct volume index [59].

9. Role of ST2 in Other Clinical Settings

sST2 role in predicting mortality was recently demonstrated in 588 outpatients referred for echocardiograms. The optimal cut-point of sST2 for predicting 1-year mortality was 28.25 ng/mL. Patients with high ST2 levels had an increased risk of death ($p = 0.02$); and if both ST2 and B-type natriuretic peptide levels were elevated, the risk resulted even higher ($p = 0.01$) [60].

In the Framingham Heart Study, sST2 concentrations were related to systolic blood pressure ($p = 0.006$), antihypertensive medication use ($p = 0.03$), and diabetes ($p = 0.001$) [61]. By adjusting for CAD, HF, atrial fibrillation, diabetes, hypertension, obesity, valvular disease, LV systolic dysfunction, and pulmonary and renal dysfunction, the Framingham Heart Study showed that male sex ($p < 0.0001$) and old age ($p = 0.004$) were related to higher sST2 concentrations [61].

Nevertheless, the measurement of sST2 in 348 patients presenting to the emergency department with chest pain sST2 was found to be increased in those suffering from pulmonary disease (obstructive sleep apnea, chronic obstructive pulmonary disease, asthma, pulmonary embolism, and pulmonary hypertension), systemic infection or inflammation, and alcohol abuse [62].

10. Conclusions

The ST2/IL-33 pathway is involved in pathophysiology of myocardial dysfunction by potentially attenuating the cardioprotective effects of IL-33. It seems to reduce the extent of cardiac damage after myocardial infarct, cardiomyocyte apoptosis, inflammatory cardiac activation, fibrosis, adverse myocardial remodeling. sST2, by acting as a decoy receptor, could prevent the beneficial effects of IL-33/ST2L interaction. sST2 measurements in blood samples could be a clinical prognostic biomarker useful in risk stratification of patients suffering from myocardial infarction, HF and dyspnea. Further studies are needed in order to better point out the evidence for a routine use of sST2 evaluation in the clinical evaluation of a patient suffering from myocardial infarction and HF.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Fauci, A.S.; Touchette, N.A.; Folkers, G.K. Emerging infectious diseases: A 10-year perspective from the national institute of allergy and infectious diseases. *Emerg. Infect. Dis.* **2005**, *11*, 519–525.

2. Ahern, R.M.; Lozano, R.; Naghavi, M.; Foreman, K.; Gakidou, E.; Murray, C.J. Improving the public health utility of global cardiovascular mortality data: The rise of ischemic heart disease. *Popul. Health Metr.* **2011**, *9*, 8.
3. Ciccone, M.M.; Scicchitano, P.; Zito, A.; Agati, L.; Gesualdo, M.; Mandolesi, S.; Carbonara, R.; Ciciarello, F.; Fedele, F. Correlation between coronary artery disease severity, left ventricular mass index and carotid intima media thickness, assessed by radio-frequency. *Cardiovasc. Ultrasound* **2011**, *9*, 32.
4. Ciccone, M.M.; Iacoviello, M.; Puzzovivo, A.; Scicchitano, P.; Monitillo, F.; de Crescenzo, F.; Caragnano, V.; Sassara, M.; Quistelli, G.; Guida, P.; *et al*. Clinical correlates of endothelial function in chronic heart failure. *Clin. Res. Cardiol.* **2011**, *100*, 515–521.
5. Ciccone, M.M.; Scicchitano, P.; Gesualdo, M.; Zito, A.; Carbonara, R.; Locorotondo, M.; Mandurino, C.; Masi, F.; Boccalini, F.; Lepera, M.E. Serum osteoprotegerin and carotid intima-media thickness in acute/chronic coronary artery diseases. *J. Cardiovasc Med. (Hagerstown).* **2013**, *14*, 43–48.
6. Thygesen, K.; Mair, J.; Mueller, C.; Huber, K.; Weber, M.; Plebani, M.; Hasin, Y.; Biasucci, L.M.; Giannitsis, E.; Lindahl, B.; *et al.* Recommendations for the use of natriuretic peptides in acute cardiac care: A position statement from the study group on biomarkers in cardiology of the ESC working group on acute cardiac care. *Eur. Heart J.* **2012**, *33*, 2001–2006.
7. Schmitz, J.; Owyang, A.; Oldham, E.; Song, Y.; Murphy, E.; McClanahan, T.K.; Zurawski, G.; Moshrefi, M.; Qin, J.; Li, X.; *et al.* IL-33, an interleukin-1- like cytokine that signals via the IL-1 receptor related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **2005**, *23*, 479–490.
8. Kakkar, R.; Lee, R.T. The IL-33/ST2 pathway: Therapeutic target and novel biomarker. *Nat. Rev. Drug Discov.* **2008**, *7*, 827–840.
9. Baba, Y.; Maeda, K.; Yashiro, T.; Inage, E.; Kasakura, K.; Suzuki, R.; Niyonsaba, F.; Hara, M.; Tanabe, A.; Ogawa, H.; *et al.* GATA2 is a critical transactivator for the human IL1RL1/ST2 promoter in mast cells/basophils: Opposing roles for GATA2 and GATA1 in human IL1RL1/ST2 gene expression. *J. Biol. Chem.* **2012**, *287*, 32689–32696.
10. Caporali, A.; Meloni, M.; Miller, A.M.; Vierlinger, K.; Cardinali, A.; Spinetti, G.; Nailor, A.; Faglia, E.; Losa, S.; Gotti, A.; *et al.* Soluble ST2 is regulated by p75 neurotrophin receptor and predicts mortality in diabetic patients with critical limb ischemia. *Arterioscler. Thromb. Vasc. Biol.* **2012**, *32*, e149–e160.
11. Weinberg, E.O. ST2 protein in heart disease: From discovery to mechanisms and prognostic value. *Biomark. Med.* **2009**, *3*, 495–511.
12. Schmieder, A.; Multhoff, G.; Radons, J. Interleukin-33 acts as a pro-inflammatory cytokine and modulates its receptor gene expression in highly metastatic human pancreatic carcinoma cells. *Cytokine* **2012**, *60*, 514–521.
13. Sweet, M.J.; Leung, B.P.; Kang, D.; Sogaard, M.; Schulz, K.; Trajkovic, V.; Campbell, C.C.; Xu, D.; Liew, F.Y. A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. *J. Immunol.* **2001**, *166*, 6633–6639.

14. Weinberg, E.O.; Shimpo, M.; de Keulenaer, G.W.; MacGillivray, C.; Tominaga, S.; Solomon, S.D.; Rouleau, J.L.; Lee, R.T. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation* **2002**, *106*, 2961–2966.
15. Meisel, C.; Bonhagen, K.; Löhning, M.; Coyle, A.J.; Gutierrez-Ramos, J.C.; Radbruch, A.; Kamradt, T. Regulation and function of T1/ST2 expression on CD4+ T cells: Induction of type 2 cytokine production by T1/ST2 cross-linking. *J. Immunol.* **2001**, *166*, 3143–3150.
16. Kakkar, R.; Hei, H.; Dobner, S.; Lee, R.T. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J. Biol. Chem.* **2012**, *287*, 6941–6948.
17. Sanada, S.; Hakuno, D.; Higgins, L.J.; Schreiter, E.R.; McKenzie, A.N.; Lee, R.T. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J. Clin. Invest.* **2007**, *117*, 1538–1549.
18. Ho, J.E.; Chen, W.Y.; Chen, M.H.; Larson, M.G.; McCabe, E.L.; Cheng, S.; Ghorbani, A.; Coglianese, E.; Emilsson, V.; Johnson, A.D.; *et al.* Common genetic variation at the IL1RL1 locus regulates IL-33/ST2 signaling. *J. Clin. Invest.* **2013**, *123*, 4208–4218.
19. Manzano-Fernández, S.; Mueller, T.; Pascual-Figal, D.; Truong, Q.A.; Januzzi, J.L. Usefulness of soluble concentrations of interleukin family member st2 as predictor of mortality in patients with acutely decompensated heart failure relative to left ventricular ejection fraction. *Am. J. Cardiol.* **2011**, *107*, 259–267.
20. Ky, B.; French, B.; Levy, W.C.; Sweitzer, N.K.; Fang, J.C.; Wu, A.H.; Goldberg, L.R.; Jessup, M.; Cappola, T.P. Multiple biomarkers for risk prediction in chronic heart failure. *Circ. Heart Fail.* **2012**, *5*, 183–190.
21. Chen, L.Q.; de Lemos, J.A.; Das, S.R.; Ayers, C.R.; Rohatgi, A. Soluble ST2 is associated with all-cause and cardiovascular mortality in a population-based cohort: The Dallas Heart Study. *Clin. Chem.* **2013**, *59*, 536–546.
22. Pascual-Figal, D.A.; Manzano-Fernández, S.; Boronat, M.; Casas, T.; Garrido, I.P.; Bonaque, J.C.; Pastor-Perez, F.; Valdés, M.; Januzzi, J.L. Soluble ST2, high-sensitivity troponin T- and N-terminal pro-B-type natriuretic peptide: Complementary role for risk stratification in acutely decompensated heart failure. *Eur. J. Heart Fail.* **2011**, *13*, 718–725.
23. Sabatine, M.S.; Morrow, D.A.; Higgins, L.J.; MacGillivray, C.; Guo, W.; Bode, C.; Rifai, N.; Cannon, C.P.; Gerszten, R.E.; Lee, R.T. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. *Circulation* **2008**, *117*, 1936–1944.
24. McMurray, J.J.; Adamopoulos, S.; Anker, S.D.; Auricchio, A.; Böhm, M.; Dickstein, K.; Falk, V.; Filippatos, G.; Fonseca, C.; Gomez-Sanchez, M.A.; *et al.* ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The task force for the diagnosis and treatment of acute and chronic heart failure 2012 of the european society of cardiology. Developed in collaboration with the heart failure association (HFA) of the ESC. *Eur. Heart J.* **2012**, *33*, 1787–1847.
25. Thygesen, K.; Alpert, J.S.; Jaffe, A.S.; Simoons, M.L.; Chaitman, B.R.; White, H.D.; Katus, H.A.; Apple, F.S.; Lindahl, B.; Morrow, D.A.; *et al.* Third universal definition of myocardial infarction. *J. Am. Coll. Cardiol.* **2012**, *60*, 1581–1598.
26. Januzzi, J.L., Jr.; Peacock, W.F.; Maisel, A.S.; Chae, C.U.; Jesse, R.L.; Baggish, A.L.; O'Donoghue, M.; Sakhuja, R.; Chen, A.A.; van Kimmenade, R.R.; *et al.* Measurement of the

interleukin family member ST2 in patients with acute dyspnea: Results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J. Am. Coll. Cardiol.* **2007**, *50*, 607–613.

27. Zwaan, L.; Thijs, A.; Wagner, C.; Timmermans, D.R. Does inappropriate selectivity in information use relate to diagnostic errors and patient harm? The diagnosis of patients with dyspnea. *Soc. Sci. Med.* **2013**, *91*, 32–38.
28. Januzzi, J.L.; Rehman, S.; Mueller, T.; van Kimmenade, R.R.J.; Lloyd-Jones, D.M. Importance of biomarkers for long-term mortality prediction in acutely dyspneic patients. *Clin. Chem.* **2010**, *56*, 1814–1821.
29. Socrates, T.; de Filippi, C.; Reichlin, T.; Twerenbold, R.; Breidhardt, T.; Noveanu, M.; Potocki, M.; Reiter, M.; Arenja, N.; Heinisch, C.; *et al.* Interleukin family member ST2 and mortality in acute dyspnoea. *J. Intern. Med.* **2010**, *268*, 493–500.
30. Shah, K.B.; Kop, W.J.; Christenson, R.H.; Diercks, D.B.; Henderson, S.; Hanson, K.; Li, S.Y.; deFilippi, C.R. Prognostic utility of ST2 in patients with acute dyspnea and preserved left ventricular ejection fraction. *Clin. Chem.* **2011**, *57*, 874–882.
31. Januzzi, J.L., Jr.; Camargo, C.A.; Anwaruddin, S.; Baggish, A.L.; Chen, A.A.; Krauser, D.G.; Tung, R.; Cameron, R.; Nagurney, J.T.; Chae, C.U.; *et al.* The *N*-terminal pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. *Am. J. Cardiol.* **2005**, *95*, 948–954.
32. Januzzi, J.L., Jr.; Sakhuja, R.; O'donoghue, M.; Baggish, A.L.; Anwaruddin, S.; Chae, C.U.; Cameron, R.; Krauser, D.G.; Tung, R.; Camargo, C.A., Jr.; *et al.* Utility of aminoterminal pro-brain natriuretic peptide testing for prediction of 1-year mortality in patients with dyspnea treated in the emergency department. *Arch. Intern. Med.* **2006**, *166*, 315–320.
33. Dieplinger, B.; Gegenhuber, A.; Kaar, G.; Poelz, W.; Haltmayer, M.; Mueller, T. Prognostic value of established and novel biomarkers in patients with shortness of breath attending an emergency department. *Clin. Biochem.* **2010**, *43*, 714–719.
34. Shah, R.V.; Chen-Tournoux, A.A.; Picard, M.H.; van Kimmenade, R.R.; Januzzi, J.L. Serum levels of the interleukin-1 receptor family member ST2, Cardiac structure and function, and long-term mortality in patients with acute dyspnea. *Circ. Heart Fail.* **2009**, *2*, 311–319.
35. Boisot, S.; Beede, J.; Isakson, S.; Chiu, A.; Clopton, P.; Januzzi, J.; Maisel, A.S.; Fitzgerald, R.L. Serial sampling of ST2 predicts 90-day mortality following destabilized HF. *J. Card. Fail.* **2008**, *14*, 732–738.
36. Bayes-Genis, A.; Pascual-Figal, D.; Januzzi, J.L.; Maisel, A.; Casas, T.; Valdés-Chávarri, M.; Ordóñez-Llanos, J. SST2monitoring provides additional risk stratification for outpatients with decompensated HF. *Rev. Esp. Cardiol.* **2010**, *63*, 1171–1178.
37. Mueller, T.; Dieplinger, B.; Gegenhuber, A.; Poelz, W.; Pacher, R.; Haltmayer, M. Increased plasma concentrations of sST2are predictive for 1-year mortality in patients with acute destabilized heart failure. *Clin. Chem.* **2008**, *54*, 752–756.
38. Rehman, S.U.; Mueller, T.; Januzzi, J.L., Jr. Characteristics of the novel interleukin family biomarker ST2 in patients with acute HF. *J. Am. Coll. Cardiol.* **2008**, *52*, 1458–1465.
39. Diez, J. Serum soluble ST2 as a biochemical marker of acute heart failure. *J. Am. Coll. Cardiol.* **2008**, *52*, 1466–1467.

40. Weinberg, E.O.; Shimpo, M.; Hurwitz, S.; Tominaga, S.; Rouleau, J.L.; Lee, R.T. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* **2003**, *107*, 721–726.
41. Ky, B.; French, B.; McCloskey, K.; Rame, J.E.; McIntosh, E.; Shahi, P.; Dries, D.L.; Tang, W.; Wu, A.H.B.; Fang, J.C.; *et al.* Sensitivity ST2 for prediction of adverse outcomes in chronic heart failure. *Circ. Heart Fail.* **2011**, *4*, 180–187.
42. Bartunek, J.; Delrue, L.; van Durme, F.; Muller, O.; Casselman, F.; de Wiest, B.; Croes, R.; Verstreken, S.; Goethals, M.; de Raedt, H.; *et al.* Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. *J. Am. Coll. Cardiol.* **2008**, *52*, 2166–2174.
43. Shah, R.V.; Januzzi, J.L., Jr. ST2: A novel remodeling biomarker in acute and chronic heart failure. *Curr. Heart Fail. Rep.* **2010**, *7*, 9–14.
44. Bhardwaj, A.; Januzzi, J.L., Jr. ST2: A novel biomarker for heart failure. *Expert Rev. Mol. Diagn.* **2010**, *10*, 459–464.
45. Xanthakis, V.; Larson, M.G.; Wollert, K.C.; Aragam, J.; Cheng, S.; Ho, J.; Coglianese, E.; Levy, D.; Colucci, W.S.; Michael Felker, G.; *et al.* Association of novel biomarkers of cardiovascular stress with left ventricular hypertrophy and dysfunction: Implications for screening. *J. Am. Heart Assoc.* **2013**, *2*, e000399.
46. Seki, K.; Sanada, S.; Kudinova, A.Y.; Steinhauser, M.L.; Handa, V.; Gannon, J.; Lee, R.T. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. *Circ. Heart Fail.* **2009**, *2*, 684–691.
47. Baidya, S.G.; Zeng, Q.T. Helper T cells and atherosclerosis: The cytokine web. *Postgrad. Med. J.* **2005**, *81*, 746–752.
48. Shah, P.K.; Falk, E.; Badimon, J.J.; Fernandez-Ortiz, A.; Mailhac, A.; Villareal-Levy, G.; Fallon, J.T.; Regnstrom, J.; Fuster, V. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* **1995**, *92*, 1565–1569.
49. Dollery, C.M.; McEwan, J.R.; Henney, A.M. Matrix metalloproteinases and cardiovascular disease. *Circ. Res.* **1995**, *77*, 863–868.
50. Dominguez-Rodriguez, A.; Abreu-Gonzalez, P. Clinical implications of elevated serum interleukin-6, Soluble CD40 ligand, metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 in patients with acute ST-segment elevation myocardial infarction. *Clin. Cardiol.* **2009**, *32*, 288.
51. Inokubo, Y.; Hanada, H.; Ishizaka, H.; Fukushi, T.; Kamada, T.; Okumura, K. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. *Am. Heart J.* **2001**, *141*, 211–217.
52. Kaden, J.J.; Dempfle, C.E.; Sueselbeck, T.; Brueckmann, M.; Poerner, T.C.; Haghi, D.; Haase, K.K.; Borggrefe, M. Time-dependent changes in the plasma concentration of matrix metalloproteinase 9 after acute myocardial infarction. *Cardiology* **2003**, *99*, 140–144.
53. Higo, S.; Uematsu, M.; Yamagishi, M.; Ishibashi-Ueda, H.; Awata, M.; Morozumi, T.; Ohara, T.; Nanto, S.; Nagata, S. Elevation of plasma matrix metalloproteinase-9 in the culprit coronary artery in patients with acute myocardial infarction*:* Clinical evidence from distal protection. *Circ. J.* **2005**, *69*, 1180–1185.

54. Guzel, S.; Serin, O.; Guzel, E.C.; Buyuk, B.; Yılmaz, G.; Güvenen, G. Interleukin-33, Matrix metalloproteinase-9, and tissue inhibitor of matrix metalloproteinase-1 in myocardial infarction. *Korean J. Intern. Med.* **2013**, *28*, 165–173.
55. Shimpo, M.; Morrow, D.A.; Weinberg, E.O.; Sabatine, M.S.; Murphy, S.A.; Antman, E.M.; Lee, R.T. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation* **2004**, *109*, 2186–2190.
56. Eggers, K.M.; Armstrong, P.W.; Califf, R.M.; Simoons, M.L.; Venge, P.; Wallentin, L.; James, S.K. ST2 and mortality in non-ST-segment elevation acute coronary syndrome. *Am. Heart J.* **2010**, *159*, 788–794.
57. Kohli, P.; Bonaca, M.P.; Kakkar, R.; Kudinova, A.Y.; Scirica, B.M.; Sabatine, M.S.; Murphy, S.A.; Braunwald, E.; Lee, R.T.; Morrow, D.A. Role of ST2 in non-ST-elevation acute coronary syndrome in the MERLIN-TIMI 36 trial. *Clin. Chem.* **2012**, *58*, 257–266.
58. Dhillon, O.S.; Narayan, H.K.; Quinn, P.A.; Squire, I.B.; Davies, J.E.; Ng, L.L. Interleukin 33 and ST2 in non-ST-elevation myocardial infarction: Comparison with global registry of acute coronary events risk scoring and NT-proBNP. *Am. Heart J.* **2011**, *161*, 1163–1170.
59. Weir, R.A.; Miller, A.M.; Murphy, G.E.; Clements, S.; Steedman, T.; Connell, J.M.; McInnes, I.B.; Dargie, H.J.; McMurray, J.J. Serum soluble ST2: A potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction. *J. Am. Coll. Cardiol.* **2010**, *55*, 243–250.
60. Daniels, L.B.; Clopton, P.; Iqbal, N.; Tran, K.; Maisel, A.S. Association of ST2 levels with cardiac structure and function and mortality in outpatients. *Am. Heart J.* **2010**, *160*, 721–728.
61. Coglianese, E.E.; Larson, M.G.; Vasan, R.S.; Ho, J.E.; Ghorbani, A.; McCabe, E.L.; Cheng, S.; Fradley, M.G.; Kretschman, D.; Gao, W.; *et al.* Distribution and clinical correlates of the interleukin receptor family member sST2in the framingham heart study. *Clin. Chem.* **2012**, *58*, 1673–1681.
62. Brown, A.M.; Wu, A.H.; Clopton, P.; Robey, J.L.; Hollander, J.E. ST2 in emergency department chest pain patients with potential acute coronary syndromes. *Ann. Emerg. Med.* **2007**, *50*, 153–158.

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NICE National Institute for Health and Care Excellence

Hepatitis B (chronic)

Diagnosis and management of chronic hepatitis B in children, young people and adults

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NICE clinical guideline 165

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NICE has accredited the process used by the Centre for Clinical Practice at NICE to produce guidelines. Accreditation is valid for 5 years from September 2009 and applies to guidelines produced since April 2007 using the processes described in NICE's 'The guidelines manual' (2007, updated 2009). More information on accreditation can be viewed at www.nice.org.uk/accreditation

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Introduction

This guideline updates and replaces recommendations in NICE technology appraisal 96 and incorporates recommendations from NICE technology appraisals 96, 153, 154 and 173. See About this guideline for details.

Chronic hepatitis B describes a spectrum of disease usually characterised by the presence of detectable hepatitis B surface antigen (HBsAg) in the blood or serum for longer than 6 months. In some people, chronic hepatitis B is inactive and does not present significant health problems, but others may progress to liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). The progression of liver disease is associated with hepatitis B virus (HBV) DNA levels in the blood. Without antiviral treatment, the 5-year cumulative incidence of cirrhosis ranges from 8 to 20%. People with cirrhosis face a significant risk of decompensated liver disease if they remain untreated. Five-year survival rates among people with untreated decompensated cirrhosis can be as low as 15%. Chronic hepatitis B can be divided into e antigen- (HBeAg) positive or HBeAg-negative disease based on the presence or absence of e antigen. The presence of HBeAg is typically associated with higher rates of viral replication and therefore increased infectivity.

The goal of treatment for chronic hepatitis B is to prevent cirrhosis, HCC and liver failure. In clinical practice surrogate markers are used to monitor progression of disease and treatment response, and include normalisation of serum alanine aminotransferase (ALT) levels, decrease in inflammation scores with no worsening or improvement in fibrosis on liver biopsies, suppression of serum HBV DNA to undetectable levels, loss of HBeAg and seroconversion to HBe antibody (anti-HBe), and loss of HBsAg and seroconversion to HBs antibody (anti-HBs).

Antiviral therapy suppresses HBV replication and decreases hepatic inflammation and fibrosis, thereby reducing the likelihood of serious clinical disease. Since the introduction of effective treatment in the form of interferon alfa, several nucleoside and nucleotide analogues are now approved for use in adults with chronic hepatitis B, together with a pegylated form of interferon alfa. With multiple treatment options that are efficacious and safe, the key questions are which patients need immediate treatment and what sequence and combination of drug regimens should be used, and which patients can be monitored and delay treatment.

In this guideline we cover the following:

- information needs of people with chronic hepatitis B and their carers

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- where children, young people and adults with chronic hepatitis B should be assessed
- assessment of liver disease, including the use of non-invasive tests and genotype testing
- criteria for offering antiviral treatment
- the efficacy, safety and cost effectiveness of currently available treatments
- selection of first-line therapy
- management of treatment failure or drug resistance
- whether there is a role for combination therapy
- when it is possible to stop treatment
- managing the care of pregnant and breastfeeding women and prevention of vertical transmission
- prophylactic treatment during immunosuppressive therapy
- monitoring for treatment response, severity of fibrosis and development of HCC.

The spontaneous mutation rate of HBV DNA is high. Exposure of HBV to nucleoside or nucleotide analogues selects for mutations in the polymerase gene that confer resistance or decreased susceptibility to the drugs. The relative risk of drug resistance must be taken into account when considering treatment with nucleoside or nucleotide analogues, including the level of cross resistance between different agents.

Figure 1 depicts the natural history of chronic HBV infection. The immune-tolerance phase is seen in HBeAg-positive disease and is characterised by high levels of HBV replication with normal ALT levels and limited liver necroinflammation. Because there is minimal immune response to the virus it is unusual for spontaneous HBeAg loss to occur. This phase is commonly seen in children. It is followed by an immune-clearance or immune-reactive phase in which the immune system recognises and starts to clear the virus. ALT levels are typically elevated or fluctuating, and there is a higher risk of liver fibrosis. This tends to be the initial phase in people infected with HBV as adults. It lasts from weeks to years and ends with HBeAg seroconversion.

With the loss of HBeAg the person may enter an immune-control phase with very low or undetectable HBV DNA levels, normal ALT and minimal fibrosis progression. However, some

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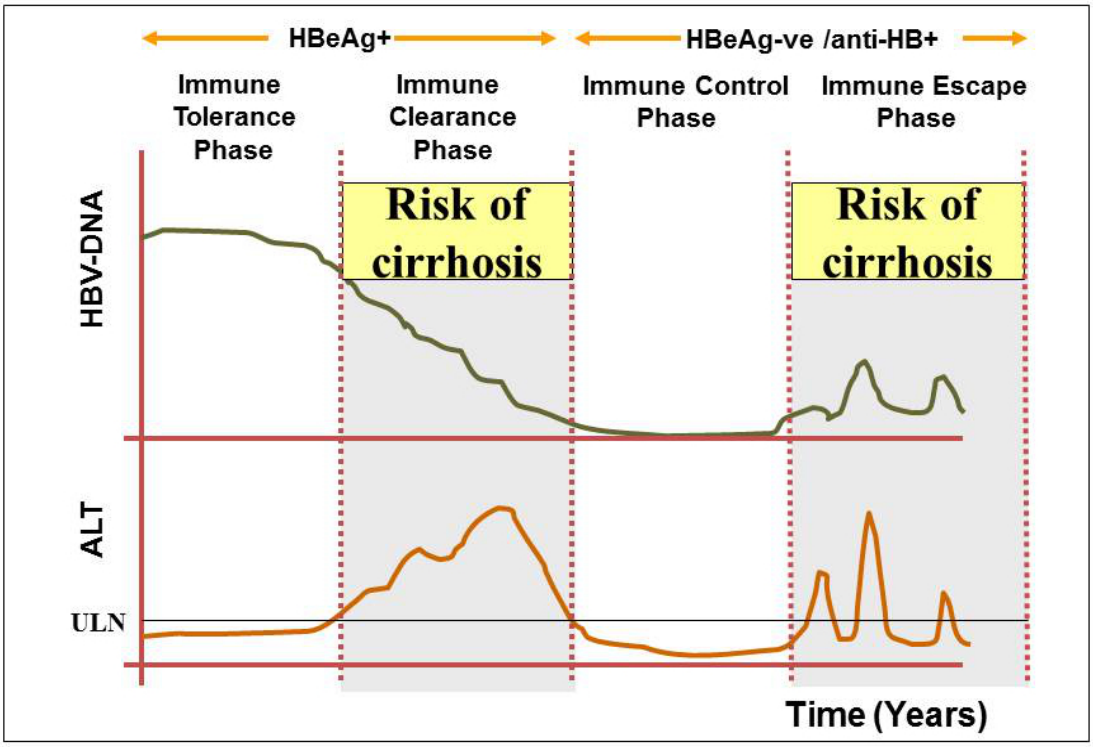
people may experience rising HBV DNA levels despite HBeAg negativity. This is caused by virions that do not express HBeAg because of genetic mutations. This immune-escape phase can lead to active necroinflammation and progression of fibrosis.

Figure 1. Natural history of chronic HBV infection



After Chu et al, Hepatology 1985;5:431-34 ULN: upper limit of normal of ALT

Substantial progress has been made in the treatment of chronic hepatitis B in the past decade but the appropriate time for starting treatment remains a topic of debate. Although currently available treatment is effective in suppressing HBV replication, it fails to eradicate the virus necessitating long treatment duration and perhaps lifelong treatment.

The guideline will assume that prescribers will use a drug's summary of product characteristics to inform decisions made with individual patients.

This guideline recommends some drugs for indications for which they do not have a U marketing authorisation at the date of publication, if there is good evidence to support that use. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. The patient (or those with authority to give consent on their behalf) should provide informed consent, which should be documented. See the General Medical Council's Good

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practice in prescribing medicines □ guidance for doctors for further information. Where recommendations have been made for the use of drugs outside their licensed indications ('off-label use'), these drugs are marked with a footnote in the recommendations.

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Patient-centred care

This guideline offers best practice advice on the care of children, young people and adults with chronic hepatitis B.

Patients and healthcare professionals have rights and responsibilities as set out in the NHS Constitution for England – all NICE guidance is written to reflect these. Treatment and care should take into account individual needs and preferences. Patients should have the opportunity to make informed decisions about their care and treatment, in partnership with their healthcare professionals. If the patient is under 16, their family or carers should also be given information and support to help the child or young person to make decisions about their treatment. Healthcare professionals should follow the Department of Health's advice on consent. If someone does not have capacity to make decisions, healthcare professionals should follow the code of practice that accompanies the Mental Capacity Act and the supplementary code of practice on deprivation of liberty safeguards. In Wales, healthcare professionals should follow advice on consent from the Welsh Government.

NICE has produced guidance on the components of good patient experience in adult NHS services. All healthcare professionals should follow the recommendations in Patient experience in adult NHS services.

If a young person is moving between paediatric and adult services, care should be planned and managed according to the best practice guidance described in the Department of Health's Transition: getting it right for young people.

Adult and paediatric healthcare teams should work jointly to provide assessment and services to young people with chronic hepatitis B. Diagnosis and management should be reviewed throughout the transition process, and there should be clarity about who is the lead clinician to ensure continuity of care.

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Terms used in this guidance

Chronic hepatitis B

Chronic hepatitis B is defined as persistence of hepatitis B surface antigen (HBsAg) for 6 months or more after acute infection with hepatitis B virus (HBV).

HBV DNA

HBV DNA level, or 'viral load', is an indicator of viral replication. Higher HBV DNA levels are usually associated with an increased risk of liver disease and hepatocellular carcinoma. HBV DNA level typically falls in response to effective antiviral treatment.

Hepatitis B surface antigen (HBsAg)

Hepatitis B surface antigen (HBsAg) is a viral protein detectable in the blood in acute and chronic hepatitis B infection.

HBsAg seroconversion

The development of antibodies against HBsAg is known as HBsAg seroconversion. It signifies clearance of HBsAg and resolution of the chronic infection.

Hepatitis B e antigen (HBeAg)

Hepatitis B e antigen (HBeAg) is an indicator of viral replication, although some variant forms of the virus do not express HBeAg (see 'HBeAg-negative chronic hepatitis B' below). Active infection can be described as HBeAg-positive or HBeAg-negative according to whether HBeAg is secreted.

HBeAg-negative chronic hepatitis B

HBeAg-negative hepatitis B is a form of the virus that does not cause infected cells to secrete HBeAg. People can be infected with the HBeAg-negative form of the virus from the beginning, or

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the viral mutation can emerge later in the course of infection in people initially infected with the HBeAg-positive form of the virus.

HBeAg seroconversion

HBeAg seroconversion occurs when people infected with the HBeAg-positive form of the virus develop antibodies against the 'e' antigen. The seroconverted disease state is referred to as the 'inactive HBV carrier state' when HBeAg has been cleared, anti-HBe is present and HBV DNA is undetectable or less than 2000 IU/ml. Once seroconversion has taken place, most people remain in the inactive HBV carrier state (the immune-control phase; see figure 1). However, increasing HBV DNA and recurrent hepatitis after seroconversion indicate the emergence of the HBeAg-negative strain of the virus (the immune-escape phase; see figure 1).

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Key priorities for implementation

The following recommendations have been identified as priorities for implementation.

Assessment and referral

- Arrange the following tests in primary care for adults who are hepatitis B surface antigen (HBsAg) positive:
  - hepatitis B e antigen (HBeAg)/antibody (anti-HBe) status
  - HBV DNA level
  - IgM antibody to hepatitis B core antigen (anti-HBc IgM)
  - hepatitis C virus antibody (anti-HCV)
  - hepatitis delta virus antibody (anti-HDV)
  - HIV antibody (anti-HIV)
  - IgG antibody to hepatitis A virus (anti-HAV)
  - additional laboratory tests including alanine aminotransferase (ALT) or aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), serum albumin, total bilirubin, total globulins, full blood count and prothrombin time
  - tests for hepatocellular carcinoma (HCC), including hepatic ultrasound and alpha-fetoprotein testing.
- Include the results of the initial tests with the referral (see recommendation 1.2.1).

Treatment sequence in adults with HBeAg-positive chronic hepatitis B and compensated liver disease

- Offer a 48-week course of peginterferon alfa-2a as first-line treatment in adults with HBeAg-positive chronic hepatitis B and compensated liver disease[1].

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- Offer tenofovir disoproxil as second-line treatment to people who do not undergo HBeAg seroconversion or who relapse (revert to being HBeAg positive following seroconversion) after first-line treatment with peginterferon alfa-2a.
- Offer entecavir as an alternative second-line treatment to people who cannot tolerate tenofovir disoproxil or if it is contraindicated.

Treatment sequence in adults with HBeAg-negative chronic hepatitis B and compensated liver disease

- Offer a 48-week course of peginterferon alfa-2a as first-line treatment in adults with HBeAg-negative chronic hepatitis B and compensated liver disease[1].
- Offer entecavir or tenofovir disoproxil as second-line treatment to people with detectable HBV DNA after first-line treatment with peginterferon alfa-2a.

Women who are pregnant or breastfeeding

- Offer tenofovir disoproxil to women with HBV DNA greater than 10^7 IU/ml in the third trimester to reduce the risk of transmission of HBV to the baby[2].

Prophylactic treatment during immunosuppressive therapy

- In people who are HBsAg positive and have HBV DNA greater than 2000 IU/ml, offer prophylaxis with entecavir or tenofovir disoproxil[3].
    - Start prophylaxis before beginning immunosuppressive therapy and continue for a minimum of 6 months after HBeAg seroconversion and HBV DNA is undetectable.
- In people who are HBsAg positive and have HBV DNA less than 2000 IU/ml, offer prophylaxis:
    - consider lamivudine[3] if immunosuppressive therapy is expected to last less than 6 months
        - monitor HBV DNA monthly in people treated with lamivudine and change to tenofovir disoproxil if HBV DNA remains detectable after 3 months

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- consider entecavir or tenofovir disoproxil[3] if immunosuppressive therapy is expected to last longer than 6 months
- start prophylaxis before beginning immunosuppressive therapy and continue for a minimum of 6 months after stopping immunosuppressive therapy.

[1] Avoid use of peginterferon alfa-2a in pregnancy unless the potential benefit outweighs risk. Women of childbearing potential must use effective contraception throughout therapy.

[2] At the time of publication (June 2013), tenofovir disoproxil did not have a U□ marketing authorisation for this indication. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. Informed consent should be obtained and documented. See the General Medical Council's Good practice in prescribing medicines □guidance for doctors for further information.

[3] At the time of publication (June 2013), entecavir, lamivudine and tenofovir disoproxil did not have a U□ marketing authorisation for this indication. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. Informed consent should be obtained and documented. See the General Medical Council's Good practice in prescribing medicines □guidance for doctors for further information.

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1 Recommendations

The following guidance is based on the best available evidence. The full guideline gives details of the methods and the evidence used to develop the guidance.

The wording used in the recommendations in this guideline (for example, words such as 'offer' and 'consider') denotes the certainty with which the recommendation is made (the strength of the recommendation). See About this guideline for details.

In this guideline, children and young people are defined as aged up to 18 years. Please follow the recommendations for women who are pregnant for young people with chronic hepatitis B who are pregnant.

1.1 Patient information

1.1.1 Provide information on the following topics to people with chronic hepatitis B and to family members or carers (if appropriate) before assessment for antiviral treatment:

- the natural history of chronic hepatitis B, including stages of disease and long-term prognosis
- lifestyle issues such as alcohol, diet and weight
- family planning
- monitoring
- routes of hepatitis B virus (HBV) transmission
- the benefits of antiviral treatment, including reduced risk of serious liver disease and death and reduced risk of transmission of HBV to others
- treatment options and contraindications based on the patient's circumstances, including peginterferon alfa-2a and nucleoside or nucleotide analogues
- short- and long-term treatment goals

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- causes of treatment failure, including non-adherence to prescribed medicines, and options for re-treatment
- risks of treatment, including adverse effects and drug resistance.

1.1.2 Offer a copy of the personalised care plan to people with chronic hepatitis B and to family members or carers (if appropriate) outlining proposed treatment and long-term management, for example, a copy of the hospital consultation summary.

1.1.3 Provide information on self-injection techniques to people beginning peginterferon alfa-2a or to family members or carers.

1.1.4 NICE has produced public health guidance on ways to promote and offer testing to people at increased risk of infection with hepatitis B. All healthcare professionals should follow the recommendations in Hepatitis B and C: ways to promote and offer testing to people at increased risk of infection (NICE public health guidance 43).

1.1.5 NICE has produced guidance on the components of good patient experience in adult NHS services. All healthcare professionals should follow the recommendations in Patient experience in adult NHS services (NICE clinical guidance 138).

1.2 Assessment and referral in primary care

Adults who are HBsAg positive

1.2.1 Arrange the following tests in primary care for adults who are hepatitis B surface antigen (HBsAg) positive:

- hepatitis B e antigen (HBeAg)/antibody (anti-HBe) status
- HBV DNA level
- IgM antibody to hepatitis B core antigen (anti-HBc IgM)
- hepatitis C virus antibody (anti-HCV)

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- hepatitis delta virus antibody (anti-HDV)
- HIV antibody (anti-HIV)
- IgG antibody to hepatitis A virus (anti-HAV)
- additional laboratory tests including alanine aminotransferase (ALT) or aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), serum albumin, total bilirubin, total globulins, full blood count and prothrombin time
- tests for hepatocellular carcinoma (HCC), including hepatic ultrasound and alpha-fetoprotein testing.

1.2.2 Refer all adults who are HBsAg positive to a hepatologist or to a gastroenterologist or infectious disease specialist with an interest in hepatology.

1.2.3 Include the results of the initial tests with the referral (see recommendation 1.2.1).

Pregnant women who test HBsAg positive at antenatal screening

1.2.4 Refer pregnant women who are HBsAg positive to a hepatologist, or to a gastroenterologist or infectious disease specialist with an interest in hepatology, for assessment within 6 weeks of receiving the screening test result and to allow treatment in the third trimester (see recommendation 1.5.39).

Adults with decompensated liver disease

1.2.5 Refer adults who develop decompensated liver disease immediately to a hepatologist or to a gastroenterologist with an interest in hepatology. Symptoms of decompensated liver disease include (but are not limited to) ascites, encephalopathy and gastrointestinal haemorrhage.

Children and young people who are HBsAg positive

1.2.6 Arrange the following tests for children and young people who are HBsAg positive:

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- HBeAg/anti-HBe status
- HBV DNA level
- anti-HBc IgM
- anti-HCV
- anti-HDV
- anti-HIV
- anti-HAV
- additional laboratory tests, including ALT or AST, GGT, serum albumin, total bilirubin, total globulins, full blood count and prothrombin time
- tests for HCC, including hepatic ultrasound and alpha-fetoprotein testing.

1.2.7 Refer all children and young people who are HBsAg positive to a paediatric hepatologist or to a gastroenterologist or infectious disease specialist with an interest in hepatology.

1.2.8 Include the results of the initial tests with the referral (see recommendation 1.2.6).

1.3 Assessment of liver disease in secondary specialist care

Adults with chronic hepatitis B

Please refer to recommendations 1.5.3 to 1.5.7 for detailed guidance on offering antiviral treatment.

1.3.1 Ensure all healthcare professionals who refer adults for non-invasive tests for liver disease are trained to interpret the results and aware of co-factors that influence liver elasticity (for example, fatty liver caused by obesity or alcohol misuse).

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1.3.2 Discuss the accuracy, limitations and risks of the different tests for liver disease with the patient.

1.3.3 Offer transient elastography as the initial test for liver disease in adults newly referred for assessment.

1.3.4 Offer antiviral treatment without a liver biopsy to adults with a transient elastography score greater than or equal to 11 kPa[4], in line with recommendation 1.5.6.

1.3.5 Consider liver biopsy to confirm the level of fibrosis in adults with a transient elastography score between 6 and 10 kPa[5]. Offer antiviral treatment in line with recommendations 1.5.3 to 1.5.7.

1.3.6 Offer liver biopsy to adults with a transient elastography score less than 6 kPa if they are younger than 30 years and have HBV DNA greater than 2000 IU/ml and abnormal ALT (greater than or equal to 30 IU/L for males and greater than or equal to 19 IU/L for females) on 2 consecutive tests conducted 3 months apart[6]. Offer antiviral treatment in line with recommendations 1.5.3 to 1.5.7.

1.3.7 Do not offer liver biopsy to adults with a transient elastography score less than 6 kPa who have normal ALT (less than 30 IU/L in males and less than 19 IU/L in females) and HBV DNA less than 2000 IU/ml as they are unlikely to have advanced liver disease or need antiviral treatment (see recommendations 1.5.3 to 1.5.7)[6].

1.3.8 Offer an annual reassessment of liver disease using transient elastography to adults who are not taking antiviral treatment.

Children and young people with chronic hepatitis B

1.3.9 Discuss the accuracy, limitations and risks of liver biopsy in determining the need for antiviral treatment with the child or young person and with parents or carers (if appropriate).

1.3.10 Consider liver biopsy to assess liver disease and the need for antiviral treatment in children and young people with HBV DNA greater than 2000 IU/ml

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and abnormal ALT (greater than or equal to 30 IU/L for males and greater than or equal to 19 IU/L for females) on 2 consecutive tests conducted 3 months apart. Offer biopsy under a general anaesthetic to children who are too young to tolerate the procedure under a local anaesthetic.

1.4 Genotype testing

1.4.1 Do not offer genotype testing to determine initial treatment in people with chronic hepatitis B.

1.5 Antiviral treatment

Adults with chronic hepatitis B

Recommendations 1.5.8 to 1.5.12 are reproduced from existing NICE technology appraisals on options for the treatment of chronic hepatitis B, and 1.5.13 to 1.5.15 update guidance in NICE technology appraisal 96[7]. The GDG has reviewed the evidence and has made recommendations on treatment sequences and combination drug regimens (see recommendations 1.5.16 to 1.5.28).

Recommendations 1.5.8 to 1.5.43 do not apply to people with chronic hepatitis B who also have hepatitis C, hepatitis D or HIV.

1.5.1 Discuss treatment options, adverse effects and long-term prognosis with the patient before starting treatment.

1.5.2 Re-assess the person's risk of exposure to HIV before starting treatment and offer repeat testing if needed.

1.5.3 Offer antiviral treatment to adults aged 30 years and older who have HBV DNA greater than 2000 IU/ml and abnormal ALT (greater than or equal to 30 IU/L in males and greater than or equal to 19 IU/L in females) on 2 consecutive tests conducted 3 months apart.

1.5.4 Offer antiviral treatment to adults younger than 30 years who have HBV DNA greater than 2000 IU/ml and abnormal ALT (greater than or equal to 30 IU/L in

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males and greater than or equal to 19 IU/L in females) on 2 consecutive tests conducted 3 months apart if there is evidence of necroinflammation or fibrosis on liver biopsy or a transient elastography score greater than 6 kPa.

1.5.5 Offer antiviral treatment to adults who have HBV DNA greater than 20,000 IU/ml and abnormal ALT (greater than or equal to 30 IU/L in males and greater than or equal to 19 IU/L in females) on 2 consecutive tests conducted 3 months apart regardless of age or the extent of liver disease.

1.5.6 Offer antiviral treatment to adults with cirrhosis and detectable HBV DNA, regardless of HBeAg status, HBV DNA and ALT levels.

1.5.7 Consider antiviral treatment in adults with HBV DNA greater than 2000 IU/ml and evidence of necroinflammation or fibrosis on liver biopsy.

1.5.8 Peginterferon alfa-2a is recommended as an option for the initial treatment of adults with chronic hepatitis B (HBeAg-positive or HBeAg-negative), within its licensed indications. [This recommendation is from Adefovir dipivoxil and peginterferon alfa-2a for the treatment of chronic hepatitis B (NICE technology appraisal guidance 96).]

1.5.9 Entecavir, within its marketing authorisation, is recommended as an option for the treatment of people with chronic HBeAg-positive or HBeAg-negative hepatitis B in whom antiviral treatment is indicated. [This recommendation is from Entecavir for the treatment of chronic hepatitis B (NICE technology appraisal guidance 153).]

1.5.10 Tenofovir disoproxil, within its marketing authorisation, is recommended as an option for the treatment of people with chronic HBeAg-positive or HBeAg-negative hepatitis B in whom antiviral treatment is indicated. [This recommendation is from Tenofovir disoproxil fumarate for the treatment of hepatitis B (NICE technology appraisal guidance 173).]

1.5.11 Telbivudine is not recommended for the treatment of chronic hepatitis B. [This recommendation is from Telbivudine for the treatment of chronic hepatitis B (NICE technology appraisal guidance 154).]

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1.5.12 People currently receiving telbivudine should have the option to continue therapy until they and their clinicians consider it appropriate to stop. [This recommendation is from Telbivudine for the treatment of chronic hepatitis B (NICE technology appraisal guidance 154).]

1.5.13 Do not offer adefovir dipivoxil for treatment of chronic hepatitis B.

1.5.14 People currently receiving adefovir dipivoxil should be offered the option to switch to a different treatment. Offer tenofovir disoproxil or entecavir, depending on previous antiviral exposure:

- offer tenofovir disoproxil to people with a history of lamivudine resistance.

1.5.15 Antiviral treatment should be initiated only by an appropriately qualified healthcare professional with expertise in the management of viral hepatitis. Continuation of therapy under shared-care arrangements with a GP is appropriate.

Treatment sequence in adults with HBeAg-positive chronic hepatitis B and compensated liver disease

1.5.16 Offer a 48-week course of peginterferon alfa-2a as first-line treatment in adults with HBeAg-positive chronic hepatitis B and compensated liver disease[8].

1.5.17 Consider stopping peginterferon alfa-2a 24 weeks after starting treatment if HBV DNA level has decreased by less than 2 $\log_{10}$ IU/ml and/or if HBsAg is greater than 20,000 IU/ml, and offer second-line treatment in line with recommendations 1.5.18 and 1.5.19.

1.5.18 Offer tenofovir disoproxil as second-line treatment to people who do not undergo HBeAg seroconversion or who relapse (revert to being HBeAg positive following seroconversion) after first-line treatment with peginterferon alfa-2a.

1.5.19 Offer entecavir as an alternative second-line treatment to people who cannot tolerate tenofovir disoproxil or if it is contraindicated.

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1.5.20 Review adherence in people taking tenofovir disoproxil who have detectable HBV DNA at 48 weeks of treatment and, if appropriate, provide support in line with Medicines adherence (NICE clinical guidance 76).

- If HBV DNA remains detectable at 96 weeks, and there is no history of lamivudine resistance, consider adding lamivudine to tenofovir disoproxil.
- In people with a history of lamivudine resistance, consider adding entecavir to tenofovir disoproxil.

1.5.21 Consider stopping nucleoside or nucleotide analogue treatment 12 months after HBeAg seroconversion in people without cirrhosis.

1.5.22 Do not stop nucleoside or nucleotide analogue treatment 12 months after HBeAg seroconversion in people with cirrhosis.

Treatment sequence in adults with HBeAg-negative chronic hepatitis B and compensated liver disease

1.5.23 Offer a 48-week course of peginterferon alfa-2a as first-line treatment in adults with HBeAg-negative chronic hepatitis B and compensated liver disease[8].

1.5.24 Consider stopping peginterferon alfa-2a 24 weeks after starting treatment if HBV DNA level has decreased by less than 2 $\log_{10}$ IU/ml and HBsAg has not decreased, and consider second-line treatment in line with recommendation 1.5.25.

1.5.25 Offer entecavir or tenofovir disoproxil as second-line treatment to people with detectable HBV DNA after first-line treatment with peginterferon alfa-2a.

1.5.26 Consider switching from tenofovir disoproxil to entecavir, or from entecavir to tenofovir disoproxil, as third-line treatment in people who have detectable HBV DNA at 48 weeks of treatment.

1.5.27 Consider stopping nucleoside or nucleotide analogue treatment 12 months after achieving undetectable HBV DNA and HBsAg seroconversion in people without cirrhosis.

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1.5.28 Do not stop nucleoside or nucleotide analogue treatment after achieving undetectable HBV DNA and HBsAg seroconversion in patients with cirrhosis.

Children and young people with chronic hepatitis B and compensated liver disease

1.5.29 Discuss treatment options, adverse effects and long-term prognosis with the child or young person and with parents or carers (if appropriate) before starting treatment.

1.5.30 Re-assess the child or young person's risk of exposure to HIV before starting treatment and offer repeat testing if necessary.

1.5.31 Offer antiviral treatment if there is evidence of significant fibrosis (METAVIR stage greater than or equal to F2 or Ishak stage greater than or equal to 3) or abnormal ALT (greater than or equal to 30 IU/L for males and greater than or equal to 19 IU/L for females) on 2 consecutive tests conducted 3 months apart.

1.5.32 Consider a 48-week course of peginterferon alfa-2a as first-line treatment in children and young people with chronic hepatitis B and compensated liver disease[8],[9].

1.5.33 Consider stopping peginterferon alfa-2a 24 weeks after starting treatment if HBV DNA level has decreased by less than 2 $\log_{10}$ IU/ml and/or if HBsAg is greater than 20,000 IU/ml.

1.5.34 Consider a nucleoside or nucleotide analogue as second-line treatment in children and young people with detectable HBV DNA after first-line treatment with peginterferon alfa-2a[10].

Adults with decompensated liver disease

1.5.35 Manage decompensated liver disease in adults in conjunction with a liver transplant centre.

1.5.36 Do not offer peginterferon alfa-2a to people with chronic hepatitis B and decompensated liver disease.

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1.5.37 Offer entecavir as first-line treatment in people with decompensated liver disease if there is no history of lamivudine resistance.

- Offer tenofovir disoproxil to people with a history of lamivudine resistance.
- Reduce the dose of tenofovir disoproxil in people with renal impairment, in line with guidance in the summary of product characteristics.

Women who are pregnant or breastfeeding

1.5.38 Discuss with pregnant women the benefits and risks of antiviral treatment for them and their baby.

1.5.39 Offer tenofovir disoproxil to women with HBV DNA greater than 10^7 IU/ml in the third trimester to reduce the risk of transmission of HBV to the baby[11].

1.5.40 Monitor quantitative HBV DNA 2 months after starting tenofovir disoproxil and ALT monthly after the birth to detect postnatal HBV flares in the woman.

1.5.41 Stop tenofovir disoproxil 4 to 12 weeks after the birth unless the mother meets criteria for long-term treatment (see recommendations 1.5.4 to 1.5.8).

1.5.42 Offer active and passive hepatitis B immunisation in infants and follow up in line with the guidance below:

- Hepatitis B antenatal screening and newborn immunisation programme: best practice guidance
- Immunisation against infectious disease (the Green book)
- Hepatitis B and C: ways to promote and offer testing. NICE public health guidance 43 (2012)
- Reducing differences in the uptake of immunisations. NICE public health guidance 21 (2009).

1.5.43 Advise women that there is no risk of transmitting HBV to their babies through breastfeeding if guidance on hepatitis B immunisation has been followed, and that they may continue antiviral treatment while they are breastfeeding.

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Adults who are co-infected with hepatitis C

1.5.44 Offer peginterferon alfa and ribavirin in adults co-infected with chronic hepatitis B and C[8].

Adults who are co-infected with hepatitis D

1.5.45 Offer a 48-week course of peginterferon alfa-2a in people co-infected with chronic hepatitis B and hepatitis delta infection who have evidence of significant fibrosis (METAVIR stage greater than or equal to F2 or Ishak stage greater than or equal to 3)[6].

1.5.46 Consider stopping peginterferon alfa-2a if there is no decrease in HDV RNA after 6 months to 1 year of treatment. Otherwise, continue treatment and re-evaluate treatment response annually.

1.5.47 Stop treatment after HBsAg seroconversion.

Prophylactic treatment during immunosuppressive therapy

1.5.48 Perform the following tests in people who are HBsAg and/or anti-HBc positive before starting immunosuppressive therapy for autoimmune or atopic diseases, chemotherapy, bone marrow or solid organ transplantation:

- antibody to hepatitis B surface antigen (anti-HBs)
- plasma or serum HBV DNA level
- ALT.

1.5.49 In people who are HBsAg positive and have HBV DNA greater than 2000 IU/ml, offer prophylaxis with entecavir or tenofovir disoproxil[12].

- Start prophylaxis before beginning immunosuppressive therapy and continue for a minimum of 6 months after HBeAg seroconversion and HBV DNA is undetectable.

1.5.50 In people who are HBsAg positive and have HBV DNA less than 2000 IU/ml, offer prophylaxis.

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- Consider lamivudine[12] if immunosuppressive therapy is expected to last less than 6 months.
  - Monitor HBV DNA monthly in people treated with lamivudine and change to tenofovir disoproxil if HBV DNA remains detectable after 3 months.
- Consider entecavir or tenofovir disoproxil[12] if immunosuppressive therapy is expected to last longer than 6 months.
- Start prophylaxis before beginning immunosuppressive therapy and continue for a minimum of 6 months after stopping immunosuppressive therapy.

1.5.51 In people who are HBsAg negative and anti-HBc positive (regardless of anti-HBs status) and are starting rituximab or other B cell-depleting therapies:

- offer prophylaxis with lamivudine[12]
- start prophylaxis before beginning immunosuppressive therapy and continue for a minimum of 6 months after stopping immunosuppressive therapy.

1.5.52 In people who are HBsAg negative, anti-HBc positive and anti-HBs negative and are not taking rituximab or other B cell-depleting therapies:

- monitor HBV DNA monthly and offer prophylaxis to people whose HBV DNA becomes detectable
  - consider lamivudine[12] in people with HBV DNA less than 2000 IU/ml and for whom immunosuppressive therapy is expected to last less than 6 months; change to tenofovir disoproxil if HBV DNA remains detectable after 6 months
  - consider entecavir or tenofovir disoproxil[12] in people with HBV DNA greater than 2000 IU/ml and for whom immunosuppressive therapy is expected to last longer than 6 months
  - continue antiviral therapy for a minimum of 6 months after stopping immunosuppressive therapy.

1.5.53 Do not offer prophylaxis to people who are HBsAg negative and anti-HBc and anti-HBs positive who are not taking rituximab or other B cell-depleting therapies.

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1.6 Monitoring

Monitoring in people who do not meet criteria for antiviral treatment

Further information on the progression of chronic hepatitis B can be found in the Introduction (see Figure 1).

Adults with HBeAg-positive disease in the immune-tolerant and immune clearance phases

1.6.1 Monitor ALT levels every 24 weeks in adults with HBeAg-positive disease who are in the immune-tolerant phase (defined by active viral replication and normal ALT levels [less than 30 IU/L in males and less than 19 IU/L in females]).

1.6.2 Monitor ALT every 12 weeks on at least 3 consecutive occasions if there is an increase in ALT levels.

Adults with inactive chronic hepatitis B (immune-control phase)

1.6.3 Monitor ALT and HBV DNA levels every 48 weeks in adults with inactive chronic hepatitis B infection (defined as HBeAg negative on 2 consecutive tests with normal ALT [less than 30 IU/L in males and less than 19 IU/L in females] and HBV DNA less than 2000 IU/ml).

- Consider monitoring more frequently (for example, every 12–24 weeks) in people with cirrhosis who have undetectable HBV DNA.

Children and young people

1.6.4 Monitor ALT levels every 24 weeks in children and young people with HBeAg-positive disease who have normal ALT levels (less than 30 IU/L for males and less than 19 IU/L for females) and no evidence of significant fibrosis (METAVIR stage less than F2 or Ishak stage less than 3).

1.6.5 Review annually children and young people with HBeAg-negative disease who have normal ALT (less than 30 IU/L for males and less than 19 IU/L for

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females), no evidence of significant fibrosis (METAVIR stage less than F2 or Ishak stage less than 3) and HBV DNA less than 2000 IU/ml.

1.6.6 Review every 12 weeks children and young people with HBeAg-negative disease who have abnormal ALT (greater than or equal to 30 IU/L for males and greater than or equal to 19 IU/L for females) and HBV DNA greater than 2000 IU/ml.

Children, young people and adults with HBeAg or HBsAg seroconversion after antiviral treatment

1.6.7 In people with HBeAg seroconversion after antiviral treatment, monitor HBeAg, anti-HBe, HBV DNA level and liver function at 4, 12 and 24 weeks after HBeAg seroconversion and then every 6 months.

1.6.8 Monitor HBsAg and anti-HBs annually in people with HBsAg seroconversion after antiviral treatment and discharge people who are anti-HBs positive on 2 consecutive tests.

Monitoring in people taking antiviral treatment

Children, young people and adults taking peginterferon alfa-2a

1.6.9 Review injection technique and adverse effects weekly during the first month of treatment in people taking peginterferon alfa-2a[10].

1.6.10 Monitor full blood count, liver function (including bilirubin, albumin and ALT), renal function (including urea and electrolyte levels) and thyroid function (and in children, weight and height) before starting peginterferon alfa-2a and 2, 4, 12, 24, 36 and 48 weeks after starting treatment to detect adverse effects[10].

1.6.11 Monitor HBV DNA and quantitative HBsAg levels and HBeAg status before starting peginterferon alfa-2a at 12, 24 and 48 weeks after starting treatment to determine treatment response[10].

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Children, young people and adults with compensated liver disease taking entecavir or lamivudine

1.6.12 Monitor full blood count, liver function (including bilirubin, albumin and ALT) and renal function (including urea and electrolyte levels) in people with compensated liver disease before starting entecavir or lamivudine, 4 weeks after starting treatment and then every 3 months to detect adverse effects[10].

1.6.13 Monitor HBV DNA and quantitative HBsAg levels and HBeAg status before starting entecavir or lamivudine, 12, 24 and 48 weeks after starting treatment and then every 6 months to determine treatment response and medicines adherence[10].

1.6.14 Monitor HBV DNA levels every 12 weeks in people with HBeAg-negative disease who have been taking lamivudine for 5 years or longer[10].

Children, young people and adults with compensated liver disease taking tenofovir disoproxil

1.6.15 Monitor full blood count, liver function (including bilirubin, albumin and ALT), renal function (including urea and electrolyte levels and urine protein/creatinine ratio), and phosphate levels in people with compensated liver disease before starting tenofovir disoproxil, 4 weeks after starting treatment and then every 3 months to detect adverse effects[10].

1.6.16 Monitor HBV DNA and quantitative HBsAg levels and HBeAg status before starting tenofovir disoproxil, 12, 24 and 48 weeks after starting treatment and then every 6 months to determine treatment response and medicines adherence[10].

Children, young people and adults with decompensated liver disease who are taking entecavir or lamivudine

1.6.17 Monitor full blood count, liver function (including bilirubin, albumin and ALT), renal function (including urea and electrolyte levels and urine protein/creatinine ratio), blood clotting, HBV DNA level and HBeAg status in people with decompensated liver disease before starting entecavir or lamivudine and

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weekly after starting treatment to assess treatment response and adverse effects. When the person is no longer decompensated, follow the recommendations in 'Children, young people and adults with compensated liver disease taking entecavir or lamivudine'[10].

Children, young people and adults with decompensated liver disease who are taking tenofovir disoproxil

1.6.18 Monitor full blood count, liver function (including bilirubin, albumin and ALT), renal function (including urea and electrolyte levels and urine protein/creatinine ratio) and phosphate, blood clotting, HBV DNA level and HBeAg status in people with decompensated liver disease before starting tenofovir disoproxil and weekly after starting treatment to assess treatment response and adverse effects. When the person is no longer decompensated, follow the recommendations in 'Children, young people and adults with compensated liver disease taking tenofovir disoproxil'[10].

1.7 Surveillance testing for hepatocellular carcinoma in adults with chronic hepatitis B

1.7.1 Perform 6-monthly surveillance for HCC by hepatic ultrasound and alpha-fetoprotein testing in people with significant fibrosis (METAVIR stage greater than or equal to F2 or Ishak stage greater than or equal to 3) or cirrhosis.

1.7.2 In people without significant fibrosis or cirrhosis (METAVIR stage less than F2 or Ishak stage less than 3), consider 6-monthly surveillance for HCC if the person is older than 40 years and has a family history of HCC and HBV DNA greater than or equal to 20,000 IU/ml.

1.7.3 Do not offer surveillance for HCC in people without significant fibrosis or cirrhosis (METAVIR stage less than F2 or Ishak stage less than 3) who have HBV DNA less than 20,000 IU/ml and are younger than 40 years.

[4] Adults with a transient elastography score greater than or equal to 11 kPa are very likely to have cirrhosis and confirmation by liver biopsy is not needed.

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[5] The degree of fibrosis cannot be accurately predicted in adults with a transient elastography score between 6 to 10 kPa. Some people may choose to have a liver biopsy in these circumstances to confirm the extent of liver disease.

[6] Adults with a transient elastography score less than 6 kPa are unlikely to have significant fibrosis.

[7] Adefovir dipivoxil and peginterferon alfa-2a for the treatment of chronic hepatitis B (NICE technology appraisal guidance 96), Entecavir for the treatment of chronic hepatitis B (NICE technology appraisal guidance 153), Telbivudine for the treatment of chronic hepatitis B (NICE technology appraisal guidance 154) and Tenofovir disoproxil fumarate for the treatment of hepatitis B (NICE technology appraisal guidance 173).

[8] Avoid use of peginterferon alfa-2a in pregnancy unless the potential benefit outweighs risk. Women of childbearing potential must use effective contraception throughout therapy.

[9] At the time of publication (June 2013), peginterferon alfa-2a did not have a U□ marketing authorisation for use in children for this indication. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. Informed consent should be obtained and documented. See the General Medical Council's Good practice in prescribing medicines □guidance for doctors for further information.

[10] At the time of publication (June 2013), peginterferon alfa-2a and entecavir did not have a U□ marketing authorisation for use in children for this indication, and tenofovir disoproxil did not have a U□ marketing authorisation for use in children younger than 12 years for this indication. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. Informed consent should be obtained and documented. See the General Medical Council's Good practice in prescribing medicines □guidance for doctors for further information.

[11] At the time of publication (June 2013), tenofovir disoproxil did not have a U□ marketing authorisation for this indication. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. Informed consent should be obtained and documented. See the General Medical Council's Good practice in prescribing medicines □guidance for doctors for further information.

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[12] At the time of publication (June 2013), entecavir, lamivudine and tenofovir disoproxil did not have a U□ marketing authorisation for this indication. The prescriber should follow relevant professional guidance, taking full responsibility for the decision. Informed consent should be obtained and documented. See the General Medical Council's Good practice in prescribing medicines □guidance for doctors for further information.

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2 Research recommendations

The Guideline Development Group has made the following recommendations for research, based on its review of evidence, to improve NICE guidance and patient care in the future.

2.1 Stopping antiviral treatment in HBeAg-negative disease

Further research should be undertaken to evaluate the clinical and cost effectiveness of hepatitis B surface antigen (HBsAg) quantitative assays in determining treatment duration in hepatitis B e antigen- (HBeAg) negative disease.

Why this is important

In HBeAg-positive disease, HBeAg seroconversion is a predictor of durable response to antiviral treatment and can be used as a milestone after which treatment can be stopped. At present, similar parameters have not been defined in HBeAg-negative disease. □uantitative HBsAg may have a role in determining treatment duration in this setting. Establishing threshold levels for HBsAg titre associated with durable off-treatment control in HBeAg-negative disease would transform current treatment strategies. People on long-term nucleoside or nucleotide analogues could safely stop treatment once they achieved a threshold level of HBsAg. Further research is needed to define these levels of HBsAg and to determine when treatment in HBeAg-negative disease can be safely stopped.

2.2 ALT values for children and young people

Further research should be undertaken to examine whether the upper limit of normal ALT values for adults (below 30 IU/L for males and below 19 IU/L for females) are appropriate for use in children and young people with chronic hepatitis B when making decisions on when to initiate treatment.

Why this is important

Recent studies have highlighted the imprecision of using biochemical activity as a measure of immune activity in children and young people with chronic hepatitis B. Researchers have found T-cell exhaustion and even HBV-specific immune responses in children and young people considered to have immune-tolerant disease. These findings need to be validated in larger

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studies to see if upper limit of normal ALT values derived from adults accurately reflect disease activity in children and young people. Further research is needed to investigate whether there is a genuine state of immune tolerance in children and young people reflected in lower levels of biochemical activity and a lower upper limit of normal ALT value.

2.3 Long-term safety of tenofovir disoproxil in chronic hepatitis B

Further research should be undertaken to determine the long-term safety of tenofovir disoproxil, including the risk of clinically significant hypophosphataemia and related bone toxicity, in people with chronic hepatitis B. The cost effectiveness of routine monitoring for phosphate loss and bone disease in people with chronic hepatitis B who are receiving tenofovir disoproxil treatment needs further evaluation.

Why this is important

Tenofovir disoproxil is recommended as an option for treatment of people with chronic hepatitis B, and is typically prescribed for long-term use. □idney dysfunction has been reported in people treated with tenofovir disoproxil, including rare cases of proximal renal tubular dysfunction that appear related to long-term exposure but are not well understood. Adverse renal effects such as hypophosphataemia may have an impact on bone architecture which could result in clinical problems such as fragility fractures. Monitoring for phosphate loss and bone disease could have a role in preventing clinically significant bone problems in people with chronic hepatitis B receiving long-term tenofovir disoproxil. However, the cost effectiveness and clinical utility of routine monitoring needs to be established before recommendations can be made about its use.

2.4 Prophylactic treatment in people receiving immunosuppressive therapy

Further research should be undertaken to determine whether long-term use of mild immunosuppressive agents for autoimmune and allergic problems presents a risk for reactivation of HBV infection in people with previous or current chronic hepatitis B, including occult HBV infection. The cost effectiveness of routine tests for HBV in this population, including HBV DNA for occult HBV infection, and the need for prophylactic treatment with nucleoside or nucleotide analogues needs further evaluation.

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Why this is important

Reactivation of HBV may occur spontaneously or arise during immunosuppression. Solid organ transplantation, chemotherapy and immunosuppressive drugs used to treat autoimmune diseases are key causes of HBV reactivation. Antiviral agents can be used as prophylaxis to prevent reactivation of HBV infection in people receiving immunosuppressive therapy but the optimal treatment and duration of therapy are unknown. Decision-making and cost-effectiveness studies are needed to determine optimal screening strategies to identify people at risk of HBV reactivation. People with occult HBV (including people coming from high endemicity regions) might carry a low, but not negligible, risk of viral reactivation. Prospective studies are needed to assess the risk of HBV reactivation in people receiving mild immunosuppressants or biological treatment for autoimmune diseases, to identify risk factors that predict HBV reactivation in this population, and evaluate treatment or pre-emptive strategies using existing nucleoside and nucleotide analogues.

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3 Other information

3.1 Scope and how this guideline was developed

NICE guidelines are developed in accordance with a scope that defines what the guideline will and will not cover.

How this guideline was developed

NICE commissioned the National Clinical Guideline Centre to develop this guideline. The Centre established a Guideline Development Group (see section 4), which reviewed the evidence and developed the recommendations.

The methods and processes for developing NICE clinical guidelines are described in The guidelines manual.

3.2 Related NICE guidance

Details are correct at the time of consultation on the guideline (January 2013). Further information is available on the NICE website.

Published

General

- Patient experience in adult NHS services. NICE clinical guidance 138 (2012).
- Medicines adherence. NICE clinical guidance 76 (2011).
- Looked-after children and young people. NICE public health guidance 28 (2010)

Condition-specific

- Hepatitis B and C: ways to promote and offer testing to people at increased risk of infection. NICE public health guidance 43 (2012).
- Increasing the uptake of HIV testing among men who have sex with men. NICE public health guidance 34 (2011).

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- Increasing the uptake of HIV testing among black Africans in England. NICE public health guidance 33 (2011).
- Alcohol-use disorders. NICE clinical guideline 115 (2011).
- Reducing differences in the uptake of immunisations. NICE public health guidance 21 (2009).
- Tenofovir disoproxil fumarate for the treatment of hepatitis B. NICE technology appraisal guidance 173 (2009).
- Antenatal care. NICE clinical guideline 62 (2008).
- Entecavir for the treatment of chronic hepatitis B. NICE technology appraisal guidance 153 (2008).
- Telbivudine for the treatment of chronic hepatitis B. NICE technology appraisal guidance 154 (2008).
- Adefovir dipivoxil and peginterferon alfa-2a for the treatment of chronic hepatitis B.NICE technology appraisal guidance 96 (2006).
- Obesity. NICE clinical guideline 43 (2006).

□nder development

NICE is developing the following guidance (details available from the NICE website):

- Hepatitis C. NICE clinical guideline. Publication date to be confirmed.

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Changes after publication

January 2014: A correction has been made to the units used for abnormal alanine aminotransferase (ALT) in men and women. The abnormal ALT levels should read greater than or equal to 30 IU/L for males and greater than or equal to 19 IU/L for females, not IU/ml. This has been changed in recommendations 1.3.6, 1.3.7, 1.3.10, 1.5.3□5, 1.5.31, 1.6.1, 1.6.3□6, and 2.2.

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About this guideline

NICE clinical guidelines are recommendations about the treatment and care of people with specific diseases and conditions in the NHS in England and Wales.

NICE guidelines are developed in accordance with a scope that defines what the guideline will and will not cover.

This guideline was developed by the National Clinical Guideline Centre, which is based at the Royal College of Physicians. The Centre worked with a Guideline Development Group, comprising healthcare professionals (including consultants, GPs and nurses), patients and carers, and technical staff, which reviewed the evidence and drafted the recommendations. The recommendations were finalised after public consultation.

The methods and processes for developing NICE clinical guidelines are described in The guidelines manual.

Update information

In this guideline, recommendations 1.5.13 to 1.5.15 update and replace recommendations 1.2 to 1.4 on the use of adefovir dipivoxil for treating chronic hepatitis B in Adefovir dipivoxil and peginterferon alfa-2a for the treatment of chronic hepatitis B (NICE technology appraisal guidance 96). NICE technology appraisal guidance 153, 154, 173 and recommendation 1.1 of NICE technology appraisal guidance 96 have been incorporated into this guideline and remain extant.

Strength of recommendations

Some recommendations can be made with more certainty than others. The Guideline Development Group makes a recommendation based on the trade-off between the benefits and harms of an intervention, taking into account the quality of the underpinning evidence. For some interventions, the Guideline Development Group is confident that, given the information it has looked at, most patients would choose the intervention. The wording used in the recommendations in this guideline denotes the certainty with which the recommendation is made (the strength of the recommendation).

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For all recommendations, NICE expects that there is discussion with the patient about the risks and benefits of the interventions, and their values and preferences. This discussion aims to help them to reach a fully informed decision (see also Patient-centred care).

Interventions that must (or must not) be used

We usually use 'must' or 'must not' only if there is a legal duty to apply the recommendation. Occasionally we use 'must' (or 'must not') if the consequences of not following the recommendation could be extremely serious or potentially life threatening.

Interventions that should (or should not) be used □ a 'strong' recommendation

We use 'offer' (and similar words such as 'refer' or 'advise') when we are confident that, for the vast majority of patients, an intervention will do more good than harm, and be cost effective. We use similar forms of words (for example, 'Do not offer□ ') when we are confident that an intervention will not be of benefit for most patients.

Interventions that could be used

We use 'consider' when we are confident that an intervention will do more good than harm for most patients, and be cost effective, but other options may be similarly cost effective. The choice of intervention, and whether or not to have the intervention at all, is more likely to depend on the patient's values and preferences than for a strong recommendation, and so the healthcare professional should spend more time considering and discussing the options with the patient.

Other versions of this guideline

The full guideline, 'Hepatitis B (chronic): Diagnosis and management of chronic hepatitis B in children, young people and adults' contains details of the methods and evidence used to develop the guideline. It is published by the National Clinical Guideline Centre.

The recommendations from this guideline have been incorporated into a NICE Pathway.

We have produced information for the public about this guideline.

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Implementation

Implementation tools and resources to help you put the guideline into practice are also available.

Your responsibility

This guidance represents the view of NICE, which was arrived at after careful consideration of the evidence available. Healthcare professionals are expected to take it fully into account when exercising their clinical judgement. However, the guidance does not override the individual responsibility of healthcare professionals to make decisions appropriate to the circumstances of the individual patient, in consultation with the patient and/or guardian or carer, and informed by the summaries of product characteristics of any drugs.

Implementation of this guidance is the responsibility of local commissioners and/or providers. Commissioners and providers are reminded that it is their responsibility to implement the guidance, in their local context, in light of their duties to have due regard to the need to eliminate unlawful discrimination, advance equality of opportunity and foster good relations. Nothing in this guidance should be interpreted in a way that would be inconsistent with compliance with those duties.

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SIGN 133 • Management of hepatitis C

A national clinical guideline

July 2013

Evidence

KEY TO EVIDENCE STATEMENTS AND GRADES OF RECOMMENDATIONS

LEVELS OF EVIDENCE	
1^{++}	High quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1^{+}	Well conducted meta-analyses, systematic reviews, or RCTs with a low risk of bias
1^{-}	Meta-analyses, systematic reviews, or RCTs with a high risk of bias
2^{++}	High quality systematic reviews of case control or cohort studies High quality case control or cohort studies with a very low risk of confounding or bias and a high probability that the relationship is causal
2^{+}	Well conducted case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is causal
2^{-}	Case control or cohort studies with a high risk of confounding or bias and a significant risk that the relationship is not causal
3	Non-analytic studies, eg case reports, case series
4	Expert opinion

GRADES OF RECOMMENDATION	
Note: The grade of recommendation relates to the strength of the evidence on which the recommendation is based. It does not reflect the clinical importance of the recommendation.	
A	At least one meta-analysis, systematic review, or RCT rated as 1^{++}, and directly applicable to the target population; or A body of evidence consisting principally of studies rated as 1^{+}, directly applicable to the target population, and demonstrating overall consistency of results
B	A body of evidence including studies rated as 2^{++}, directly applicable to the target population, and demonstrating overall consistency of results; *or* Extrapolated evidence from studies rated as 1^{++} or 1^{+}
C	A body of evidence including studies rated as 2^{+}, directly applicable to the target population and demonstrating overall consistency of results; *or* Extrapolated evidence from studies rated as 2^{++}
D	Evidence level 3 or 4; or Extrapolated evidence from studies rated as 2^{+}

GOOD PRACTICE POINTS	
✓	Recommended best practice based on the clinical experience of the guideline development group

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NHS Evidence has accredited the process used by **Scottish Intercollegiate Guidelines Network** to produce guidelines. Accreditation is applicable to guidance produced using the processes described in SIGN 50: a guideline developer's handbook, 2008 edition (**www.sign.ac.uk/guidelines/fulltext/50/index.html**). More information on accreditation can be viewed at **www.evidence.nhs.uk**

Healthcare Improvement Scotland (HIS) is committed to equality and diversity and assesses all its publications for likely impact on the six equality groups defined by age, disability, gender, race, religion/belief and sexual orientation.

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Scottish Intercollegiate Guidelines Network

Management of hepatitis C

A national clinical guideline

July 2013

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1 Introduction

1.1 THE NEED FOR A GUIDELINE

The hepatitis C virus (HCV) was first identified in 1989 and HCV infection has become a major health problem worldwide.[1] Approximately 0.8% of the Scottish population are thought to be chronically infected with HCV (around 37,500 individuals). The prevalence of infection varies between population groups, ranging from 50% in injecting drug users (IDU) to less than 0.04% among new blood donors.[1]

Up to 80% of patients infected with HCV become chronically infected and most of these patients will show evidence of chronic hepatitis.[2]

Hepatitis C is usually slowly progressive over a period of many years. Five to fifteen per cent of patients with chronic hepatitis may progress to liver cirrhosis over 20 years.[3] Four to nine per cent of patients with cirrhosis will develop liver failure; and two to five per cent of patients with cirrhosis will develop hepatocellular carcinoma (HCC) per annum.

In the UK the two major routes of transmission of HCV have been sharing of drug injecting equipment by IDU and transfusion of infected blood or blood products. Virus inactivation treatment of blood products began in 1987 and from 1991 blood has been screened for hepatitis C, virtually eliminating blood products as a source of HCV infection.

HCV infection can be effectively treated with combination drug therapy (pegylated interferon alfa and ribavirin) with sustained viral response (SVR) rates in 50-80% of patients. Although there are existing guidelines for the selection of patients for treatment[4-7] there are no national guidelines for screening, testing, diagnosis, service configuration, care during treatment or post-treatment follow up in adults or children.

1.1.1 UPDATING THE EVIDENCE

This guideline updates SIGN 92: Management of hepatitis C to reflect the most recent evidence around protease inhibitor treatment as an adjunct to current standard dual therapy treatment.

This update was limited in scope and covered treatment of chronic hepatitis C (CHC), provision of information for patients and carers and several other minor updates (*see section 1.2.3 and Annex 1*. In the sections not updated text and recommendations are reproduced verbatim from SIGN 92. The original supporting evidence was not re-appraised by the current guideline development group.

1.2 REMIT OF THE GUIDELINE

1.2.1 OVERALL OBJECTIVES

The guideline provides evidence based recommendations covering all stages of the patient care pathway; screening, testing, diagnosis, referral, treatment, care and follow up of infants, children and adults with, or exposed to, HCV infection. The remit encompasses prevention of secondary transmission of the virus but specifically excludes primary prevention of HCV infection. Primary prevention of hepatitis C infection is an important public health concern but is outwith the remit of this guideline. The principles and evidence for the prevention of blood borne viruses are generalisable and while reviewing this large body of evidence would have been beyond the capacity of the guideline development group, reviewing the HCV evidence alone would have produced a distorted view.

1.2.2 TARGET USERS OF THE GUIDELINE

This guideline will be of interest to all health professionals in primary and secondary care involved in the management of people with hepatitis C infection.

1.2.3 SUMMARY OF UPDATES TO THE GUIDELINE, BY SECTION

2	Key recommendations	New
4	Prevention of secondary transmission	Minor update at 4.1
6	Children and hepatitis C	Minor update at 6.4
8	Assessment of liver disease	Minor update at 8.2
10	Treatment of chronic hepatitis C	Completely revised at 10.1, 10.2, 10.3.1, 10.3.3, 10.3.4, 10.3.6, 10.6.2, 10.6.4, 10.6.10 and 10.7.1
11	Treatment of advanced infection	Minor update at 11.1.1
13	Provision of information	Completely revised
14	Implementing the guideline	Minor update

1.3 STATEMENT OF INTENT

This guideline is not intended to be construed or to serve as a standard of care. Standards of care are determined on the basis of all clinical data available for an individual case and are subject to change as scientific knowledge and technology advance and patterns of care evolve. Adherence to guideline recommendations will not ensure a successful outcome in every case, nor should they be construed as including all proper methods of care or excluding other acceptable methods of care aimed at the same results. The ultimate judgement must be made by the appropriate healthcare professional(s) responsible for clinical decisions regarding a particular clinical procedure or treatment plan. This judgement should only be arrived at following discussion of the options with the patient, covering the diagnostic and treatment choices available. It is advised, however, that significant departures from the national guideline or any local guidelines derived from it should be fully documented in the patient's case notes at the time the relevant decision is taken.

1.3.1 PRESCRIBING OF LICENSED MEDICINES OUTWITH THEIR MARKETING AUTHORISATION

Recommendations within this guideline are based on the best clinical evidence. Some recommendations may be for medicines prescribed outwith the marketing authorisation (MA) also known as product licence. This is known as 'off label' use.

Medicines may be prescribed off label in the following circumstances:

- for an indication not specified within the marketing authorisation
- for administration via a different route
- for administration of a different dose
- for a different patient population.

An unlicensed medicine is a medicine which does not have MA for medicinal use in humans.

Generally the off label use of medicines becomes necessary if the clinical need cannot be met by licensed medicines within the marketing authorisation. Such use should be supported by appropriate evidence and experience. [8]

"Prescribing medicines outside the conditions of their marketing authorisation alters (and probably increases) the prescribers' professional responsibility and potential liability". [8]

The General Medical Council (GMC) recommends that when prescribing a medicine off-label, doctors should:

- be satisfied that such use would better serve the patient's needs than an authorised alternative (if one exists)
- be satisfied that there is sufficient evidence/experience of using the medicines to show its safety and efficacy, seeking the necessary information from appropriate sources
- record in the patient's clinical notes the medicine prescribed and, when not following common practice, the reasons for the choice
- take responsibility for prescribing the medicine and for overseeing the patient's care, including monitoring the effects of the medicine.

Non-medical prescribers should ensure that they are familiar with the legislative framework and their own professional prescribing standards.

Prior to any prescribing, the licensing status of a medication should be checked in the current version of the British National Formulary (BNF). [8] The prescriber must be competent, operate within the professional code of ethics of their statutory bodies and the prescribing practices of their employers. [9]

1.3.2 ADDITIONAL ADVICE TO NHSSCOTLAND FROM HEALTHCARE IMPROVEMENT SCOTLAND AND THE SCOTTISH MEDICINES CONSORTIUM

Healthcare Improvement Scotland processes multiple technology appraisals (MTAs) for NHSScotland that have been produced by the National Institute for Health and Care Excellence (NICE) in England and Wales.

The Scottish Medicines Consortium (SMC) provides advice to NHS Boards and their Area Drug and Therapeutics Committees about the status of all newly licensed medicines and any major new indications for established products.

SMC advice relevant to this guideline is summarised in section 14.

2 Key recommendations

The following recommendations were highlighted by the guideline development group as the key clinical recommendations that should be prioritised for implementation. The grade of recommendation relates to the strength of the supporting evidence on which the recommendation is based. It does not reflect the clinical importance of the recommendation.

2.1 TREATMENT OF CHRONIC HEPATITIS C

A **All treatment-naive patients infected with HCV genotype 1 should be considered for treatment with pegylated IFN and weight-based ribavirin with the addition of a protease inhibitor as triple therapy.**

A **All treatment-experienced patients infected with HCV genotype 1 should be considered for treatment with pegylated IFN and weight-based ribavirin with the addition of a protease inhibitor as triple therapy.**

B **Treatment-naive patients co-infected with HIV and HCV genotype 1 who are unsuitable for treatment with a regimen which includes HCV protease inhibitors should be considered for treatment with pegylated IFN and weight-based ribavirin for 48-72 weeks depending on viral response.**

The recent availability of protease inhibitors to use with pegylated IFN and ribavirin in triple therapy, has significantly improved the SVR rates for patients with genotype 1 HCV infection with the prospect of reducing the overall length of treatment depending on response to therapy. Genotype 2 and 3 HCV infected patients continue to achieve high SVR rates with pegylated IFN and ribavirin dual therapy. These combination treatments should be discussed and offered as standard of care to all suitable patients with HCV infection.

2.2 CHILDREN AND HEPATITIS C

A **Children infected with all genotypes of hepatitis C with evidence of moderate or severe liver disease should be considered for treatment with pegylated IFN and ribavirin.**

B **Children infected with HCV genotypes 2 and 3 should be considered for treatment with pegylated IFN and ribavirin irrespective of disease stage.**

C **In children with mild disease and infection with other genotypes, benefits of treatment need to be balanced against risks of side effects.**

The outcome in children after treatment with pegylated IFN and ribavirin is equivalent to that in adults.[10] Side effects of treatment are seen with similar frequency and weekly injections cause distress. The advantages of achieving SVR early in life, eliminating the risk of onward transmission (particularly before girls reach child bearing age) and before the onset of chronic liver disease, will outweigh these disadvantages in many children infected with favourable genotypes. However, for those with less favourable genotypes and no evidence of chronic liver disease, it is appropriate to wait until more effective and acceptable treatment becomes available.

2.3 TESTING

D **Dried blood spot testing should be considered as a convenient and cost-effective method of accessing some target populations.**

D **There should be consideration given to methods to raise awareness and highlight information regarding hepatitis C amongst at-risk groups and the general public. The targeting of awareness campaigns to particular audiences is recommended. Staff should have access to appropriate training.**

D **Anyone who has a negative test but remains at risk of infection should be offered further testing on an annual basis.**

D **Testing for HCV should be offered to migrants from countries with a medium or high prevalence of HCV.**

The NICE guidance *Hepatitis B and C: Ways to promote and offer testing to people at increased risk of infection* [11] contains a number of key recommendations on testing. Although designed for the NHS in England and Wales it has direct applicability to Scotland on testing for HCV.

3 Testing

3.1 CLINICAL AND COST-EFFECTIVE TESTING FOR HEPATITIS C VIRUS

National and international guidelines recommend that individuals who have an excess risk of being infected and might benefit from knowing their HCV status should be offered an HCV test.[5, 12-14] This recommendation is based primarily on the need to diagnose an often silent infection, allowing the initiation of prompt antiviral treatment if appropriate.[15] Since treatment cannot be offered unless a diagnosis of chronic HCV infection is made, the offering, and uptake, of testing among populations at risk of HCV will convey a degree of clinical benefit. **4**

Further benefits of diagnosing people infected with HCV include the opportunity to convey information aimed at slowing the rate of HCV disease progression (such as advice about the dangers of excess alcohol consumption) and reducing the chances of infection being transmitted to others. No robust, consistent evidence to indicate the effectiveness of these interventions was identified.

UK guidelines consistently recommend that people who may convey an HCV risk to patients in the healthcare setting should undergo HCV testing.[5, 12-14] Several instances of healthcare worker to patient and blood/organ donor to recipient transmission of HCV have been recorded.[16, 17] **4**

Controlled trials or cohort studies to gauge the cost effectiveness of offering an HCV test to different population groups have not been undertaken. Limited evidence from economic modelling work indicates that offering an HCV test to former injecting drug users (IDU) in drug treatment and perhaps other settings would convey cost-effective clinical benefits.[18] Former IDU are more likely to have a higher prevalence of HCV and comply with therapy than current IDU. Models of best practice for the identification and testing of former IDU have not been developed and evaluated. Expert opinion suggests that general practices, particularly those that serve areas with a high prevalence of drug use, may constitute environments where focused, well supported testing initiatives might be successful. Prisons may also offer similar opportunities.[19] Targeted and generalised HCV awareness/testing campaigns have been conducted but no evaluations of their success in encouraging people (including former IDU) at high risk of HCV to engage with services have been reported. **4**

In populations where prevalence of HCV is low (eg genitourinary medicine clinic attendees), economic modelling indicates that universal testing does not convey cost-effective clinical benefit.[18] **4**

D **The following groups should be tested for HCV:**

- **blood/tissue donors**
- **patients on haemodialysis**
- **healthcare professionals who intend to pursue a career in a specialty that requires them to perform exposure prone procedures.**

D **The following groups should be offered an HCV test:**

- **patients with an otherwise unexplained persistently elevated alanine aminotransferase**
- **people with a history of injecting drug use**
- **people who are human immunodeficiency virus (HIV) positive**
- **recipients of blood clotting factor concentrates prior to 1987**
- **recipients of blood and blood components before September 1991 and organ/tissue transplants in the UK before 1992**
- **children whose mother is known to be infected with HCV**
- **healthcare professionals following percutaneous or mucous membrane exposure to blood which is, or is suspected to be, infected with HCV**
- **people who have received medical or dental treatment in countries where HCV is common and infection control may be poor**
- **people who have had tattoos or body piercing in circumstances where infection control procedure is, or is suspected to be, suboptimal**
- **people who have had a sexual partner or household contact who is HCV infected.**

The NICE guidance *Hepatitis B and C: Ways to promote and offer testing to people at increased risk of infection* [11] contains a number of key recommendations on testing. Although designed for the NHS in England and Wales it has direct applicability to Scotland on testing for HCV. | **4**

D **Dried blood spot testing should be considered as a convenient and cost-effective method of accessing some target populations.**

D **There should be consideration given to methods to raise awareness and highlight information regarding hepatitis C amongst at-risk groups and the general public. The targeting of awareness campaigns to particular audiences is recommended. Staff should have access to appropriate training.**

D **Anyone who has a negative test but remains at risk of infection should be offered further testing on an annual basis.**

D **Testing for HCV should be offered to migrants from countries with a medium or high prevalence of HCV.**

3.2 HEPATITIS C VIRUS DIAGNOSTIC TESTING

3.2.1 PRINCIPLES OF TESTING

Detection of viral ribonucleic acid (RNA) by nucleic acid tests (NAT, usually using reverse transcriptase polymerase chain reaction; RT PCR) indicates current infection. Detection of antibodies indicates resolved or current infection. The testing algorithm suggested in Figure 1 is based on:

Diagnostic assays are most reliable when used on plasma or serum.[20] | **2++**

Assays for antibody in saliva are very sensitive if optimum salivary collection devices and modified enzyme linked immunosorbent assays (ELISA) are used, but NAT for viral RNA is unreliable.[20-22] | **2++**

Nucleic acid testing sensitive enough to detect 50-100 IU/ml of virus must be performed to detect current infection.[23] | **2+**

Viral RNA can be detected as early as one to two weeks after infection, whereas antibody can be detected at seven to eight weeks after infection.[24] | **4**

Antibody to infection may not be generated, particularly if the individual is immune-suppressed.[25] | **4**

Following acute infection, HCV RNA may oscillate between positive and negative for several months. Results from samples taken at this time may be misleading.[24] In an individual positive for HCV antibody, but negative for HCV RNA, a second sample should be tested to confirm the initial diagnosis, especially as the date of infection is unknown in most cases. **4**

Individuals with a positive HCV antibody test and repeatedly negative RNA do not require further active management of hepatitis C infection.[25] **4**

Since hepatitis C is a serious communicable disease, after an initial laboratory diagnosis, a second sample should be taken from the patient to confirm correct identification of the original sample.[26] **4**

Genotyping of individuals with proven HCV infection is required to determine likely response to treatment. Those with genotype 1 infection require longer duration of treatment than those with genotype 2 and 3 (*see section 10.2.1*).[27] **1++**

Expert guidance suggests that healthcare professionals who have, or might have, sustained an occupational exposure to HCV should be offered RNA testing at 6, 12 and 24 weeks, with anti-HCV testing at 12 and 24 weeks.[28] **4**

B **Diagnostic testing for HCV should be performed on serum or plasma where possible.**

D **HCV genotyping should be undertaken if antiviral therapy is being considered.**

D **Following an isolated acute percutaneous exposure to blood infected, or strongly suspected of being infected, with HCV, healthcare professionals should be offered HCV RNA testing at six, 12 and 24 weeks and anti-HCV testing at 12 and 24 weeks.**

4 Prevention of secondary transmission

Secondary transmission is defined as the onward transmission of infection from individuals who are known to be HCV infected.

4.1 TRANSMISSION THROUGH SEXUAL AND HOUSEHOLD CONTACT

Observational studies indicate that there is a very small risk of people with diagnosed HCV infection transmitting infection to their family, or close contacts, and sexual partners. Cohort studies of couples discordant for HCV indicated an HCV incidence of 0-2 per 1,000 years of sexual contact.[29-31] Those with HIV co-infection, particularly men who have sex with men, may be more likely to transmit HCV to their sexual partners.[32, 33] The findings suggest that transmission may occur through exposure to blood as a consequence of, for example, the sharing of razors and toothbrushes (ie activities which might result in percutaneous or mucous membrane exposure to infected blood), and through unprotected sexual intercourse. **2+ 1-**

No studies were identified to ascertain if interventions such as educational initiatives, including the promotion of condom use, aimed at people diagnosed with HCV infection, are effective in reducing the frequency of such risk behaviours and/or preventing associated secondary transmission of HCV. Expert opinion suggests that people infected with HCV should be advised that the use of condoms and the avoidance of activities which could lead to percutaneous or mucous membrane exposure to infected blood will eliminate the albeit very small risk of them transmitting the virus to others.[14, 34] **4**

✓ After being advised of the low risk of HCV being transmitted sexually, individuals infected with HCV should be asked to consider using condoms for sexual intercourse if they are men who have sex with men or if either partner is infected with HIV.

D **Individuals co-infected with HIV/HCV should be advised always to practice safe sex and use condoms.**

D **Individuals infected with HCV should be advised to avoid activities which could result in percutaneous or mucous membrane exposure to their infected blood, such as the sharing of razors and toothbrushes.**

4.2 TRANSMISSION THROUGH INJECTING DRUG USE

The sharing of injecting equipment by drug users is the principal means through which infection is transmitted in developed countries.[14, 34] Observational data demonstrate that interventions such as needle and syringe exchange and methadone maintenance therapy are likely to have reduced, though not controlled, HCV transmission among IDU in a number of countries including Scotland.[35] Studies of interventions aimed specifically at preventing IDU known to be infected with HCV transmitting their infection to others through the sharing of injecting equipment, were not identified. **2+**

No robust consistent evidence on the influence of knowledge of HCV infection status among IDU on their injecting risk behaviour was identified. Expert opinion suggests that advising current IDU with chronic HCV on how to prevent transmission of their infection to other IDU, through for example safe injecting practice, may be an effective intervention.[14,34] **4**

D **Injecting drug users known to be infected with HCV should be given advice on how they can prevent transmission of infection to other injecting drug users.**

4.3 TRANSMISSION BETWEEN HEALTHCARE PROFESSIONALS AND PATIENTS

4.3.1 RISK OF HEALTHCARE PROFESSIONAL INFECTION

Expert opinion suggests that infection control precautions should be standard and universal and not determined by knowledge of patients' blood borne virus status.[36] **4**

Estimates of transmission risk following needlestick injury vary, with one large prospective study of 4,403 exposed healthcare professionals finding an overall transmission rate of 0.31%, whilst a review of 25 smaller studies reported a combined rate of 1.9% from 2,357 exposures.[16, 37] The relative risk is higher when injuries are deep and from blood filled needles. Risk arising from superficial or mucocutatenous exposures is likely to be much lower, though difficult to quantify, while transmission from solid needles is extremely unlikely.[37] Transmission occurs only from RNA positive sources. **2+ 4**

✓ Standard infection control precautions against blood borne virus transmission should be undertaken by all healthcare professionals regardless of the patient's known or suspected infective status.

✓ Healthcare professionals sustaining needlestick injuries from HCV infected sources should be advised that:

- the overall risk of transmission is probably less than 2% and may be much lower
- the risk is higher from deep injuries and from blood filled needles
- transmission from solid needles is very unlikely.

4.3.2 RISK OF PATIENT INFECTION

Several reports have shown that HCV can be transmitted from healthcare professionals to patients.[17] Most of these occurred after exposure-prone procedures usually after deep-cavity surgery. Estimates of transmission rates to patients in two retrospective analyses involving infected cardiothoracic surgeons were 2.3% and 0.36%, whilst the risk of transmission from an infected gynaecologist was only 0.04%.[38-40] UK health departments advise that healthcare professionals who are HCV RNA positive should not undertake exposure prone procedures.[17, 41] **3 4**

D **Healthcare professionals who are aware they are HCV RNA positive should not undertake exposure-prone procedures.**

5 Referral

Referral to specialist care should be considered for all patients with active HCV infection (HCV RNA positive) and not be restricted to potential candidates for antiviral therapy. Specialist clinics are often a source of information for patients and relatives, including health promotion and methods of avoiding secondary transmission of the virus.

Modelling suggests that 90% of individuals with HCV in Scotland are current or former IDU.[1] Factors associated with injecting drug use (eg poverty, chaotic lifestyle, comorbidity, including alcohol dependence) can be obstacles to individuals navigating their way through and remaining in investigation, referral and treatment pathways.[15, 19] Expert consensus suggests that uptake of services may be improved by integrated multidisciplinary care which also addresses, for example, individuals' alcohol and drug use problems simultaneously with their HCV specialist care.[15] **4**

No evidence was identified supporting the view that investigation and treatment of current IDU with HCV infection should not be promoted because they are unlikely to have progressed to at least moderate hepatitis, or are unlikely to adhere to such care.

Two observational studies and one five-year follow-up study have shown that IDU, described as active at the time of enrolment and undergoing management of their drug problem, adhered to antiviral treatment to the same degree as those who had never injected drugs.[42-44] These studies were small and no details of participants' injecting behaviour were provided. **2**

All patients with acute HCV should be referred to specialist care immediately as treatment given during the acute phase is more likely to be successful (*see section 7.3*).[45] **1++**

Ideally the specialist clinic should be integrated with other services by means of outreach clinics so that the patient journey is seamless, especially for those who find it difficult to access medical care. Such integration should encourage agencies such as drug problems services and prison medical services to positively and repeatedly address the issue of HCV infection.

D **Individuals, including injecting drug users, diagnosed with chronic HCV should be offered integrated multidisciplinary care as it can maximise their uptake of, and retention in, services.**

A **Patients with acute HCV infection should be referred to specialist care immediately.**

✓ Current injecting drug users infected with HCV should not be excluded from consideration for HCV clinical management, including antiviral therapy, on the basis of their injecting status.

✓ All patients should be referred to a setting that periodically reassesses the state of infection and the progression of liver disease, to determine if further interventions or therapies are needed.

6 Children and hepatitis C

6.1 MOTHER TO CHILD TRANSMISSION

Pregnant women who are HCV RNA negative do not pose a risk of transmission to their child.[46,47] 2+

The risk of women who are HCV infected and RNA positive transmitting infection to their babies in utero or during parturition is approximately 5%; the rate is twice as high for those co-infected with HIV.[48] The baby's risk of acquiring HCV from a mother infected with HCV is not increased by mode of delivery or breast feeding.[48] One prospective study has indicated that fetal scalp monitoring may increase the risk of mother to child transmission.[49] A large retrospective study did not demonstrate any excess risk.[48] Vaginal delivery may increase the risk of HCV transmission if the mother is co-infected with detectable HIV viral load.[48] 2++

B **In pregnant women knowledge of HCV RNA positive status should not influence obstetric management or standard advice regarding breast feeding.**

6.2 HCV TESTING IN CHILDREN AND INFANTS

The aim of testing infants born to women with hepatitis C is not primarily to identify all children to whom the virus has been transmitted, but to identify those at risk of persistent infection and its long term consequences.

Infants born to women who are HCV antibody positive will test positive for HCV antibody at birth.[50] Infants who are not infected become negative for HCV antibody between six and 20 months of age. Around 80% will be negative by 12 months of age.[46, 51] Positive results for viral RNA by NAT may be obtained in the early months of life in children who subsequently become negative and lose HCV antibody.[51-54] Some infected infants may not become HCV RNA positive until 12 months of age or older.[54] One study indicates that the sensitivity of a positive reverse transcriptase polymerase chain reaction (RT PCR) result obtained on two occasions between two and six months of life in predicting infection is 81% (95% confidence interval; CI 58 to 97%).[55] 2+

Infants of mothers with HIV co-infection who are consistently positive for viral RNA may have negative HCV antibody tests between 12 and 18 months of age.[56] 3

B **Infants born to women who are HCV antibody positive and HCV RNA negative do not need to be tested.**

B **In children born to women infected with HCV, an HCV antibody test should be performed at 12 months of age or older to identify the minority of children who are infected.**

B **In children whose mothers are co-infected with HIV, and in infants found to be HCV antibody positive after 12 months, an HCV RNA test should be performed, and if positive, confirmed on a second sample.**

B **If information regarding the risk of HCV infection in an individual child is required before 12 months of age, an HCV RNA test and retest can be performed after two months of age. Further testing is still required to make a definitive diagnosis.**

6.3 NATURAL HISTORY OF HCV INFECTION IN CHILDREN

Cross-sectional studies indicate that 20-40% of children who are HCV antibody positive after 18 months of age have undetectable HCV RNA, suggesting spontaneous clearance.[57,58] In those with chronic infection who remain HCV RNA positive, subsequent spontaneous clearance is rare (3.5%).[59] **3**

Levels of alanine aminotransferase (ALT) twice the upper limit of normal are found in 50% of infected children.[59] **3**

D **Children infected with HCV should be monitored to identify the minority who are at risk of progressive fibrosis during childhood, and who may be candidates for treatment.**

✓ Children infected with HCV should be assessed clinically every 6-12 months, and have blood taken for tests of liver function. Those with clinical or ultrasound abnormalities, or with serum ALT persistently twice the upper limit of normal should be considered for liver biopsy.

6.4 TREATMENT OF CHILDREN WITH HEPATITIS C

Response rates to treatment in children are of a similar magnitude to, and show the same influences of genotype, as adults (*see section 10*).[60] Combination treatment with interferon (IFN) and ribavirin gives an overall SVR of 50-60%.[60-63] There is potential for effects on thyroid function and growth problems.[62, 63] **3**

Combination therapy with pegylated IFN and ribavirin is superior to pegylated interferon alone, and results in outcomes similar to that in adult studies (*see section 10*).[10] **1+**

Combination treatment with interferon and ribavirin gives an SVR rate of 80-93% in children with genotype 3 infection, but only 47-59% in those with genotype 1 disease, which is similar to those in adult studies.[10, 64, 65] **2+**

A **Children infected with all genotypes of hepatitis C with evidence of moderate or severe liver disease should be considered for treatment with pegylated IFN and ribavirin.**

B **Children infected with HCV genotypes 2 and 3 should be considered for treatment with pegylated INF and ribavirin irrespective of disease stage.**

C **In children with mild disease and infection with other genotypes, benefits of treatment need to be balanced against risks of side effects.**

✓ Treatment of children with genotype 1 disease using protease inhibitors should only be considered as part of a clinical trial.

✓ Children infected with HCV should be managed in consultation with a paediatric service with specialist expertise in hepatitis C.

7 Acute hepatitis C

7.1 NATURAL HISTORY

The incidence of hepatitis C is unknown but can be estimated from the prevalence of chronic hepatitis C (CHC).[66] Acute hepatitis C infection is usually asymptomatic.[67] The full clinical spectrum of acute hepatitis C symptoms can occur but is rare (<15% patients).[68] The mortality of acute hepatitis C is very low (0.1% or less) and chronic infection is the most common outcome.[67-69] **3**

Laboratory diagnosis should start with testing for anti-HCV but in early cases HCV RNA may be the only marker of infection (*see section 3.2*).[70] **2+**

Spontaneous recovery occurs in 30-50% of patients with symptomatic infection, usually within three months of diagnosis. This is most common in females with an icteric illness.[66, 67, 71, 72] **3**

D **Patients with acute hepatitis C virus infection require clinical and laboratory monitoring** (looking for spontaneous viral clearance) **for the initial three months following diagnosis as they will often have a self limiting illness.**

7.2 POST-EXPOSURE PROPHYLAXIS

No trials were identified that show whether or not immunoglobulin, IFN based therapies or antiviral agents are effective at preventing transmission when given immediately post-exposure. Two reviews which considered older studies of immunoglobulin did not establish efficacy and concluded that immunoglobulin and IFN based therapies are not recommended after HCV exposure.[28, 73] **4**

7.3 TREATMENT OF PATIENTS WITH ACUTE HEPATITIS C

7.3.1 TIMING OF TREATMENT

Most patients who spontaneously clear hepatitis C do so within 12 weeks of diagnosis.[66, 72] There are no data to suggest that delaying treatment from three to six months post-diagnosis compromises treatment response, whilst allowing for spontaneous clearance to occur.[45] Delaying treatment to one year post-acquisition compromises a sustained viral response.[45] **3** **1++**

D **Treatment should start between three and six months after diagnosis of acute hepatitis C, if the infection has not resolved spontaneously.**

7.3.2 CHOICE AND DURATION OF TREATMENT

Two systematic reviews examined the effectiveness of non-pegylated IFN for the treatment of patients with acute hepatitis C.[74, 75] In one study participants in the treatment groups had higher sustained viral response rates (62%) than those in untreated groups (12%).[74] A Cochrane review demonstrated that increasing the dose of non-pegylated IFN during the induction phase of treatment was associated with higher sustained viral response.[75] There are no data on the influence of genotype on response to treatment for acute hepatitis C infection. **1++**

No randomised controlled trials (RCTs) of pegylated IFN versus conventional IFN for patients with acute hepatitis C were identified. A case series treated 16 patients, who had not seroconverted by three months, with pegylated IFN alone for 24 weeks, and reported a sustained viral response of 94%.[66]

3

A **Patients with acute HCV infection should be treated with IFN therapy if the infection does not resolve spontaneously.**

D **Patients can be treated with either pegylated IFN or non-pegylated IFN.**

D **Patients with acute HCV infection should be treated with IFN therapy for 24 weeks irrespective of genotype.**

8 Assessment of liver disease

8.1 CLINICAL ASSESSMENT

Clinical assessment of the severity of liver disease in patients with chronic hepatitis C is inaccurate and tends to underestimate the severity of change seen on liver biopsy.[76] **3**

8.2 FIBROSIS MARKERS

Studies of non-invasive prediction of the severity of liver disease using combinations of clinical and biochemical scores have found that it may be possible to distinguish patients with cirrhosis from those with mild disease. Intermediate stages are not distinguishable.[77] **2++**

A systematic review demonstrated that surrogate markers of fibrosis either reflecting disordered liver function (alanine aminotransferase, platelets) or fibrosis metabolism (eg tissue inhibitor of matrix metalloproteinase 1, hyaluronic acid) cannot be used individually to predict fibrosis. In individual patients such markers used alone cannot differentiate the stage of fibrosis reliably. Used in panels they can determine whether an individual has high or low levels of fibrosis. The 14 studies in the systematic review used 10 different panels of markers, none of which was superior to any other in statistical comparisons. The tests were compared against the gold standard of liver biopsy as part of their validation, although liver biopsy may potentially be inaccurate due to sampling error. Comparison of surrogate markers and liver biopsy to clinical outcomes would be more relevant.[78] **2++**

B **Biochemical markers should not be used as an alternative to liver biopsy for staging of intermediate grades of fibrosis.**

B **Biochemical tests may be used as an alternative to liver biopsy to diagnose cirrhosis or to direct screening for complications of fibrosis.**

✓ Measurement of liver stiffness may be useful as a non-invasive assessment of liver fibrosis.

8.3 LIVER BIOPSY

A liver biopsy needs to be at least 25 mm long in order to report stage of fibrosis with 75% accuracy.[79] The mortality rate of liver biopsy is between 0.13 and 0.33% and the rate of significant morbidity is about 5.9%.[80] **3**

8.3.1 WHEN TO BIOPSY

Liver biopsy of patients with CHC infection can reveal additional diagnoses such as alcoholic liver disease or steatosis (10% patients) and may influence management decisions in five per cent of patients.[81] Repeat liver biopsies may be useful in identifying individuals for treatment; one third of patients with mild CHC show one stage of fibrosis progression on the Ishak scale (0-6) at a median of 30 months.[82] The frequency and timing of liver biopsy should be tailored to individual patients as progression of fibrosis is non-linear. **3**

Advanced fibrosis or cirrhosis on liver biopsy compared with milder disease predicts a modest reduction in SVR after antiviral therapy.[83] **1++**

Liver biopsy before and after successful antiviral therapy (median 20 months interval) has shown both improvement in fibrosis (277 out of 1,094 patients) and downgrading of stage in cirrhosis (75 out of 153 patients).[84] **1+**

D **Liver biopsy should be performed if there is concern about additional causes of liver disease.**

D **Repeat liver biopsies should be considered in patients with mild disease who remain untreated, if progression of liver fibrosis would influence the decision to opt for antiviral therapy.**

✓ In patients with congenital bleeding disorders liver biopsy should be performed in consultation with a haemophilia specialist.

8.3.2 BIOPSY AND GENOTYPE

The sustained viral response rate after pegylated IFN and ribavirin therapy for patients with genotype 2 and 3 infection is 76-82% and is 41-51% for patients with genotype 1 infection.[7] The UK Health Technology Assessment Centre has recommended that pre-treatment liver biopsy in patients with genotype 2 and 3 may not be required.[7]

4

D **Liver biopsy should not be considered an essential test prior to using antiviral therapy, especially in patients with genotype 2 and 3 disease.**

9 Progression of untreated disease

Chronic hepatitis C infection is associated with a significant risk of progression to cirrhosis and hepatocellular carcinoma (HCC).[3, 85] Quantifying the magnitude of risk of progression to cirrhosis and HCC with time is difficult as reported outcomes are strongly influenced by study design and the characteristics of the population sampled.[3, 85] **2++**

A systematic review of 57 studies (both cross-sectional and longitudinal) which included liver clinic, post-transfusion, blood donor and community based patients, calculated the following estimates for the risk of progressing to cirrhosis after 20 years:[3] **2++**

- liver clinic: 22% (95% CI 18 to 26%)
- post-transfusion: 24% (95% CI 11 to 37%)
- blood donor: 4% (95% CI 1 to 7%)
- community based: 7% (95% CI 4 to 10%).

Due to the selection biases inherent in the cross-sectional liver clinic data, the community based cohort studies may be the most representative of true disease progression at a population level. The community based cohorts indicate that in those who acquire HCV infection in young adulthood, less than 10% will develop cirrhosis within 20 years. Older age at HCV acquisition, male gender and heavy alcohol consumption were associated with more rapid disease progression.[3] **2++**

The mean time from HCV infection to the development of HCC also shows considerable variation between studies, ranging from nine to 31 years in one systematic review.[85] Virtually no cases of HCC occur during the first decade of HCV infection, most are detected after 20 years of infection.[85] **2++**

Patients with established HCV related cirrhosis have a seven per cent risk of developing HCC by five years follow up.[86,87] **2+**

Patients with established CHC related cirrhosis are also at risk of complications such as ascites, gastrointestinal bleeding and hepatic encephalopathy.[86,87] The cumulative probability of all forms of decompensation in cirrhotic patients who remained tumour free was 18% at five years in one study, with an overall five-year survival rate of 91%.[87] **2+**

9.1 AGE, GENDER AND ETHNICITY

Increasing age at time of infection with HCV is associated with more rapid progression of liver fibrosis and reduced time from infection to cirrhosis.[88-90] Age over 40 years at time of infection is particularly associated with more rapid progression.[89, 90] **3**

Three cohort studies reported that men infected with HCV are more likely to progress to advanced stages of hepatic fibrosis than women.[89,91,92] **3**

Variations in disease progression have been observed in patients of different race. Two cohort studies demonstrated that disease progressed less rapidly in African-American than non African-American patients.[93, 94] The likely rate of progression in these patients should be considered when deciding whether to proceed with antiviral therapy. **2+**

D **When estimating the likely rate of progression of liver disease age at infection, gender and ethnicity should be considered.**

9.2 BODY WEIGHT

Studies have identified body mass index (BMI) >25 as being associated with hepatic steatosis (*see section 12.1.3*).

9.3 TOBACCO SMOKING

Smoking is an independent risk factor for the progression of hepatic inflammation and fibrosis in patients with CHC.[95,96] No data were identified on the impact of stopping smoking. **3**

D **Patients with CHC should be advised that smoking tobacco can accelerate progression of liver disease.**

9.4 ALCOHOL

Heavy alcohol consumption in patients infected with CHC is associated with more severe liver disease including cirrhosis, endstage liver disease and HCC.[97, 98] Average alcohol intake of more than six UK units per day is associated with more rapid progression of liver fibrosis.[88, 89, 92] Even moderate amounts of alcohol (within government recommended guidelines) have been associated with increased liver fibrosis compared to those who abstain.[89, 99] **2+** **2++**

Patients who are aware of their HCV status are more likely to heed advice on reducing alcohol intake than those who perceive themselves to be uninfected.[100] **2++**

B **Patients with CHC should be advised that drinking alcohol** (even in moderation) **can accelerate progression of liver disease.**

9.5 ALANINE AMINOTRANSFERASE

Approximately 25% (range 10-40%) of patients with CHC have persistently normal serum alanine aminotransferase (PNALT). Such patients are more likely to be female and have mild disease.[101] Although there is a substantial overlap between patients with PNALT and patients with mild liver disease, the terms are not synonymous and the groups are regarded separately for treatment purposes (*see sections 10.3.1 and 10.3.3*). The definition of 'persistently normal' varies in the literature with ALT measurements made every two to three months for time periods ranging between six and 18 months.[101] Flares in ALT have been reported in 21.5% of patients after being normal for 12 months.[102] There is no association with hepatitis C genotype or viral load.[101] **1-** **2-**

Progression of liver fibrosis is slower in patients with PNALT than in patients with elevated ALT.[103] In patients with untreated mild liver disease the progression to moderate or severe disease during follow up of 5.6 years is 5% in patients with PNALT and 24% in patients with elevated ALT. **2++**

Routine liver biopsy is not believed to be indicated unless specific information is required in selected patients.[101] **1+**

D **When defining PNALT serum ALT measurement should be undertaken every two to three months to ensure that flares in ALT are not missed.**

✓ The duration of follow up to define PNALT should be 12 months.

✓ Liver biopsy should only be considered if there are clinical or other concerns about the individual patient.

9.6 HIV CO-INFECTION

There is an increased rate of progression to end-stage liver disease in patients with HIV and HCV co-infection compared to those with HCV mono-infection (relative risk; RR 6.14, 95% CI: 2.86 to 13.2).[104] Median time to cirrhosis in patients with co-infection is 26 years, compared to 38 years in those with HCV mono-infection.[104] Patients with HCV infection with mild immunodepression as a result of HIV also have more severe liver disease than those with HCV mono-infection.[105] There is a marked increase in liver related mortality in patients with CHC and HIV co-infection (RR 17.5).[106] **1+ 2+**

Effective anti-HIV therapy and the associated immune recovery may limit HCV liver disease progression.[107] **2++**

B **The increased rate of progression to decompensated liver disease in patients with HCV and HIV co-infection should prompt early consideration of antiviral therapy.**

9.7 CO-INFECTION WITH HEPATITIS A OR B VIRUSES

Vaccination against hepatitis A and B is recommended in people with HCV.[108] A consensus report on the treatment of hepatitis recommended vaccination for hepatitis B virus (HBV) but not hepatitis A.[109] One case study of patients with HCV who contracted hepatitis A reported a very high level of fulminant hepatitis.[110] **4**

Antibody response to hepatitis B vaccination is reduced in patients with chronic HCV.[111] **3**

D **Vaccination against hepatitis A and B should be considered for patients infected with hepatitis C.**

Patients who are infected with HCV who have serological evidence of current or past infection with HBV are more likely to have advanced liver disease.[91, 112, 113] **3**

D **When estimating the likely rate of progression of liver disease as a result of hepatitis C infection, active or previous hepatitis B virus infection should be considered.**

✓ Patients infected with HCV should be tested for evidence of active or previous HBV infection.

9.8 IRON STATUS

Patients with CHC can have elevated iron stores, but there is debate over whether this has any influence on the disease. Serum ferritin and transferrin saturation are increased in 20-60% of patients and correlate with serum ALT, suggesting they are markers of inflammation.There is a poor correlation with hepatic iron concentration (HIC).[114] HIC is rarely significantly elevated in pre-cirrhotic patients.Twenty to fifty per cent of patients with cirrhosis will have elevated HIC but this is also a common finding in patients with cirrhosis due to hepatitis B and alcoholic liver disease.[114] **4**

It is uncertain whether hepatic iron excess as a single factor has any influence on response to treatment with IFN alone.[114] **4**

No evidence was found that iron depletion (by venesection) has any influence on the virus or the activity of liver disease.[114] There is some evidence from four small RCTs that venesection on selected patients with markers of iron excess prior to IFN monotherapy may improve the SVR.[114, 115] **4 1-**

D **Modest iron loading does not justify specific intervention prior to antiviral therapy as it is unlikely to be of clinical importance.**

D **Patients with significant iron retention require further investigation for additional conditions known to result in iron overload.**

9.9 HCV GENOTYPE

No consistent link between HCV genotype and disease progression has been demonstrated in several cohort studies.[88, 91,92, 116,117] **2+**

9.10 CRYOGLOBULINAEMIA

A poor quality meta-analysis of the influence of cryoglobulinaemia suggested that cirrhosis is diagnosed more frequently in patients with cryoglobulinaemia.[118] **1-**

10 Treatment of chronic hepatitis C

10.1 ANTIVIRAL THERAPY

Several meta-analyses and systematic reviews confirm that a combination of pegylated IFN and ribavirin dual therapy is effective in treating patients with CHC, leading to high levels of sustained viral response (SVR).[7, 119-121] All patients should be considered as candidates for treatment. **1+**

A **All patients with chronic HCV infection should be considered for antiviral therapy.**

10.1.1 SUSTAINED VIRAL RESPONSE

Sustained viral response has become the accepted objective of treatment programmes for CHC and is currently achieved in 41-51% of patients with genotype 1 disease and 73-82% of patients with genotype 2 and 3 disease who have received a course of combination therapy with pegylated IFN and ribavirin.[122, 123] Data are available on long term outcomes after SVR but are limited in number, quality and length of follow up:

- viral relapse is uncommon after SVR (1-13% of patients)[124-126] **1+**
- mortality is reduced after SVR[127] **2+**
- patients with an SVR have a reduced risk of developing cirrhosis and primary hepatocellular carcinoma[124,128] **2++**
- occult hepatitis C may persist in macrophages, lymphocytes to hepatocytes in some patients who have achieved an SVR. There may be a small risk of future relapse in this event.[129,130] **3**

B **Sustained viral response should be used as a marker for viral clearance.**

An SVR of 80% is achieved in patients who take 80% of the dose of both pegylated IFN and ribavirin dual therapy for more than 80% of the duration. This compares with 33% in less adherent patients.[131] **2+**

10.2 TREATMENT VARIATION BY GENOTYPE

10.2.1 GENOTYPE 1 AND DURATION OF TREATMENT

A systematic review assessed adjunct treatment with the protease inhibitors boceprevir and telaprevir in patients with genotype 1 hepatitis C. In both treatment-naive and treatment-experienced patients, sustained viral response rates were achieved more often with the protease inhibitors boceprevir or telaprevir in combination as triple therapy with pegylated IFN and weight-based ribavirin compared with pegylated IFN and weight-based ribavirin alone. Both medications were well tolerated, with anaemia presenting as the most treatment-limiting adverse effect.[132] Figures 1 and 2 show treatment algorithms for patients with genotype 1 HCV infection. **1+**

Three RCTs reviewed in two systematic reviews found that telaprevir administered for at least 12 and up to 48 weeks in combination with pegylated IFN and weight-based ribavirin as triple therapy was effective in the treatment of previously untreated or treatment-experienced adults with genotype 1 chronic hepatitis C infection. The trials included treatment-experienced adults who were non-responders, partial responders and relapsers following pegylated IFN and weight-based ribavirin. Sustained viral responses were significantly better than pegylated IFN and weight-based ribavirin dual therapy alone.[133,134] **1+**

Two systematic reviews found that boceprevir administered for 24-44 weeks as triple therapy in combination with pegylated IFN and weight-based ribavirin for up to 48 weeks was effective in the treatment of previously untreated or treatment-experienced adults with genotype 1 chronic hepatitis C infection. These trials included treatment-experienced adults who were either partial responders or relapsers following pegylated IFN and weight-based ribavirin but did not include previous non-responders. Sustained virological responses were significantly better than with pegylated IFN and weight-based ribavirin dual therapy alone; 66% for fixed duration triple therapy versus 63% for response guided triple therapy versus 38% for control group receiving standard dual therapy.[134,135] **1+**

A regimen including boceprevir requires a four week lead-in of pegylated IFN and weight-based ribavirin, whereas this is not required for telaprevir. A lead-in with boceprevir, however, does not reduce SVR. The response to the lead-in period can indicate likelihood of SVR in both treatment-naive and experienced patients.

A **All treatment-naive patients infected with HCV genotype 1 should be considered for treatment with pegylated IFN and weight-based ribavirin with the addition of a protease inhibitor as triple therapy.**

A **All treatment-experienced patients infected with HCV genotype 1 should be considered for treatment with pegylated IFN and weight-based ribavirin with the addition of a protease inhibitor as triple therapy.**

✓ In treatment-experienced patients with a lower likelihood of SVR, benefits of treatment need to be weighed against potential risks and side effects.

A **Response-guided therapy can only be used in treatment-naive patients and previous treatment relapsers who are not cirrhotic.**

No direct comparison studies of boceprevir and telaprevir were identified, therefore neither drug can be recommended over the other.

No evidence was identified suggesting that a particular pegylated IFN should be used in combination with a particular protease inhibitor.

Figure 1: Algorithm for the use of protease inhibitors in treatment-naive HCV genotype 1 infected patients.

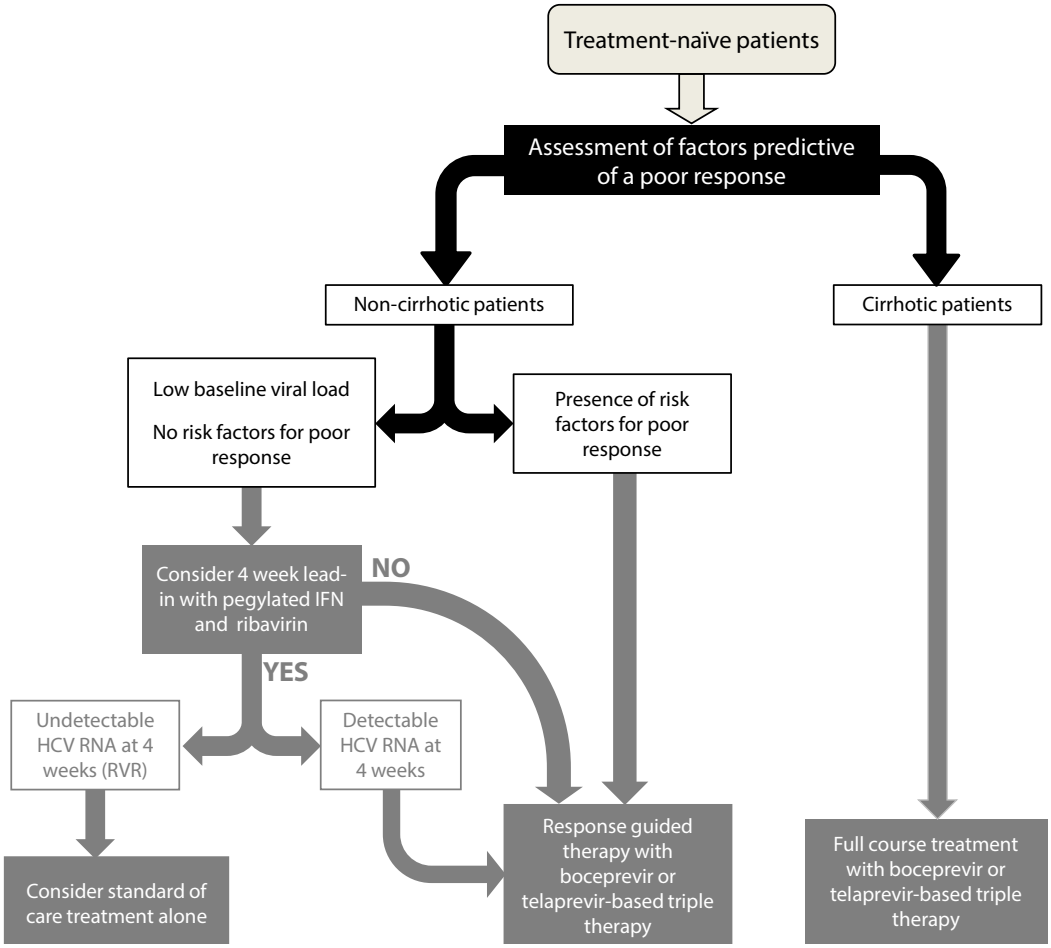

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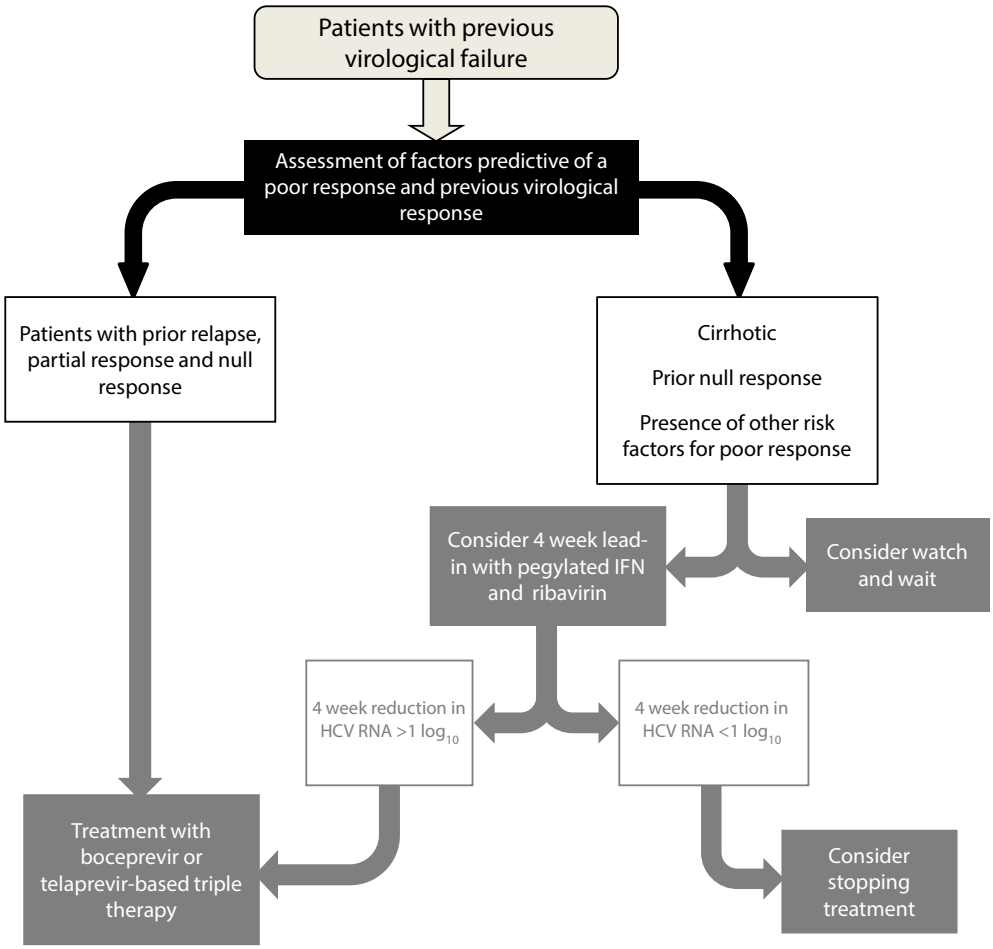
Figure 2: Algorithm for the use of protease inhibitors in HCV genotype 1 infected patients who have had prior viralogical failure on treatment.



Reprinted from Alimentary Pharmacology and Therapeutics 35, Ramachandran et al. UK consensus guidelines for the use of the protease inhibitors boceprevir and telaprevir in genotype 1 chronic hepatitis C infected patients (2012) with the permission of the authors under acknowledgement by John Wiley & Sons.

10.2.2 GENOTYPE 1 AND DURATION OF TREATMENT FOR PATIENTS UNSUITABLE FOR PROTEASE INHIBITORS

The optimal duration of treatment for patients with genotype 1 hepatitis C is 48 weeks.[7,119,120] **1++**

Patients with genotype 1 infection who fail to achieve an early viral response (EVR) at 12 weeks have a less than five per cent chance of achieving a SVR.[136] Of those genotype 1 patients who failed to achieve an EVR but continued on therapy and were still HCV RNA positive at 24 weeks, none had an SVR.[137] Following careful clinical assessment some patients infected with genotype 1 HCV may be considered unsuitable for treatment with a regimen which includes a protease inhibitor. This includes patients with potentially dangerous drug-drug interactions with prescribed or illicit medications, and those with co-morbid conditions that may impair adherence to therapy thus reducing the effectiveness of triple therapy and increasing the risk of developing drug resistance.[134] **1+**

The registration studies for telaprevir and boceprevir both demonstrate in post hoc analysis that patients with low viral loads, treated with pegylated INF and weight-based ribavirin and who achieve a rapid viral response (RVR), have the same SVR rate as those treated with the addition of a protease inhibitor.[138] A meta-analysis looked at shortened therapy for those treated with pegylated INF and weight-based ribavirin. Including all patients, relapse rates were significantly higher in patients with genotype 1 RVR if the treatment duration was reduced to 24 weeks with an overall reduction in SVR of -13.6% (95% CI -22.8% to -4.4%, p=0.004). If the viral load was less than 400,000 IU/ml there was no statistical significant reduction in SVR with 24 weeks of treatment with a mean difference of -3.1% (96% CI -8.6% to 2.4%). [138] **1+**

A
- **Patients with genotype 1 infection should be tested for an EVR at 12 weeks.**
- **Patients with genotype 1 infection who fail to achieve an EVR at 12 weeks should be considered for cessation of treatment.**
- **Patients with genotype 1 infection with an EVR at 12 weeks should continue treatment for 48 weeks. Those who are still HCV RNA positive at 24 weeks should discontinue treatment.**

B
Following informed discussion, treatment-naive patients with genotype 1 infection and:
- **minimal or no fibrosis**
- **low viral load** (less than 400,000 IU/ml)
- **who achieve an RVR following a lead in with pegylated IFN and weight-based ribavirin for four weeks can be considered for 24 weeks of treatment without the addition of a protease inhibitor.**

10.2.3 GENOTYPE 2 AND 3 AND DURATION OF TREATMENT

The optimal treatment for patients with genotype 2 and 3 infection is pegylated INF and weight-based ribavirin.[7, 119, 120] The primary outcome of one meta-analysis was comparison of SVR rates in patients with either genotype 2 or 3 infection. After 24 weeks of therapy, SVR rates were 74% and 69% respectively with an OR of 1.49 (95% CI 1.23 to 1.80) however the percentage difference was not significant (p=0.90). Among those with higher viral loads, the SVR rate in genotype 2 infection (75%) differed from genotype 3 infection (58%) with an OR of 2.36 (95% CI 1.80 to 3.09). The percentage difference of 24.9 % (95% CI 12.8 to 37.0; p=0.07) was not significant. In patients with low viral loads respective rates were 79% and 75% with an OR of 1.50 (95% CI 1.08 to 2.09) again with a non-significant difference (p=0.84). As a secondary outcome shortened therapy in patients with RVR treated for 12–16 or 24 weeks was analysed. SVR rates in genotype 2 infected patients were 83% and 84%, respectively, and in genotype 3 infected patients 84% and 86%. In patients without RVR treated for 24 weeks, the SVR was higher in those with genotype 2 infection, with a 17.8% weighted difference (95% CI: 8.7 to 27.0) and a pooled OR of 2.06 (95% CI 1.40 to 3.02). The authors concluded that 24 weeks of therapy should remain the standard duration for patients with genotype 2 or 3 infection. However, in patients who achieve an RVR, those with genotype 3 HCV respond to shortened treatment as well as those with genotype 2, irrespective of basal viraemia.[139] **1++**

The aim of a second meta-analysis was to determine the optimal length of treatment in patients with HCV genotypes 2 and 3. The results for these two genotypes were pooled. Pooled SVR data were higher for standard treatment in RCTs that randomised at baseline, with an RR of 0.88 (95% CI 0.76 to 1.01) favouring standard therapy over shortened therapy. The pooled proportion of SVR rates of RCTs that randomised at RVR were similar in the shortened treatment group (82%) and in the standard treatment (83%), with the pooled effect given by an RR of 1.00 (95% CI 0.92 to 1.09). The authors' conclusions should be treated with caution given the lack of detail about the methodology and a high level of heterogeneity between the included studies. The authors conclude that based on baseline characteristics all patients should be treated for 24 weeks with dual therapy. However patients who achieve an RVR at four weeks can have their therapy shortened to between 12-16 weeks of dual therapy with pegylated IFN and weight-based ribavirin. They found no difference between 12 or 16 weeks but numbers were small. Numbers of patients with cirrhosis were too small to allow any inference about that group. Weight-based ribavirin achieved higher SVRs than low fixed dose ribavirin.[140] **1+**

Evidence from two studies suggests that patients with genotype 2 or 3 infection who achieve a rapid viral response (HCV RNA negative) at four weeks can receive 12 or 16 weeks of pegylated IFN and weight-based ribavirin therapy with similar results to 24 weeks of treatment.[141, 142] **1+**

A
For patients with HCV genotype 2 or 3 standard treatment should be pegylated IFN and weight-based ribavirin for 24 weeks.

B
Non-cirrhotic patients, with genotype 2 or 3, who achieve an RVR at week 4 of therapy, could be considered for shortened duration of therapy of 12 to 16 weeks.

10.2.4 GENOTYPE 4, 5 AND 6 AND DURATION OF TREATMENT

The optimal duration of treatment for patients with genotype 4 hepatitis C is 48 weeks.[7, 119, 120] 1++

A meta-analysis of two large phase three/four prospective randomised clinical trials conducted in Belgium in patients with chronic hepatitis C (n = 1,073) compared the response to antiviral therapy of those with HCV genotype 5 with that of those with other HCV genotypes. The study found that genotype 5 infection responds to therapy in a similar way to genotype 1, however the genotype 5 population is older, more likely to have acquired infection by blood transfusion and more likely to have cirrhosis. The meta-analysis recommended treating patients with genotype 5 infection for 48 weeks with dual therapy.[143] 1+

A systematic review found very few studies of treatment of patients with HCV genotype 6 infection. Treatment with pegylated IFN and weight-based ribavirin is effective, with SVR rates around 70% after 48 weeks of therapy. SVR rates are similar in patients with HCV genotype 6 infection who receive 24 weeks of pegylated IFN and weight-based ribavirin, although additional studies are needed before recommending 24 weeks as the optimal treatment duration for these patients.[144] 1+

A **For patients with HCV genotype 4, 5 or 6 infection, standard treatment should be 48 weeks of pegylated IFN and weight-based ribavirin.**

10.3 PATIENT SUBGROUPS

10.3.1 PATIENTS WITH MILD CHRONIC HEPATITIS

In patients with mild CHC the efficacy and safety of non-pegylated IFN and ribavirin combination therapy is similar to that in other patients with hepatitis C. Liver biopsy to exclude patients with mild disease is therefore not required prior to considering antiviral treatment.[145] 1++

B **Patients with mild CHC should be considered for treatment.**

10.3.2 PATIENTS WITH CIRRHOSIS

See section 11.1.1 for information on patients with cirrhosis.

10.3.3 PATIENTS WITH PERSISTENTLY NORMAL ALT LEVELS

The efficacy and safety of pegylated IFN and ribavirin combination therapy in patients with CHC and persistently normal ALT level is similar to that seen in patients with elevated ALT levels (*see section 9.5*).[146] 1+

A **Patients with chronic hepatitis C and normal ALT should be considered for treatment.**

10.3.4 PATIENTS WITH HIV CO-INFECTION

Pegylated IFN and ribavirin for 48 weeks is effective in treating patients with HCV-HIV co-infection, leading to sustained viral response in 60% of patients with genotype 2 and 3 and 14-29% in patients with genotype 1. For patients with genotype 1 infection and low HCV viral load (<800,000 IU/ml), the sustained viral response rate is around 60%.[147-149] 1+

Ninety eight per cent of patients with HIV-HCV co-infection who did not have an EVR at week 12 did not achieve an SVR at week 48.[149] 1+

Patients co-infected with HIV and HCV genotypes 2 and 3 with undetectable HCV-RNA at week 4 did not benefit from prolonging therapy beyond 24 weeks.[150] 1+

Pegylated IFN plus weight-based ribavirin for the treatment of HCV was more effective than standard IFN plus weight-based ribavirin in achieving a sustained viralogical response in patients co-infected with HIV: 55% versus 26% overall, 46% versus 18% in genotype 1 and 4 and 71% versus 43% in genotype 2 and 3. The differences between cohorts were HCV-genotype dependant during the first 12 weeks of pegylated IFN therapy, regardless of HIV status. Among patients with HCV genotype 2 or 3, the study found a significantly sharper decrease in HCV RNA in HCV mono-infected patients compared with HCV-HIV co-infected patients 1+

only at week 4, whereas significant differences among patients with HCV genotype 1 were observed only after 12 weeks of therapy. Overall, longer duration of treatment (48-72 weeks) showed better treatment responses irrespective of genotype than shorter duration (24-48 weeks).[150-152] 1+

The expert opinion of the guideline development group was that recommendations could be made for treatment of patients co-infected with HIV and HCV genotype 1 by extrapolating from HCV genotype 1 mono-infected patient treatment regimens which include protease inhibitors.

A **All patients co-infected with HCV and HIV should be considered for HCV treatment.**

A **For patients with HCV genotype 1 infection and HIV, who do not achieve an EVR, treatment should be stopped.**

A
- **Co-infected non-genotype 1 patients who are considered suitable for treatment should be offered treatment with pegylated IFN and weight-based ribavirin for 48 weeks.**
- **Co-infected genotype 2 or 3 patients who achieve an RVR may be considered for 24 weeks of treatment.**

C **All patients co-infected with HIV and HCV genotype 1 should be considered for treatment with a regimen which includes an HCV protease inhibitor.**

B **Treatment-naive patients co-infected with HIV and HCV genotype 1 who are unsuitable for treatment with a regimen which includes HCV protease inhibitors should be considered for treatment with pegylated IFN and weight-based ribavirin for 48-72 weeks depending on viral response.**

✓ Genotype 1 co-infected patients who are not cured of their HCV infection should be monitored for disease progression.

10.3.5 PATIENTS WITH HEPATITIS B CO-INFECTION

Treatment outcomes with a combination of non-pegylated IFN and ribavirin in patients co-infected with chronic hepatitis B and C are similar to those achieved in patients with HCV mono-infection.[153,154] No trials were found examining pegylated IFN and ribavirin in patients co-infected with chronic hepatitis B and C. 2++

No evidence was identified to make a recommendation on treatment with protease inhibitors for patients who are genotype 1 and co-infected with hepatitis B as these patients were excluded from clinical trials.

C **Patients with chronic hepatitis B and C co-infection should be considered for treatment with pegylated IFN and weight-based ribavirin.**

✓ Patients with chronic hepatitis B and C genotype 1 co-infection could be considered for combination treatment with pegylated IFN and weight-based ribavirin with the addition of a protease inhibitor on an individual basis in centres with an expertise in management of hepatitis B.

10.3.6 PATIENTS IN DRUG TREATMENT PROGRAMMES

In patients with CHC who are on a stable drug treatment programme, management with a combination of pegylated IFN and ribavirin is effective, leading to high levels of sustained viral response. Whilst drop-out rates are higher than in other cohorts, the drop-outs occur early, within the first eight weeks. After eight weeks adherence to treatment is similar to other groups.[44,155] 2+

Recent studies suggest active drug users have comparable treatment outcomes to non-drug users.[156-158] 2+ 1+

B **Patients with CHC who are on a drug treatment programme should be considered for treatment.**

✓ Active drug users should be engaged in efforts to address their healthcare needs and in harm reduction.

✓ Active drug users should have a comprehensive assessment of their psychological needs and of their likely adherence to antiviral treatment.

10.4 FACTORS INFLUENCING EFFECTIVENESS

10.4.1 AGE, GENDER AND ETHNICITY

Antiviral therapy is less effective in patients over the age of 40 and men are less likely than women to achieve a sustained viral response.[7,119,120] Variations have been observed in the response of patients of different race to antiviral therapy. A meta-analysis of ethnic differences showed that patients of African-American or Hispanic origin had lower SVRs than Caucasian or Asian groups (16% and 24% versus 32% and 59% with genotype 1 achieved SVR).[159] **1+**

A **Patients should be advised that older age at the time of treatment leads to a lower sustained viral response.**

B **Patients should be advised about the likelihood of sustained viral response according to their ethnic origin.**

10.4.2 BODY WEIGHT

Three systematic reviews report that in patients with CHC whose weight is greater than 75 kg, treatment with a combination of pegylated IFN and ribavirin leads to a lower SVR than in patients weighing less than 75 kg.[7,119,120] Dosage of pegylated IFN and ribavirin in these studies was given at a cut-off point of 75 kg and not weight related, therefore caution should be taken when extrapolating results. Weight and diet are discussed in section 12. **1+**

10.4.3 ALCOHOL

Treatment studies in patients continuing to use alcohol are limited. Two cohort studies have shown that response rate to standard IFN treatment was inversely proportional to the amount of alcohol ingested.[160, 161] A six month abstinence from alcohol did not offset previous lifetime alcohol intake.[162] **2+**

✓ Patients should be advised that drinking alcohol (even in moderation) can reduce the response to treatment with pegylated IFN and ribavirin.

10.5 CONTRAINDICATIONS

10.5.1 PREGNANCY AND RISK OF PREGNANCY

There are no studies on the effects of antiviral therapy on human pregnancy. Studies in animals have shown that ribavirin therapy, at well below the recommended human dose, causes malformations in the fetus. The incidence and severity of the teratogenic effects increased with escalation of the ribavirin dose. Survival of the fetus and the offspring was reduced.[163] Further animal studies have shown abnormalities in sperm.[163]

There are no data on the use of pegylated IFN in pregnant women and it is not known whether pegylated IFN or ribavirin are excreted in human milk.

✓ Pegylated IFN and ribavirin must not be prescribed to women who are pregnant.

✓ Treatment with pegylated IFN and ribavirin should not be initiated until pregnancy has been excluded.

✓ Couples, with one partner receiving pegylated IFN and ribavirin, should use two forms of contraception during treatment and for six months after therapy has ended.

10.5.2 PATIENTS WITH RENAL FAILURE

Ribavirin causes a dose-dependent haemolytic anaemia and the degree of haemolysis is dependent on the severity of the renal failure.[164] Treatment with pegylated IFN monotherapy at a dose of 135 µg subcutaneously per week for patients on haemodialysis may be considered but patients need to be closely monitored.[165] **1+ 3**

D **Patients with CHC and renal failure may be treated with IFN monotherapy, with careful monitoring required.**

10.5.3 PATIENTS WITH MENTAL HEALTH PROBLEMS

Patients with mental health problems respond equally well to IFN and ribavirin therapy but their psychiatric symptoms should be managed carefully, particularly in the first four weeks of treatment.[166,167] **2+ 3**

B **Patients with stable mental health problems should not be excluded from treatment for CHC.**

B **Patients with mental health problems should have their psychiatric symptoms monitored prior to and throughout IFN treatment.**

✓ Formal psychiatric assessment should be considered for selected patients if necessary.

10.5.4 PATIENTS TAKING OTHER MEDICINES

The protease inhibitors telaprevir and boceprevir are both inhibitors and substrates of the metabolising enzyme, cytochrome P450 (CYP) 3A4. Co-administration of either telaprevir or boceprevir with drugs metabolised by this enzyme can result in clinically significant and possibly life threatening drug-drug interactions. Due to the potential for toxicity and/or suboptimal treatment particular care should be taken to ensure a full drug history and medication review of all patients (including prescribed medication, over-the-counter products, herbal and/or illicit drugs).[134,168] An assessment should be made to ensure that co-administration will not result in any clinically significant alteration to drug metabolism. Reference sources such as the summary of product characteristics for each drug and University of Liverpool Hepatitis C drug interaction website should be consulted as the main sources of information;[169] however, all possible sources of reliable information should be used to prevent problems with drug interactions. In patients with complex medication regimens, referral to a specialist pharmacist should be considered to ensure that complex interactions are explored adequately. Primary care providers should be alerted to the potential for interactions by communicating the results of the medication review. In patients whose concurrent prescribed or illicit medicines are contraindicated with protease inhibitors, treatment should be with pegylated IFN and ribavirin only after consultation with the local multidisciplinary team.[134] **4**

Caution should be taken if co-prescribing protease inhibitors with drugs known to prolong the QT interval. Prescribing information for individual drugs provides directions on ECG monitoring. Other mechanisms for drug metabolism may be involved but not yet identified and prescribers should remain vigilant. Caution and close monitoring should be adopted when co-prescribing other medicines.

D **Patients should have a full drug history taken including prescribed, over-the-counter and illicit drugs.**

D **The co-administration of any drugs should be assessed to ensure there is no unacceptable potential for toxicity or suboptimal efficacy of either agent.**

✓ Patients with complex polypharmacy may benefit from input of a specialist pharmacist.

✓ Patients should be made aware of potentially dangerous interactions between over-the-counter medicines, illicit drugs and HCV therapy, even if they are not known to use illicit drugs.

10.6 MANAGEMENT OF ADVERSE EFFECTS

10.6.1 FLU-LIKE SYMPTOMS

Virtually all patients taking pegylated IFN and ribavirin will experience flu-like symptoms such as fever, myalgia, rigors, arthralgia and headache. These tend to become less severe after the first month of treatment.[170] Simple interventions such as paracetamol use and increased fluid intake and rest can minimise these effects.[170, 171] **4**

D **Patients experiencing flu-like side effects from pegylated IFN and ribavirin can be advised to use paracetamol within manufacturers' guidelines.**

D **Patients should be advised to maintain an adequate fluid intake throughout treatment with pegylated IFN and ribavirin.**

D **Patients should be advised to coordinate their injections of pegylated IFN and ribavirin with periods of reduced activity, such as weekends and holidays.**

10.6.2 ANAEMIA AND NEUTROPENIA

In clinical trials the use of erythropoietin (EPO) in patients who developed anaemia (haemoglobin level ≤120g/l) while on pegylated IFN and ribavirin therapy improved the anaemia and lessened the need to reduce the dose of ribavirin. It also improved quality of life.[172,173] There is no direct evidence that this results in an increase in the SVR. None of the erythropoietins are currently licensed for this indication. **1+**

Haemoglobin levels should be maintained at a level that prevents a need for dose reduction or discontinuation of pegylated IFN and ribavirin therapy as this can cause a reduction in SVR.[174] Up to a third of patients receiving combination therapy develop anaemia and 13% progress to a haemoglobin of less than100 g/l. With the addition of protease inhibitors to pegylated INF and ribavirin treatment, anaemia can be more frequent and severe, occurring in the first few weeks of therapy.[174] An RCT compared the use of EPO as first line therapy to maintain haemoglobin, with ribavirin dose reduction in patients receiving boceprevir. This demonstrated that dose reduction of ribavirin maintained haemoglobin levels and provided similar SVR (71%) to patients whose treatment was supplemented with EPO. This finding was confirmed in patients with cirrhosis[175] and during phase three trials using telaprevir in triple therapy regimes.[176] **2+** **1+**

Granulocyte-colony stimulating factor (G-CSF) may relieve drug-induced neutropenia in patients receiving pegylated IFN and ribavirin therapy. It is most commonly needed in patients given antiviral therapy post liver transplant.[177] **3**

B **Erythropoietin should be considered in CHC patients receiving pegylated IFN and ribavirin therapy who develop anaemia, to prevent curtailment or dose reduction of ribavirin.**

B **For patients receiving a protease inhibitor in combination with pegylated IFN and ribavirin therapy, consider ribavirin dose reduction as an alternative to the addition of EPO for controlling anaemia.**

D **Granulocyte-colony stimulating factor should be considered on a case-by-case basis for patients who develop significant neutropenia while receiving treatment with pegylated IFN and ribavirin for CHC infection, to prevent curtailment or dose reduction of pegylated IFN.**

10.6.3 DEPRESSION

Depression is a commonly reported side effect of pegylated IFN and ribavirin therapy in both patients who have previously experienced depression and those who have not.[178] Antidepressants can be successfully used for treatment related depression and as a preventative measure prior to exposure to antiviral treatment.[166, 179] **1++** **1+** **3**

B **All patients receiving pegylated IFN and ribavirin should be monitored for signs of depression before, during and immediately post-treatment.**

B **Patients treated with pegylated IFN and ribavirin who experience depression should be considered for treatment with antidepressants and for referral to a specialist, if necessary.**

✓ A validated assessment tool (eg Hospital Anxiety and Depression score) should be used for monitoring depression.

10.6.4 SKIN REACTIONS

Severe skin reactions are uncommon during pegylated IFN or ribavirin therapy but dry skin, pruritus and diffuse eczematous lesions occur in approximately 20% of patients.[170] Psoriasis may also be exacerbated by treatment for CHC. Injection site reactions occur in over 50% of treated patients.[171] Skin lesions appear most commonly on the distal limbs and head and neck region, suggesting a predominance in sun-exposed areas.[180] Patients respond well to antihistamines, emollients and topical steroids, allowing continuation of treatment.[180] Discontinuation rates for dermatological side effects are approximately 3-4%.[181] **4** **3**

Three RCTs report on a potentially fatal rash, one of the principal adverse events of telaprevir treatment. This leads to discontinuation of telaprevir in 5-7% of patients. Once the medication is discontinued the rash resolves (although this may take several weeks). The rash is predominantly eczematous and pruritic. Fifty per cent of patients developing a rash do so within the first four weeks of treatment although it can occur at any time. Early intervention with topical steroids reduces the severity of rash. Rarely, telaprevir has been associated with Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) or Severe Cutaneous Adverse Reactions to Drugs (SCAR) syndromes, which includes Stevens Johnson syndrome and toxic epidermal necrolysis, and these should be monitored for and, if detected, therapy should be discontinued.[182-184] **1+** **1++**

D **All patients on pegylated IFN and ribavirin should be advised to ensure appropriate skin hygiene and hydration.**

D **Patients should be advised to avoid overexposure to sun.**

D **Patients should be advised to rotate injection sites.**

D **The use of emollients and topical corticosteroids can be considered for non-specific rashes.**

A **Patients taking telaprevir must be monitored closely for rash and treatment centres should have a rash management plan.**

✓ The use of antihistamines can be considered for pruritus.

✓ Severe dermatological reactions or those that do not respond to first line treatment should be referred for dermatological opinion.

✓ Patients taking telaprevir should be promptly assessed for severity according to the percentage of body surface area involved and the presence of systemic symptoms.

✓ Patients with a history of rash or skin problems should be considered for treatment with boceprevir rather than telaprevir.

10.6.5 THYROID DISFUNCTION

IFN therapy is associated with the development of thyroid dysfunction (both hypothyroid and hyperthyroid) in up to 6% of those treated.[170] Females are more at risk, especially those with thyroid autoantibodies before treatment.[185] IFN is associated with the induction and enhancement of thyroid autoimmunity, which is not always reversible.[186,187] Pre-treatment autoantibodies are not universally predictive of thyroid dysfunction during treatment.[170]

4
1-
2+

D **Thyroid function should be monitored at baseline before IFN therapy, at week 12 of treatment and at any time where there is a suspicion of thyroid dysfunction.**

✓ Patients developing thyroid dysfunction should be referred to an endocrinologist.

10.6.6 WEIGHT LOSS

Chronic hepatitis C infection causes increased basal metabolic rate in non-cirrhotic patients.[188] Weight loss is commonly reported in patients on antiviral treatment.[170,171,189] Nutritional therapy of patients with HCV is discussed in section 12.1.

2-
4

10.6.7 DYSPNOEA

Dyspnoea is a rarely reported side effect of pegylated IFN and ribavirin therapy. It may occur as a result of treatment-related anaemia but may also be caused by more serious cardiovascular or respiratory conditions.[170, 171,190]

2-
4

D **Patients treated with pegylated IFN or ribavirin who report dyspnoea that is not related to anaemia should be urgently assessed medically for cardiopulmonary problems.**

10.6.8 RETINOPATHY

Retinopathy during pegylated IFN therapy is common but generally mild and transient. It resolves spontaneously on discontinuing IFN and treatment is seldom required. The long term consequences are unknown.[191] Patients with hypertension or diabetes are at greater risk of developing retinopathy.[171] Other ophthalmic side effects are uncommon.

1+
4

D **Patients with CHC and hypertension or diabetes should have an ophthalmic examination prior to commencing treatment, paying particular attention to cotton wool spots and retinal haemorrhage.**

D **Any patient reporting visual disturbance during treatment should be examined further by an ophthalmologist.**

D **IFN should be discontinued in any patient with visual disturbance until it has resolved or an ophthalmologist has confirmed there is no retinal injury.**

10.6.9 ALOPECIA

Alopecia is a relatively common reported side effect of IFN and ribavirin therapy. Hair will grow again on cessation of treatment.[170, 171]

D **Patients should be advised that treatment related hair loss is reversible on cessation of treatment.**

10.6.10 OTHER SIDE EFFECTS

Other reported side effects of protease inhibitors include insomnia, poor concentration, oral disease, anal/rectal discomfort, gastrointestinal problems, altered taste, nausea, and post-treatment withdrawal symptoms. No evidence on their effective management was identified.

Fatigue is one of the most commonly reported side effects of IFN or ribavirin treatment and may be multifactorial with anaemia, hypothyroidism, sleep disturbance and depression all contributing.[170, 171]

4

10.7 RELAPSE OR FAILED TREATMENT

10.7.1 IFN AND RIBAVIRIN

Retreatment with a combination of pegylated IFN and ribavirin is effective in patients with CHC who have had unsuccessful treatment with non-pegylated IFN with or without ribavirin, and leads to sustained viral response in a proportion of patients. The SVR is highest in patients who had received prior treatment with non-pegylated IFN monotherapy, those infected with genotypes 2 or 3, those who had relapsed rather than not responded to previous treatment, and those who were not cirrhotic at time of retreatment.[192] **3**

Two trials which addressed retreatment of patients with genotype 1 HCV, in whom treatment had been previously unsuccessful, showed that there was significant benefit in repeating treatment with a pegylated IFN and ribavirin regimen with the addition of a protease inhibitor.[134] For telaprevir triple therapy the overall SVR were 64% (without lead-in), 66% (with a four week lead-in) and 17% in the dual therapy control group, the differences of 47% (95% CI 37 to 57; p<0.001) and 50% (95% CI 40 to 60; p<0.001) between the triple therapy and control groups were significant.[193] For boceprevir triple therapy the overall SVR were 59% (32 week triple therapy), 66% (44 week triple therapy) and 21% in the control group. The differences of 38% (95% CI 25.7 to 49.1; p<0.001) and 45% (95% CI 33.7 to 56.8; p=0.001) between triple therapy and control groups were significant.[194] **1+**

D **Patients with CHC who have had unsuccessful treatment with non-pegylated IFN and ribavirin should be considered for pegylated IFN and ribavirin retreatment.**

A **Patients with genotype 1 CHC who have had any unsuccessful treatment should be considered for treatment with a protease inhibitor based regimen.**

✓ Patients with genotype 2 or 3 CHC who have undergone a suboptimal therapy could be considered for retreatment with pegylated INF and ribavirin for 48 weeks.

10.8 MONITORING PATIENTS WHO ARE NOT RECEIVING TREATMENT

10.8.1 CLINICAL REVIEW

No evidence was identified regarding effective practice in monitoring and advising patients who are not candidates for treatment or who have received unsuccessful treatment.

✓ Patients should be encouraged to continue attending follow-up clinics for review in order to monitor their condition and discuss new therapies as they emerge.

✓ Patients should have access to counselling and specialist nurse services to provide support on lifestyle issues relating to hepatitis C.

10.8.2 ROLE OF LIVER BIOPSY

Routine liver biopsy during or after antiviral treatment is not indicated unless specific information is required in selected patients.[101] **1-**

11 Treatment of advanced infection

11.1 ANTIVIRAL THERAPY

11.1.1 PATIENTS WITH CIRRHOSIS

Patients with cirrhosis are defined as having compensated or decompensated cirrhosis. Those with decompensation have deterioration with development of one or more of the following: jaundice, ascites, variceal bleeding or encephalopathy.

There are several large well conducted RCTs of IFN and ribavirin, pegylated IFN and ribavirin, and pegylated IFN monotherapy in patients with chronic HCV who have a high likelihood of progressing to end stage liver disease.[122, 123, 195, 196] Subgroups within these trials had cirrhosis or advanced fibrosis. Randomisation distributed them evenly between the interventions so that subgroup analysis was possible. Therapy appeared no more toxic to the patients with cirrhosis compared to those without cirrhosis, but was less effective. The SVRs achieved were 50-70% for genotype 2 and 3, and 20-30% for genotype 1. A further systematic review in patients with cirrhosis have confirmed reduced risk in hepatocellular carcinoma in those who achieve SVR (RR 0.25; 95% CI 0.14 to 0.46; p=0.00001).[97] **1+**

Whilst improved over previous therapies, SVR rates are likely to remain low in previously non-responding patients with cirrhosis, particularly in the context of a poor response to lead-in pegylated IFN and ribavirin. Treating these patients with protease inhibitors is also likely to increase development of drug resistant mutants, potentially jeopardising the use of future direct-acting antivirals. Therefore, careful consideration should be given to the best management option for this group. In patients with advanced fibrosis or cirrhosis there are non-significant trends towards better SVR rates with fixed duration therapy, but there is currently insufficient evidence for the use of response-guided therapy in patients with cirrhosis.[134] Further study of the efficacy of protease inhibitors in patients with cirrhosis would be beneficial as major trials on telaprevir excluded patients with a platelet count of <90,000/mm^3.[133] **1+**

No head-to-head trials of the different pegylated IFNs were identified. Studies of long term therapy for patients with cirrhosis are ongoing.

A **Patients with compensated cirrhosis should be considered for therapy, unless contraindicated.**

Treatment with weekly maintenance low-dose pegylated IFN in patients with cirrhosis showed no significant difference in slowing disease progression or reducing clinical outcomes compared to patients receiving no treatment. There were no differences in development of HCC, decompensation or transplantation.[198,199] **1++**

One RCT showed that 48 weeks of escalating pegylated IFN monotherapy in predominantly treatment-experienced patients with cirrhosis showed a reduction in all cause mortality and non-oncological morbidity; however this study was inadequately powered and not as yet reproduced by other researchers.[200] The prospectively defined sub-analysis in another RCT of patients with portal hypertension showed that pegylated IFN delayed significant portal hypertensive events such as variceal bleeding.[201] **1-** **1+**

A **Low-dose pegylated IFN maintenance monotherapy should not be used in patients with compensated cirrhosis.**

There was insufficient evidence on the treatment of patients with HCV and decompensated cirrhosis to make a recommendation.

11.1.2 PATIENTS REFERRED FOR LIVER TRANSPLANT

Several studies have addressed the benefits of antiviral therapy given in the period leading up to, or following, orthotopic liver transplantation (OLT).[177, 202-205] In the period before transplantation many patients are excluded from therapy because of contraindications. SVR rates are low. There is little evidence for treatment in the peri-transplant period (the time period immediately before, during and after transplantation). Post-transplant therapy is poorly tolerated, due to anaemia and leucopenia, but appears safe in regard to graft failure. In those patients able to tolerate full dose therapy, high SVRs are achieved. **3** **1+**

D **Patients in whom transplant is planned should not receive antiviral therapy in the pre-transplant or peri-transplant stages, except as part of clinical trials.**

D **Patients should be considered for antiviral therapy post liver transplant to achieve HCV clearance in cases of recurrence of HCV related liver disease.**

11.2 LIVER TRANSPLANTATION

In early studies of patients transplanted for HCV cirrhosis, the five- and 10-year survival rates were equivalent to patients after OLT for non-HCV causes (68% and 60%).[205] In an analysis of the United Network for Organ Sharing (UNOS) database outcomes were studied in HCV positive (n=5,640, 43%) and HCV negative recipients (n=7,386, 56.7%). In the HCV negative and the HCV positive recipient populations, five-year patient survival rates were 83.5% versus 74.6% ($p<0.00001$) and five-year graft survival rates 80.6% *v* 69.9% ($p<0.00001$), respectively. HCV infection reduces outcome following transplantation but this effect is not sufficient to deny an individual patient transplantation.[206] **2+**

Patients with HCV and transplantable hepatocellular carcinoma (one lesion <5 cm or fewer than three lesions <3 cm, on cross-sectional imaging) have no decrease in survival benefit up to 48 months post orthotopic liver transplantation when compared to patients with HCV alone.[205, 207] **2+**

Quality of life in patients post OLT for HCV is equivalent to patients with non-HCV at three years.[208] **3**

C **Patients with hepatitis C virus and concurrent operable hepatocellular carcinoma should be offered liver transplantation.**

C **Patients with HCV associated chronic liver failure should be considered for assessment for liver transplantation.**

No studies were found on the effectiveness of retransplantation in patients with graft loss due to HCV recurrence.

11.3 SCREENING FOR HEPATOCELLULAR CARCINOMA

The results of studies evaluating the sensitivity and specificity of serum alfa-fetoprotein for detection of HCC in individuals with HCV indicate that in isolation this marker is of limited value.[209] **1+**

Annual ultrasound scanning of patients with cirrhosis and HCV does not detect tumours at a stage that permits likely curative treatment.[210] Scanning on a six-monthly basis may result in the detection of tumours at a stage that permits curative therapy.[211] **2+**

Methods of screening and surveillance other than alfa-fetoprotein and ultrasound remain experimental.

The rate of development of HCC in patients with HCV who are non-cirrhotic compared with patients who are is extremely low (7.6% versus 92.4%).[212] **2+**

A **The measurement of alfa-fetoprotein should not be used in isolation for screening or surveillance of the development of HCC in patients with hepatitis C.**

D **Surveillance using ultrasound should take place at six-monthly intervals.**

C **Surveillance should be confined to patients with cirrhosis.**

12 Nutrition, supportive care and complementary therapies

12.1 NUTRITIONAL INTERVENTIONS

12.1.1 DIETARY INTERVENTIONS

Protein energy malnutrition is common in all patients with chronic liver disease and can lead to weight loss. Chronic hepatitis C infection increases basal metabolic rate in non-cirrhotic patients.[188] Malnutrition (either under- or overweight) negatively affects nutritional status, quality of life and survival. Nutritional assessment to identify patients at risk and provision of nutritional support (enteral and parenteral) to improve clinical outcome should play an important role in patient care.[213] **2- 4**

Weight loss is commonly reported in patients on antiviral therapy.[170, 171, 189] This is possibly a result of other side effects, such as fatigue and depression, which may have a negative impact on appetite.[170, 171] **2- 3**

D

- **Nutritional care for people infected with hepatitis C should involve promotion of optimal nutrition and prevention or treatment of malnutrition or deficiencies of specific nutrients.**
- **Patients should have a nutritional screen and if needed a nutritional assessment and appropriate advice from a dietitian.**

D **Patients with advanced liver disease should be given nutritional support to minimise malnutrition.**

✓ Antiviral therapy represents a high risk period for weight loss so patients should be monitored closely and given nutritional support, as required, during treatment.

A systematic review of 11 RCTs found no conclusive evidence to support any benefit of branched chain amino acids (BCCA) in patients with cirrhosis and hepatic encephalopathy.[214] It was unclear how many of the patients included in the study were HCV positive. BCCA improve serum albumin levels in the compensated stage of cirrhosis in patients with a branch chain tyrosine ratio (BTR) <4 and serum albumin level between 35-39 g/l.[215, 216] The methodology used in these studies gave little consideration to confounding variables. **1++ 1-**

Coffee may have a protective effect against the development of hepatocellular carcinoma in patients with liver disease when consumed in quantities of three or more cups per day. It is unclear which compound in coffee causes the effect.[217, 218] **2+**

12.1.2 VITAMINS AND MINERALS

There is little evidence that individual vitamins and minerals may influence the natural history of CHC.

Zinc supplementation of 34 mg/day may have some beneficial effect on sustained viral response in patients taking IFN therapy; with genotype 1b; with viral load lower than $5x10^5$ copies/ml.[219] **2-**

Vitamin K2 may be beneficial in the prevention of development of HCC in patients with hepatitis C.[220] **2-**

Vitamin E supplementation had no beneficial effect in patients taking pegylated IFN and ribavirin. It does not appear to prevent ribavirin haemolysis or enhance virological clearance.[221] **1-**

Iron restricted to <7 mg/day in conjunction with a controlled calorie intake of 30 kcals/kg, protein intake of 1.1-1.2 g/kg, and fat at 15% of dietary intake, reduces aminotransferase levels.[222] **4**

Vitamin C supplementation of 600 mg/day is not beneficial in the prevention of retinopathy associated with IFN therapy.[191] **1-**

✓ Patients with chronic hepatitis C should be encouraged to achieve the UK recommended nutrient intake of vitamins and minerals.[223] They should be advised that there is no identified evidence to support amounts in excess of this.

✓ Patients whose serum ferritin levels are consistently high should not be advised to reduce dietary iron intake.

12.1.3 OVERWEIGHT

Studies have identified BMI>25 as being associated with hepatic steatosis, which leads to more severe fibrosis.[89, 224] Liver fibrosis, steatosis and ALT level decrease with supervised weight loss programmes of diet and regular exercise, aiming at 0.5 kg weight loss weekly.[225] **2+** **3**

C **Patients who are overweight should be advised to lose weight, within a realistic weight loss target, as this may have a beneficial effect on the degree of liver damage associated with hepatitis C infection.**

✓ Weight loss should only be considered if the patient is stable in their management of hepatitis C. Interventions aimed at weight reduction during antiviral treatment are not recommended, as side effects may lead to excessive unintentional weight loss.

✓ Patients on weight loss programmes should receive regular follow up and support.

12.2 SPECIALIST NURSE INTERVENTIONS

Specialist nursing support is key to maintaining adherence to treatment in patients with psychiatric conditions.[226] Specialist hepatology nursing has a significant role to play in helping patients to attain and maintain SVR.[227] **2-**

✓ Clinical nurse specialists should be an integral member of the clinical team caring for patients with chronic hepatitis C.

12.3 PSYCHOSOCIAL INTERVENTIONS

Two studies on psychological interventions for patients with hepatitis C showed no evidence of benefit. One small non-randomised trial showed some benefit, but the other, an RCT which tested individually tailored interventions, showed no difference in outcome from standard care.[228, 229] **2-** **1+**

12.4 EXERCISE

Light to moderate exercise programmes have been recommended for patients receiving treatment for hepatitis C.[170] A small cohort study found that patients on antiviral therapy have a reduced exercise tolerance.[230] **4** **2-**

D **Patients with hepatitis C should be encouraged to take mild to moderate exercise. Those on antiviral therapy should be advised that they may find their capacity for exercise reduced.**

12.5 COMPLEMENTARY THERAPIES

Two meta-analyses have concluded that there is no evidence to support the use of complementary or alternative medicines in the treatment of patients with hepatitis C.[231, 232] **1++**

None of the trials identified ran for a long enough period to show the long term safety or harm of herbal remedies.

✓ Patients should be made aware that there is a potential for harm associated with some complementary preparations.

12.6 PALLIATIVE CARE

No evidence was identified looking specifically at palliative care for patients with HCV.

13 Provision of information

To find out patients' main information needs, interviews and focus groups were held with patients, and questionnaires sent to people with hepatitis C across the UK by the Scottish Hepatitis C support network and the UK Hepatitis C resource centre. The results have been translated into questions and suggested answers (*see section 13.2*), which could be used to encourage discussions between patients and health professionals. Several of the organisations listed in section 13.1 produce good-quality patient leaflets.

13.1 SOURCES OF FURTHER INFORMATION

British Liver Trust
Portman House
44 High Street
Ringwood BH24 1AG
Tel: 01425 463080 • Fax: 01425 470706
www.britishlivertrust.org.uk

The British Liver Trust provides a range of publications on individual liver conditions and offers support to patients with liver disease and those who care for them.

Haemophilia Scotland
4b Gayfield Lane
Gayfield Place Lane
Edinburgh EH7 4AB
Tel: 0131 557 5953
Email: dan@haemophiliia.org.uk

Haemophilia Scotland provides services for people with haemophilia and von Willebrand's affected by HIV and viral hepatitis.

Hepatitis C Trust
5 Charlotte Square
Edinburgh EH2 4DR
Tel: 0131 777 0989 • Helpline: 0845 223 4424
www.hepctrust.org.uk • Email: helpline@hepctrust.org.uk

The Hepatitis C Trust was set up by people with the illness and runs a range of services that provide support, information and representation for people with hepatitis C.

Hepatitis New South Wales
www.hep.org.au

This Australian website has a wealth of excellent interesting and helpful information and resources on hepatitis, however some resources are specific to Australian healthcare systems.

Hepatitis Scotland
1/91 Mitchell Street
Glasgow G1 3LN

Tel: 0141 225 0419 • Fax: 0141 248 6414
www.hepatitisscotland.org.uk • Email: enquiries@hepatitisscotland.org.uk

Hepatitis Scotland is the national voluntary sector organisation funded by the Scottish Government to help improve responses to viral hepatitis prevention, treatment and support.

13.2 INFORMATION ABOUT HEPATITIS C FOR PATIENTS AND CARERS

WHAT IS HEPATITIS C?

Hepatitis C is an illness caused by a virus which can be passed through blood from one person to another. It mainly affects the liver.

When your liver becomes inflamed it can become damaged and eventually has difficulty carrying out its various and vital functions. Over a long period of time, this can progress to serious liver damage (such as fibrosis and cirrhosis) or, in some people, liver cancer (hepatocellular carcinoma).

HOW DOES IT AFFECT PEOPLE?

Hepatitis C is a potentially life threatening condition that can affect you physically and emotionally. It can affect your quality of life. Treatment is available which can cure hepatitis C in the majority of people.

WHAT ARE THE SYMPTOMS OF HEPATITIS C?

Some people have no symptoms at all for many years while others may feel extreme tiredness, have sweats (especially at night), aches and pains, loss of appetite and concentration problems. Symptoms may come and go. In the later stages of the infection if the liver is more seriously damaged, there may be symptoms such as jaundice, itchiness, internal bleeding and a swollen abdomen.

HAVE I BEEN AT RISK?

Times when blood from someone infected with hepatitis C may get into the bloodstream of another person include:

- having a blood transfusion or surgical treatment abroad or in the UK before 1991, or blood products in the UK before about 1987 (transfusions and blood products are now safe from HCV infection in the UK);
- having medical or dental treatment in countries where hepatitis C is common and infection control may be poor;
- sharing any equipment when injecting or snorting drugs;
- sharing items such as razors, toothbrushes or any item that can scratch the skin;
- piercing, tattooing and cosmetic injection procedures (eg botox) if any equipment is reused;
- sexual activity, although the risk is low except where there is a risk of bleeding;
- being exposed to blood at work, for example, a needlestick injury, cuts, cleaning up blood, and dealing with violent incidents where blood is involved;
- transmission from mother to child around the time of birth.

SHOULD I BE TESTED?

If you think you may have been at risk, then you should get tested. The earlier treatment starts the more likely it is to provide a cure for the infection. If you know you are positive you can avoid infecting others.

WHAT DOES THE TEST TELL YOU ABOUT BEING POSITIVE OR NEGATIVE?

There are three types of test. The first type of test (the HCV antibody test) tells you if you have ever had the virus. Some people get rid of the virus naturally without medical help. The second type (PCR test) tells you if you still have the virus in your body (that is if you are infected with hepatitis C). If the PCR test is positive a further test will reveal the genotype (strain) of virus you have. The genotype will determine the treatment you receive.

WHAT ABOUT CONFIDENTIALITY?

Confidential testing is available in GP surgeries and at other sites. Your test results are confidential and will not be shared with others without your permission. GPs will only pass on information about positive tests to insurance companies if you have applied for insurance and given your consent for release of medical information. Negative tests will not be disclosed.

You can find information on hepatitis services across Scotland by visiting the Hepatitis Scotland website (*see section 13.1*)

ARE MY FAMILY AND FRIENDS AT RISK? SHOULD I TELL THEM?

The support of your family and friends is important and you should consider telling them about your diagnosis and what the effects and side effects of treatment are likely to be. You cannot infect your family and friends through everyday activities, such as sharing utensils, hugging or kissing.

To avoid infecting others,

- do not share items such as toothbrushes or razors
- clean up any blood spills with diluted bleach
- do not share any drug taking equipment (eg tooters, needles and syringes, water or cookers etc).

The risk of sexual transmission is very low in the absence of other complicating factors such as:

- blood from menstruation or anal sex
- ulcers or sores on your genitalia ie from a sexually transmitted infection such as gonorrhea, herpes or genital warts.

HOW WILL IT AFFECT MY JOB AND JOB PROSPECTS?

Many people stay well enough to work but you may have to make some adjustments to your routine if you have extreme tiredness or other symptoms.

You do not usually have to tell your employer that you have hepatitis C (unless you are a healthcare worker involved in procedures that may involve exposure to blood, for example some surgeries. However side effects of treatment may mean that you feel ill and unable to work for a period of time. It may be helpful to tell them that you are having treatment. For example, they may support you by changing your working hours or reducing any physical activities associated with your job during treatment.

HOW WILL A POSITIVE DIAGNOSIS AFFECT MY LIFE INSURANCE COVER?

In common with any serious health condition, a diagnosis of hepatitis C will have an impact when taking out life insurance. There are many types of life insurance cover so it is worth checking any policies you have as you may need to tell the insurer if you have a change in your health status. It is important to remember that any information you provide to your insurer forms part of a legal contract. If the information is inaccurate or untrue the agreement may be invalid.

If you are applying for insurance and consented for release of medical information, the person who tested you is legally obliged to pass on information about a positive test result if asked to by an insurance company.

You should read the small print on current or new insurance agreements before signing.

HOW WILL A NEGATIVE TEST AFFECT MY LIFE INSURANCE COVER?

Doctors do not need to give insurers any information about a negative test. Insurers can only ask for information where someone has had a positive test or is receiving treatment.

WHAT HAPPENS DURING TESTING?

Before you have a test a healthcare professional will discuss with you what happens. This will help you to understand the testing process, the test results and confidentiality.

Blood samples will be sent for laboratory testing. If your exposure to the virus has been in the last six months, you may be asked to return for a repeat test. This is because there is a 'window' period after exposure until the test becomes positive.

If your test is negative, the healthcare team will give you advice on how to avoid putting yourself at risk in the future. If your test is positive, they will explain what this means and refer you to a specialist clinic for assessment.

WHAT HAPPENS NEXT?

Assessment will include blood tests such as liver function tests (LFT), a genotype (strain of hepatitis C virus) test, an examination, discussions about lifestyle, other medical conditions and medicines you take, ultrasound scan, fibroscan and, in rare circumstances, a liver biopsy. A liver biopsy means taking a small piece of the liver for laboratory analysis. This is done under local anaesthetic but is not required before starting treatment.

When the assessment is complete, your healthcare team will talk to you about treatment. Treatment is very effective and side effects can be managed with good care and support. Your clinic should help you to find support as part of your care while on treatment. Treatment is not suitable for everyone and depends on other medical conditions or complications you may have.

While you or your partner are on treatment, and for six to 12 months afterwards, you should both use contraception to avoid pregnancy because these drugs may be harmful to an unborn baby.

LIVING WITH HEPATITIS C

There are a number of things that you can do for yourself that make living with chronic hepatitis C easier.

- Do not drink alcohol. Alcohol and hepatitis C damage the liver, and in combination cause damage at a faster rate. Professional support is available to help you reduce or stop drinking.
- Cut down on rich, fatty and sugary foods as a balanced diet is crucial. Losing excess weight puts less strain on your liver. However, weight loss can be a side effect of treatment and food supplements may be required. Professional help to reach a healthy balanced diet is available.
- If you have a poor appetite, try and eat smaller meals more often.
- Try to exercise regularly. It can reduce stress and depression, increase energy levels and help boost your immune system.
- Follow the dosage instructions for prescribed and over-the-counter medications. Both over-the-counter and illicit drugs are processed by the liver and may put extra strain on it. Illicit drugs may also contain impurities which are harmful.
- Stop smoking if you have hepatitis C. Stopping smoking reduces the risk of getting cancer. Help is available to stop smoking.
- Speak to your GP about vaccinations against hepatitis A and B. Other types of liver infection, especially when you already have hepatitis C, put much more strain on your liver and can slow or stop recovery for all types of hepatitis. You should also make sure you get the seasonal flu vaccination as an extra precaution when your immune system may be weakened.

There is very little evidence that complementary medicines are effective, but many patients find them helpful in dealing with the many different symptoms associated with this disease. It is important to get professional advice before starting these.

WHAT SUPPORT CAN I GET?

There are various specialist support services for people with hepatitis C, such as counselling, peer support from other people with hepatitis C, and support to help with making decisions.

Hepatitis C can make people feel very isolated and emotional support is important. You should expect:

- comprehensive team services providing good communication;
- good quality information;
- involvement at all stages of your care and treatment;
- access to specialist services as you require them, and
- regular assessment of your support needs.

14 Implementing the guideline

14.1 IMPLEMENTATION STRATEGY

Implementation of national clinical guidelines is the responsibility of each NHS Board and is an essential part of clinical governance. Mechanisms should be in place to review care provided against the guideline recommendations. The reasons for any differences should be assessed and addressed where appropriate. Local arrangements should then be made to implement the national guideline in individual hospitals, units and practices.

14.2 RESOURCE IMPLICATIONS OF KEY RECOMMENDATIONS

No recommendations were identified as having significant budgetary impact.

14.3 AUDITING CURRENT PRACTICE

There are two mechanisms for auditing practice around the testing, treatment care and support provided by hepatitis C services in Scotland. The first is the Healthcare Improvement Scotland Hepatitis C Quality Indicators which are self audited by health boards. The second is the performance indicators contained within the Blood Borne Virus and Sexual Health framework which are audited nationally by the Scottish Government.

14.4 ADDITIONAL ADVICE TO NHS SCOTLAND FROM HEALTHCARE IMPROVEMENT SCOTLAND AND THE SCOTTISH MEDICINES CONSORTIUM

In September 2011, the Scottish Medicines Consortium (SMC) advised that boceprevir was accepted for use within NHSScotland for the treatment of CHC genotype 1 infection, in combination with pegylated IFN and ribavirin, in adult patients with compensated liver disease who have failed previous therapy or who have been previously untreated.

In September 2011, the Scottish Medicines Consortium (SMC) advised that telaprevir in combination with pegylated IFN and ribavirin, is indicated for the treatment of genotype 1 CHC in adult patients with compensated liver disease (including cirrhosis) who have previously been treated with interferon alfa (pegylated or non-pegylated) alone or in combination with ribavirin, including relapsers, partial responders and null responders or patients who are treatment naive.

15 The evidence base

15.1 SYSTEMATIC LITERATURE REVIEW

The evidence base for this guideline was synthesised in accordance with SIGN methodology. A systematic review of the literature was carried out using an explicit search strategy devised by a SIGN Evidence and Information Scientist. Databases searched include Medline, Embase, Cinahl, PsycINFO and the Cochrane Library. The year range covered was 2006-2012. Internet searches were carried out on various websites including the US National Guidelines Clearinghouse. The main searches were supplemented by material identified by individual members of the development group. Each of the selected papers was evaluated by one member of the group and one SIGN staff member using standard SIGN methodological checklists before conclusions were considered as evidence.

15.1.1 LITERATURE SEARCH FOR PATIENT ISSUES

At the start of the guideline development process, a SIGN Evidence and Information Scientist conducted a literature search for qualitative and quantitative studies that addressed patient issues of relevance to early management of patients with hepatitis C. Databases searched include Medline, Embase, Cinahl and PsycINFO, and the results were summarised and presented to the guideline development group.

15.2 RECOMMENDATIONS FOR RESEARCH

The guideline development group was not able to identify sufficient evidence to answer all of the key questions asked in this guideline (*see Annex 1*). The following areas for further research have been identified:

- management of hepatitis C in children
- treatment in active drug users
- care pathways for the management of hepatitis C (older patients, prisoners, transplant patients, decompensated cirrhotic patients)
- testing and screening approaches.

15.3 REVIEW AND UPDATING

This guideline was issued in 2013 and will be considered for review in three years. Any updates to the guideline in the interim period will be noted on the SIGN website: **www.sign.ac.uk**

16 Development of the guideline

16.1 INTRODUCTION

SIGN is a collaborative network of clinicians, other healthcare professionals and patient organisations and is part of Healthcare Improvement Scotland. SIGN guidelines are developed by multidisciplinary groups of practising clinicians using a standard methodology based on a systematic review of the evidence. Further details about SIGN and the guideline development methodology are contained in SIGN 50: A Guideline Developer's Handbook, available at **www.sign.ac.uk**

16.2 THE GUIDELINE DEVELOPMENT GROUP

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Ms Petra Wright	*Scottish Officer, Hepatitis C Trust, Edinburgh*
Mr Leon Wylie	*Director, Hepatitis Scotland, Glasgow*
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The membership of the guideline development group was confirmed following consultation with the member organisations of SIGN. All members of the guideline development group made declarations of interest and further details of these are available on request from the SIGN Executive.

Guideline development and literature review expertise, support and facilitation were provided by the SIGN Executive. All members of the SIGN Executive make yearly declarations of interest and further details of these are available on request.

Mrs Karen Graham	*Patient Involvement Officer*
Mr Stuart Neville	*Publications Designer*
Miss Gaynor Rattray	*Guideline Coordinator*
Mr Campbell Reynolds	*Distribution and Office Coordinator*

16.2.1 ACKNOWLEDGEMENTS

SIGN would like to acknowledge the guideline development group responsible for the development of SIGN 92: Management of hepatitis C, on which this guideline is based.

16.3 CONSULTATION AND PEER REVIEW

16.3.1 SPECIALIST REVIEWERS INVITED TO COMMENT ON THIS DRAFT

This guideline was reviewed in draft form by the following independent expert referees, who were asked to comment primarily on the comprehensiveness and accuracy of interpretation of the evidence base supporting the recommendations in the guideline. The guideline group addresses every comment made by an external reviewer, and must justify any disagreement with the reviewers' comments. All expert referees made declarations of interest and further details of these are available on request from the SIGN Executive.

SIGN is very grateful to all of these experts for their contribution to the guideline.

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Dr David Bell	*Consultant in Infectious Diseases, Brownlee Centre, Gartnavel General Hospital, Glasgow*
Mr. Charles Gore	*Director of the Hep C Trust*
Dr Magdalena Harris	*Lecturer, London School of Hygiene and Tropical Medicine*
Dr Carole Hunter	*Lead Pharmacist, Addiction Services, NHS Greater Glasgow and Clyde*
Professor William Irving	*Professor and Honorary Consultant in Virology, University of Nottingham and Nottingham University Hospitals NHS Trust.*
Dr Janice Main	*Reader and Consultant Physician in Infectious Diseases and General Medicine, Imperial College at St Mary's Hospital, London*
Dr Claire McIntosh	*Consultant Addiction Psychiatrist, Community Alcohol and Drug Service, Falkirk Community Hospital*
Dr Ewan Stewart	*Clinical Lead, Lothian Hepatitis MCN, NHS Lothian*

16.3.2 SIGN EDITORIAL GROUP

As a final quality control check, the guideline is reviewed by an editorial group comprising the relevant specialty representatives on SIGN Council to ensure that the specialist reviewers' comments have been addressed adequately and that any risk of bias in the guideline development process as a whole has been minimised. The editorial group for this guideline was as follows. All members of the SIGN Editorial group make yearly declarations of interest and further details of these are available on request from the SIGN Executive.

Professor Keith Brown	*Chair of SIGN; Co-Editor*
Dr Roberta James	*SIGN Programme Lead; Co-Editor*
Dr Sara Twaddle	*Director of SIGN; Co-Editor*

Abbreviations

ALT	alanine aminotransferase
BBV	blood borne virus
BCCA	branched chain amino acids
BMI	body mass index
BNF	British National Formulary
BTR	branch chain tyrosine ratio
CHC	chronic hepatitis C
CI	confidence interval
CYP	cytochrome P450
DDI	drug-drug interaction
DRESS	Drug Reaction (or rash) with Eosinophilia and Systemic Symptoms
ELISA	enzyme linked immunosorbent assay
EPP	exposure prone procedures
EPO	erythropoietin
EVR	early viral response
G-CSF	granulocyte-colony stimulating factor
GMC	General Medical Council
GUM	genitourinary medicine
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HIC	hepatic iron concentration
HIV	human immunodeficiency virus
IDU	injecting drug users
IFN	interferon
IU	international units
LFT	liver function tests
MA	marketing authorisation
MTA	medical technology assessment
NAT	nucleic acid test
NICE	National Institute for Health and Care Excellence
OLT	orthotopic liver transplantation
PCR	polymerase chain reaction
PNALT	persistently normal serum alanine aminotransferase
QOL	quality of life
RCT	randomised controlled trial

RNA	ribonucleic acid
RR	relative risk
RT PCR	reverse transcriptase polymerase chain reaction
RVR	rapid viral response
SCAR	Severe Cutaneous Adverse Reaction to Drugs
SEM	standard error of the mean
SIGN	Scottish Intercollegiate Guidelines Network
SMC	Scottish Medicines Consortium
SVR	sustained viral response
UK	United Kingdom
UNOS	United Network for Organ Sharing

Annex 1

Key questions addressed in this update

This guideline is based on a series of structured key questions that define the target population, the intervention, diagnostic test, or exposure under investigation, the comparison(s) used and the outcomes used to measure efficacy, effectiveness, or risk. These questions form the basis of the systematic literature search strategy (*see section 15.1)*.

Key question
1. What is the evidence for treating patients with acute hepatitis C in terms of: • viral genotype • optimal timing of treatment • duration of treatment • choice of therapy – interferon versus pegylated interferon versus. pegylated interferon + ribavirin Outcome: sustained viral response/symptoms/Quality of Life (QOL)/adverse effects.
2. What is the evidence for the benefit of antiviral therapy (pegylated interferon + ribavirin) in patients with chronic hepatitis C in terms of: • viral genotype • duration of therapy • early viral response • viral kinetics Outcome: sustained viral response/symptoms/QOL/adverse effects How effective is treatment in the following subgroups? Consider: a) Age/age of acquisition b) Gender c) Different ethnic groups (South East Asian, Indian, Pakistani, Bangladeshi, Caucasian) d) Severity of disease – mild disease/cirrhosis e) Normal serum transaminases f) HIV co-infection g) Hepatitis B co-infection h) Obesity i) Smoking j) Alcohol intake above recommended guidelines k) Iron overload l) Viral genotype m) Active drug addiction or use n) Oral methadone/buprenorphine o) Patients with extrahepatic disease – fatigue, arthralgia, cryoglobulinaemia, glomerulonephritis, sicca syndrome, porphyria cutanea tarda, lichen planus, B cell non-Hodgkin lymphoma p) Non-response/relapse after previous interferon monotherapy or interferon/ribavirin combination therapy

3. What is the evidence that standard antiviral treatment is best avoided in the following subgroups (absolute and relative contraindications)?

   Consider:

   a) Pregnancy, breast feeding, inability to use effective contraception

   b) Chronic renal failure

   c) Age

   d) Alcohol intake above recommended guidelines

   e) Active drug addiction and use
      - ongoing injecting drug use
      - ongoing drug use (non-injecting)
      - substitute prescribing

   f) Mental health problems – depression, psychosis, learning disability

   g) Epilepsy or history of seizures

   h) Neoplasia

   i) Congestive cardiac failure/respiratory failure

   j) Psoriasis

   k) Haemolytic anaemia

   l) Anaemia, leucopenia, thrombocytopaenia

   m) Retinopathy – diabetic, hypertensive

   n) Obesity – body mass index ≥ 30

   o) Organ transplantation – liver, renal, bone marrow

   p) Autoimmune conditions – autoimmune hepatitis, hyperthyroidism

   q) Normal serum transaminases

   r) Mild chronic hepatitis C

   s) Relapse/non-response after previous antiviral therapy

   t) Hypersensitivity to interferon or ribavirin

   u) Other medications – immunosuppressants, myelosuppressants, theophylline, nucleoside reverse transcriptase inhibitors.

4. For patients who relapse following treatment or who are non-responders to standard antiviral treatment what evidence is there for additional drug therapies?

Consider:

Amantadine, thymosin, histamine, long term interferon treatment.

References

1. Scottish Executive. Hepatitis C: Proposed action plan in Scotland. [cited 11 Jun 2013]. Available from url: http://www.scotland.gov.uk/Publications/2005/06/14134528/45302

2. Hutchinson S, Bird, S and Goldberg, D. Modelling the current and future disease burden of hepatitis C among injecting drug users in Scotland. Hepatology 2005;42(3):711-23.

3. Freeman AJ, Dore GJ, Law MG, Thorpe M, Von Overbeck J, Lloyd AR, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. Hepatology 2001;34(4 Pt 1):809-16.

4. NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements 2002;19(3):1-46.

5. EASL International Consensus Conference on Hepatitis C. Paris, 26-28 February 1999. Consensus Statement. J Hepatol 1999;30(5):956-61.

6. Booth JCL, O'Grady J, Neuberger J. Clinical guidelines on the management of Hepatitis C. [cited 11 Jun 2013]. Available from url: http://www.bsg.org.uk/clinical-guidelines/liver/clinical-guidelines-on-the-management-of-hepatitis-c.html

7. Shepherd J, Brodin H, Cave C, Waugh N, Price A, Gabbay J. Pegylated interferon alfa 2a and -2b in combination with ribavirin in the treatment of chronic hepatitis C: a systematic review and economic evaluation. Health Technol Assess 2004;8(39):1-125.

8. Guidance on Prescribing. In: The British National Formulary. London: British Medical Association and Royal Pharmaceutical Society of Great Britain; 2013.

9. Medicines and Healthcare products Regulatory Agency. Off-label or unlicensed use of medicines: prescribers' responsibilities. Drug safety update 2009;2(9):6-7.

10. Schwarz KB, Gonzalez-Peralta RP, Murray KF, Molleston JP, Haber BA, Jonas MM, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. Gastroenterology 2011;140(2):450-8.e1.

11. NICE. Hepatitis B and C: Ways to promote and offer testing to people at increased risk of infection 2012.

12. Department of Health. Hepatitis C action plan for England. London: Department of Health; 2004. [cited 11 Jun 2013]. Available from url: http://www.nhs.uk/hepatitisc/hcp/further-information/Pages/action-plan-for-england.aspx

13. Department of Health. Hepatitis C strategy for England. London: Department of Health; 2002. [cited 11 Jun 2013]. Available from url: http://www.nhs.uk/hepatitisc/SiteCollectionDocuments/pdf/hepatitis-c-strategy-for-england.pdf

14. NHS Scotland. Scottish Needs Assessment Programme (SNAP). Hepatitis C. Glasgow: Office for Public Health in Scotland; 2000. [cited 17 Jun 2013]. Available from url: http://www.hps.scot.nhs.uk/Search/pubdetail.aspx?id=27117

15. Royal College of Physicians of Edinburgh (RCPE). Consensus conference on Hepatitis C: Final consensus statement. Edinburgh: Royal College of Physicians of Edinburgh; 2004. [cited 11 Jun 2013]. Available from url: http://www.rcpe.ac.uk/clinical-standards/standards/hep_c_04.php

16. Henderson D. Managing occupational risks for hepatitis C transmission in the health care setting. Clin Microbiol Rev 2003;16(3):546-68.

17. Department of Health. Getting Ahead of the Curve: A strategy for combating infectious diseases (including other aspects of health protection). 2002. [cited 11 Jun 2013]. Available from url: http://antibiotic-action.com/wp-content/uploads/2011/07/DH-Getting-ahead-of-the-curve-v2002.pdf

18. Stein K, Dalziel K, Walker A, McIntyre L, Jenkins B, Horne J, et al. Screening for hepatitis C among injecting drug users and in genitourinary medicine clinics: Systematic reviews of effectiveness, modelling study and national survey of current practice. Health Technol Assess 2002;6(31):1-122.

19. Goldberg D, Hutchinson S, Anderson E, Stein K. Screening for hepatitis C virus infection: a prioritised approach. In: Bassendine M, Foster G, Miles A, editors. The Effective Management of Hepatitis C Infection. 3rd edition ed. London: Aesculapius Medical Press; 2003.

20. Judd A, Parry J, Hickman M, McDonald T, Jordan L, Lewis K, et al. Evaluation of a modified commercial assay in detecting antibody to hepatitis C virus in oral fluids and dried blood spots. J Med Virol 2003;71(1):49-55.

21. Hermida M, Ferreiro MC, Barral S, Laredo R, Castro A, Diz Dios P. Detection of HCV RNA in saliva of patients with hepatitis C virus infection by using a highly sensitive test. J Virol Methods 2002;101(1-2):29-35.

22. De Cock L, Hutse V, Verhaegen E, Quoilin S, Vandenberghe H, Vranckx R. Detection of HCV antibodies in oral fluid. J Virol Methods 2004;122(2):179-83.

23. Schirm J, van Loon AM, Valentine-Thon E, Klapper PE, Reid J, Cleator GM. External quality assessment program for qualitative and quantitative detection of hepatitis C virus RNA in diagnostic virology. J Clin Microbiol 2002;40(8):2973-80.

24. Pawlotsky JM. Diagnostic tests for hepatitis C. J Hepatol 1999;31(Suppl 1):71-9.

25. Centers for Disease Control and Prevention. Recommendations for preventing transmission of infections among chronic hemodialysis patients. 2001. (MMWR 2001;50(No. RR-5)). [cited 07 Jun 2013]. Available from url: http://www.cdc.gov/mmwr/PDF/rr/rr5005.pdf

26. General Medical Council. Serious Communicable Diseases. [cited 12 Jun 2013]. Available from url: http://www.gmc-uk.org/serious_communicable_diseases_1997.pdf_25416216.pdf

27. Shepherd J, Brodin, H et al. Pegylated interferon alfa 2a and -2b in combination with ribavirin in the treatment of chronic hepatitis C: a systematic review and economic evaluation. Health Technology Assessment 2004;8(39).

28. Ramsay ME. Guidance on the investigation and management of occupational exposure to Hepatitis C. Commun Dis Public Health 1999;2(4):258-62.

29. Kao JH, Liu CJ, Chen PJ, Chen W, Lai MY, Chen DS. Low incidence of hepatitis C virus transmission between spouses: a prospective study. J Gastroenterol Hepatol 2000;15(4):391-5.

30. Vandelli C, Renzo F, Romano L, Tisminetzky S, De Palma M, Stroffolini T, et al. Lack of evidence of sexual transmission of hepatitis C among monogamous couples: Results of a 10-year prospective follow-up study. Am J Gastroenterol 2004;99(5):855-9.

31. Ackerman Z, Ackerman E, Paltiel O. Intrafamilial transmission of hepatitis C virus: A systematic review. J Viral Hepat 2000;7(2):93-103.

32. Rauch A, Rickenbach M, Weber R, Hirschel B, Tarr PE, Bucher HC, et al. Unsafe sex and increased incidence of hepatitis C virus infection among HIV-infected men who have sex with men: the Swiss HIV Cohort Study. Clin Infect Dis 2005; 41(3):395-402.

33. Clarke A, Kulasegaram R. Hepatitis C transmission - where are we now? Int J STD AIDS 2006;17(2):74-80.

34. Scottish Executive. Hepatitis C: Essential information for professionals. [cited 12 Jun 2013]. Available from url: http://www.scotland.gov.uk/Publications/2002/07/15074/8613

35. Hutchinson SJ, McIntyre PG, Molyneaux P, Cameron S, Burns S, Taylor A, et al. Prevalence of hepatitis C among injectors in Scotland 1989-2000: declining trends among young injectors halt in the late 1990s. Epidemiol Infect 2002;128(3):473-7.

36. Department of Health. Guidance for clinical health care workers: protection against infection with bloodborne viruses. Recommendations of the Expert Advisory Group on AIDS and the Advisory Group on Hepatitis.: The Stationery Office; 1998. [cited 17 Jun 2013]. Available from url: http://webarchive.nationalarchives.gov.uk/+/www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4002766

37. De Carli G, Puro V, Ippolito G. Risk of hepatitis C virus transmission following percutaneous exposure in healthcare workers. Infection 2003;31(Suppl 2):22-7.

38. Esteban JI, Gómez J, Martell M, Cabot B, Quer J, Camps J, et al. Transmission of hepatitis C virus by a cardiac surgeon. N Engl J Med 1996;334(9):555-60.

39. Duckworth GJ, Heptonstall J, Aitken C. Transmission of hepatitis C virus from a surgeon to a patient. The Incident Control Team. Commun Dis Public Health 1999;2(3):188-92.

40. Ross RS, Viazov S, Thormählen M, Bartz L, Tamm J, Rautenberg P, et al. Risk of hepatitis C virus transmission from an infected gynecologist to patients. Results of a 7 year retrospective investigation. Arch Intern Med 2002;162(7):805-10.

41. Department of Health. HSG (93)40: Protecting health care workers and patients from hepatitis B. Recommendations of the advisory group on hepatitis. [cited Available from url: http://webarchive.nationalarchives.gov.uk/+/www.dh.gov.uk/en/Publicationsandstatistics/Lettersandcirculars/Healthserviceguidelines/DH_4084234

42. Backmund M, Meyer K, Von Zielonka M, Eichenlaub D. Treatment of hepatitis C infection in injection drug users. Hepatology 2001;34(1):188-93.

43. Cournot M, Glibert A, Castel F, Druart F, Imani K, Lauwers-Cances V, et al. Management of hepatitis C in active drugs users: experience of an addiction care hepatology unit. Gastroenterol Clin Biol 2004;28(6-7 Pt 1):533-9.

44. Dalgard O, Bjoro K, Hellum K, Myrvang B, Skaug K, Gutigard B, et al. Treatment of chronic hepatitis C in injecting drug users: 5 years' follow-up. Eur Addict Res 2002;8(1):45-9.

45. Nomura H, Sou S, Tanimoto H, Nagahama T, Kimura Y, Hayashi J, et al. Short-term interferon-alfa therapy for acute hepatitis C: a randomized controlled trial. Hepatology 2004;39(5):1213-9

46. Resti M, Azzari C, Mannelli F, Moriondo M, Novembre E, de Martino M, et al. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to seronegative women for HIV-1. BMJ 1998;317(7156):437-41.

47. Thomas SL, Newell ML, Peckham CS, Ades AE, Hall AJ. A review of hepatitis C Virus (HCV) vertical transmission: risks of transmission to infants born to mothers with and without HCV viaemia or human immunodeficiency virus infection. Int J Epidemiol 1998;27(1):108-17.

48. European Paediatric Hepatitis C Virus Network. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. European Paediatric Hepatitis C Virus Network. BJOG 2001;108(4):371-7.

49. Mast EE, Hwang LY, Seto DS, Nolte FS, Nainan OV, Wurtzel H, et al. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. J Infect Dis 2005;192(11):1880-9.

50. Spencer Jea. Transmission of HCV to infants of HIV negative intravenous drug using mothers : rate of infection and assessment of risk factors for transmission. J. Viral Hepatitis 1997; 4:395-409.

51. Healy CM, Cafferkey MT, Conroy A, Dooley S, Hall WW, Beckett M, et al. Hepatitis C infection in an Irish antenatal population. Ir J Med Sci 2000;169(3):180-2.

52. Spencer JD, Latt N, Beeby PJ, Collins E, Saunders JB, McCaughan GW, et al. Transmission of HCV to infants of HIV negative intravenous drug using mothers : rate of infection and assessment of risk factors for transmission. J Viral Hepat 1997; 4(6):395-409.

53. Ceci O, Margiotta M, Marello F, Francavilla R, Loizzi P, Francavilla A, et al. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV seronegative pregnant women: a 24 month prospective study. J Pediatr Gastroenterol Nutr 2001;33(5):570-5.

54. Mok J, Pembrey L, Tovo PA, Newell ML. When does mother to child transmission of hepatitis C virus occur? Arch Dis Child Fetal Neonatal Ed 2005;90(2):F156-F60.

55. Polywka S, Pembrey L, Tovo PA, Newell ML. Accuracy of HCV-RNA PCR tests for diagnosis or exclusion of vertically acquired HCV infection. J Med Virol 2006;78(2):305-10.

56. Granovsky MO, Minkoff HL, Tess BH, Waters D, Hatzakis A, Devoid DE, et al. Hepatitis C virus infection in the mothers and infants cohort study. Pediatrics 1998;102(2 Pt 1):355-9.

57. Gibb DM, Neave PE, Tookey PA, Ramsay M, Harris H, Balogun K, et al. Active surveillance of hepatitis C infection in the UK and Ireland. Arch Dis Child 2000;82(4):286-91.

58. Vogt M, Lang T, Frosner G, Klingler C, Sendl AF, Zeller A, et al. Prevalence and clinical outcome of hepatitis C infection in cildren who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341(12):866-70.

59. Jara P, Resti M, Hierro L, Giacchino R, Barbera C, Zancan L, et al. Chronic hepatitis C virus infection in childhood: clinical patterns and evolution in 224 white children. Clin Infect Dis 2003;36(3):275-80.

.

60. Wirth S, Pieper-Boustani H, Lang T, Ballauff A, Kullmer U, Gerner P, et al. Peginterferon alpha 2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. Hepatology 2005;41(5):1013-8.

61. Süoğlu DOD, Elkabes B, Sökücü S, Saner G. Does interferon and ribavirin combination treatment increase the rate of treatment response in children with hepatitis C? J Pediatr Gastroenterol Nutr 2002;34(2):199-206.

62. Wirth S, Lang T, Gehring S, Gerner P. Recombinant alpha interferon plus ribavirin in children and adolescents with chronic hepatitis C. Hepatology 2002;36(5):1280-4.

63. Christensson B, Wiebe T, Akesson A, Widell A. Interferon alpha and ribavirin treatment of hepatitis C in children with malignancy in remission. Clin Infect Dis 2000;30(3):585-6.

64. Wirth S, Ribes-Koninckx C, Calzado MA, Bortolotti F, Zancan L, Jara P, et al. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. Journal of Hepatology 2010;52(4):501-7.

65. Sokal EM, Bourgois A, Stephenne X, Silveira T, Porta G, Gardovska D, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in children and adolescents. Journal of Hepatology 2010;52(6):827-31.

66. Santantonio T, Fasano M, Sinisi E, Guastadisegni A, Casalino C, Mazzola M, et al. Efficacy of a 24-week course of PEG-interferon alpha-2b monotherapy in patients with acute hepatitis C after failure of spontaneous clearance. J Hepatol 2005;42(3):329-33.

67. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 1999;340(16):1228-33.

68. Villano SA, Vlahov D, Nelson KE, Cohn S, Thomas DL. Persistence of viremia and the importance of long-term follow-up after acute hepatitis C infection. Hepatology 1999;29(3):908-14.

69. Bianco E, Stroffolini T, Spada E, Szklo A, Marzolini F, Ragni P, et al. Case fatality rate of acute viral hepatitis in Italy: 1995-2000. An update. Dig Liver Dis 2003;35(6):404-8.

70. Moller JM, Krarup HB. Diagnosis of acute hepatitis C: anti-HCV or HCV-RNA? Scand J Gastroenterol 2003;38(5):556-8.

71. Larghi A, Zuin M, Crosignani A, Ribero ML, Pipia C, Battezzati PM, et al. Outcome of an outbreak of acute hepatitis C among healthy volunteers participating in pharmacokinetics studies. Hepatology 2002;36(4 Pt 1):993-1000.

72. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 2003;125(1):80-8.

73. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. 2002. (MMWR 2002;51(No. RR-6)). [cited 07 Jun 2013]. Available from url: http://www.cdc.gov/std/treatment/rr5106.pdf

74. Licata A, Di Bona D, Schepis F, Shahied L, Craxi A, Camma C. When and how to treat acute hepatitis C? J Hepatol 2003;39(6):1056-62.

75. Myers RP, Regimbeau C, Thevenot T, Leroy V, Mathurin P, Opolon P, et al. Interferon for acute hepatitis C. Cochrane Database of Systematic Reviews 2004, Issue 2.

76. Bain VG, Bonacini M, Govindarajan S, Ma M, Sherman M, Gibas A, et al. A multicentre study of the usefulness of liver biopsy in hepatitis C. J Viral Hepat 2004;11(4):375-82.

77. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001;357(9262):1069-75.

78. Parkes J, Guha IN, Roderick P, Rosenberg W. Performance of serum marker panels for liver fibrosis in chronic hepatitis C. J Hepatol 2006;44(3):462-74.

79. Bedossa P, Dargere, D, Paradis, V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38(6):1449-57.

80. Gilmore IT, Burroughs A, Murray-Lyon IM, Williams R, Jenkins D, Hopkins A. Indications, methods, and outcomes of percutaneous liver biopsy in England and Wales: an audit by the British Society of Gastroenterology and the Royal College of Physicians of London. Gut 1995;36(3):437-41.

81. Spycher C, Zimmermann A, Reichen J. The diagnostic value of liver biopsy. BMC Gastroenterol 2001;1:12.

82. Ryder SD, Irving WL, Jones DA, Neal KR, Underwood JC, Trent Hepatitis C Study Group. Progression of hepatic fibrosis in patients with hepatitis C: A prospective repeat liver biopsy study. Gut 2004;53(3):451-5.

83. Gebo KA, Herlong HF, Torbenson MS, Jenckes MW, Chander G, Ghanem KG, et al. Role of liver biopsy in management of chronic hepatitis C: A systematic review. Hepatology 2002;36(5 Suppl 1):S161-S72.

84. Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002;122(5):1303-13.

85. Goodgame B, Shaheen NJ, Galanko J, El-Serag HB. The risk of end stage liver disease and hepatocellular carcinoma among persons infected with hepatitis C virus: publication bias? Am J Gastroenterol 2003;98(11):2535-42.

86. Benvegnu L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: A prospective study on the incidence and hierarchy of major complications. Gut 2004;53(5):744-9.

87. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997;112(2):463-72.

88. Wright M, Goldin R, Fabre A, Lloyd J, Thomas H, Trepo C, et al. Measurement and determinants of the natural history of liver fibrosis in hepatitis C virus infection: a cross sectional and longitudinal study. Gut 2003;52(4):574-9.

89. Zarski JP, Mc Hutchison J, Bronowicki JP, Sturm N, Garcia-Kennedy R, Hodaj E, et al. Rate of natural disease progression in patients with chronic hepatitis C. J Hepatol 2003;38(3):307-14.

90. Minola E, Prati D, Suter F, Maggiolo F, Caprioli F, Sonzogni A, et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. Blood 2002;99(12):4588-91.

91. Mohsen AH, Trent HCV Study Group. The epidemiology of hepatitis C in a UK health regional population of 5.12 million. Gut 2001;48(5):707-13.

92. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997; 349(9055):825-32.

93. Wiley TE, Brown J, Chan J. Hepatitis C infection in African Americans: Its natural history and histological progression. Am J Gastroenterol 2002;97(3):700-6.

94. Sterling RK, Stravitz RT, Luketic VA, Sanyal AJ, Contos MJ, Mills AS, et al. A comparison of the spectrum of chronic hepatitis C virus between Caucasians and African Americans. Clin Gastroenterol Hepatol 2004;2(6):469-73.

95. Pessione F, Ramond MJ, Njapoum C, Duchatelle V, Degott C, Erlinger S, et al. Cigarette smoking and hepatic lesions in patients with chronic hepatitis C. Hepatology 2001;34(1):121-5.

96. Hezode C, Lonjon I, Roudot-Thoraval F, Mavier JP, Pawlotsky JM, Zafrani ES, et al. Impact of smoking on histological liver lesions in chronic hepatitis C. Gut 2003;52(1):126-9.

97. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. Cancer 2000;89(1):53-9.

98. Khan KN, Yatsuhashi H. Effect of alcohol consumption on the progression of hepatitis C virus infection and risk of hepatocellular carcinoma in Japanese patients. Alcohol Alcohol 2000;35(3):286-95.

99. Hezode C, Lonjon I, Roudot-Thoraval F, Pawlotsky JM, Zafrani ES, Dhumeaux D. Impact of moderate alcohol consumption on histological activity and fibrosis in patients with chronic hepatitis C, and specific influence of steatosis: a prospective study. Aliment Pharmacol Ther 2003;17(8):1031-7.

100. McCusker M. Influence of hepatitis C status on alcohol consumption in opiate users in treatment. Addiction 2001;96(7):1007-14.

101. Puoti C, Guido M, Mangia A, Persico M, Prati D, Committee on HCVcarriers with normal alanine aminotransferase levels of the Italian Association for the Study of the Liver. Clinical management of HCV carriers with normal aminotransferase levels. Dig Liver Dis 2003;35(5):362-9.

102. Puoti C, Castellacci R, Montagnese F, Zaltron S, Stornaiuolo G, Bergami N, et al. Histological and virological features and follow-up of hepatitis C virus carriers with normal aminotransferase levels: the Italian prospective study of the asymptomatic C carriers (ISACC). J Hepatol 2002;37(1):117-23.

103. Hui C, Belaye, T, Montegrande, K, Wright ,TL. A comparison in the progression of liver fibrosis in chronic hepatitis C between persistently normal and elevated transaminase. J Hepatol 2003;38(4):511-7.

104. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: A meta-analysis. Clin Infect Dis 2001;33(4):562-9.

105. Rullier A, Trimoulet P, Neau D, Bernard PH, Foucher J, Lacoste D, et al. Fibrosis is worse in HIV-HCV patients with low-level immunodepression referred for HCV treatment than in HCV-matched patients. Hum Pathol 2004;35(9):1088-94.

106. Yee TT, Griffioen A, Sabin CA, Dusheiko G, Lee CA. The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. Gut 2000;47(6):845-51.

107. Brau N, Salvatore M, Rios-Bedoya CF, Fernandez-Carbia A, Paronetto F, Rodriguez-Orengo JF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. J Hepatol 2006;44(1):47-55.

108. Clinical Effectiveness Group. United Kingdom National Guideline on the Management of the Viral Hepatitides A, B & C. London: British Association for Sexual Health and HIV (BASHH); 2005. [cited 07 Jun 2013]. Available from url: http://www.bashh.org/documents/117/117.pdf

109. Agence Nationale d'Accreditation et d'Evaluation en Sante. Treatment of hepatitis C. Consensus conference (in French). Presse Med 2002;31(21 Pt 1):988-98.

110. Vento S, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, Ferraro T,, E. C. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. N Engl J Med 1998;338(5):286-90.

111. Chlabicz S, Lapinski TW, Grzeszczuk A, Prokopowicz D. Four-year follow up of hepatitis C patients vaccinated against hepatitis B virus. World J Gastroenterol 2005;11(12):1798-801.

112. Sakhuja P, Malhotra V, Gondal R, Sarin SK, Thakur V. Histological profile of liver disease in patients with dual hepatitis B and C virus infection. Indian J Pathol Microbiol 2003;46(4):555-8.

113. Mariscal LF, Rodriguez-Inigo E, Bartolome J, Castillo I, Ortiz-Movilla N, Navacerrada C, et al. Hepatitis B infection of the liver in chronic hepatitis C without detectable hepatitis B virus DNA in serum. J Med Virol 2004;73(2):177-86.

114. Ioannou GN, Tung BY, Kowdley KV. Iron in hepatitis C: villain or innocent bystander? Semin Gastrointest Dis 2002;13(2):95-108.

115. Carlo C, Daniela P, Giancarlo C. Iron depletion and response to interferon in chronic hepatitis C. Hepatogastroenterology 2003;50(53):1467-71.

116. Roffi L, Redaelli A, Colloredo G, Minola E, Donada C, Picciotto A, et al. Outcome of liver disease in a large cohort of histologically proven chronic hepatitis C: influence of HCV genotype. Eur J Gastroenterol Hepatol 2001;13(5):501-6.

117. Rumi MG, De Filippi F, La Vecchia C, Donato MF, Gallus S, Del Ninno E, et al. Hepatitis C reactivation in patients with chronic infection with genotypes 1b and 2c: a retrospective cohort study of 206 untreated patients. Gut 2005;54(3):402-6.

118. Kayali Z, Buckwold VE, Zimmerman B, Schmidt WN. Hepatitis C, cryoglobulinaemia, and cirrhosis: a meta-analysis. Hepatology 2002;36(4 Pt 1):978-85.

119. Gebo KA, Jenckes MW, Chander G, Torbenson MS, Ghanem KG, Herlong HF, et al. Management of Chronic Hepatitis C. Rockville, MD: Agency for Healthcare Research and Quality; 2002. (Evidence Report/Technology Assessment No. 60). [cited 07 Jun 2013]. Available from url: http://www.ncbi.nlm.nih.gov/books/NBK36665/

120. Strader DB, Wright T, Thomas D, L., Seeff LB. Diagnosis, Management, and Treatment of Hepatitis C. Hepatology 2004;39(4):1147-71.

121. Khuroo MS, Khuroo MS, Dahab ST. Meta-analysis: a randomized trial of peginterferon plus ribavirin for the initial treatment of chronic hepatitis C genotype 4. Aliment Pharmacol Ther 2004;20(9):931-8.

122. Hadziyannis SJ, Sette H, Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140(5):346-55.

123. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b with ribavirin compared with interferon alfa-2b plus ribavirin for intial treatment of chronic hepatitis C: results of a randomised trial. Lancet 2001;358(9286):958-65.

124. Almasio PL, Venezia G, Craxi A. The impact of antiviral therapy on the course of chronic HCV infection. A systematic review. Panminerva Medica 2003;45(3):175-82.

125. Swain M, Lai MY, Shiffman ML, Cooksley WG, Abergel A, Diago M, et al. Treatment of patients with chronic hepatitis C (CHC) with peginterferon alfa-2a (40KD) (Pegasys) alone or in combination with ribavirin (copegus) results in longlasting sustained virological response. J Hepatol 2003;38(Suppl 2):175.

126. Veldt BJ, Saracco G, Boyer N, Camma C, Bellobuono A, Hopf U, et al. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. Gut 2004;53(10):1504-8.

127. Yoshida H, Arakawa Y, Sata M, Nishiguchi S, Yano M, Fujiyama S, et al. Interferon therapy prolonged life expectancy among chronic hepatitis C patients. Gastroenterology 2002;123(2):483-91.

128. Gebo KA, Chander G, Jenckes MW, Ghanem KG, Herlong HF, Torbenson MS, et al. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: a systematic review. Hepatology 2002;36(5 Suppl 1):S84-92.

129. Tsuda N, Yuki N, Mochizuki K, Nagaoka T, Yamashiro M, Omura M, et al. Long-term clinical and virological outcomes of chronic hepatitis C after successful interferon therapy. J Med Virol 2004;74(3):406-13.

130. Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, et al. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. Hepatology 2005;41(1):106-14.

131. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123(4):8.

132. Wilby KJ, Partovi N, Ford J-AE, Greanya E, Yoshida EM. Review of boceprevir and telaprevir for the treatment of chronic hepatitis C. Canadian Journal of Gastroenterology 2012;26(4):205-10.

133. Perry CM. Telaprevir: a review of its use in the management of genotype 1 chronic hepatitis C. Drugs 2012;72(5):619-41.

134. Ramachandran P, Fraser A, Agarwal K, Austin A, Brown A, Foster GR, et al. UK Consensus guidelines for the use of the protease inhibitors boceprevir and telaprevir in genotype 1 chronic hepatitis C infected patients. Aliment Pharmacol Ther 2012;35(6):647-62.

135. Trembling PM, Tanwar S, Dusheiko GM. Boceprevir: an oral protease inhibitor for the treatment of chronic HCV infection. Expert Rev Anti Infect Ther 2012;10(3):269-79.

136. Davis GL, Wong JB, McHutchison JG, Manns MP, J. H, Albrecht J. Early virological response to treatment with Peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38(3):645-52.

137. Davis G. Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002;36(5 Suppl 1):S145-51.

138. Moreno C, Deltenre P, Pawlotsky JM, Henrion J, Adler M, Mathurin P. Shortened treatment duration in treatment-naive genotype 1 HCV patients with rapid virological response: A meta-analysis. Journal of Hepatology 2010;52(1):25-31.

139. Andriulli A, Mangia A, Iacobellis A, Ippolito A, Leandro G, Zeuzem S. Meta-analysis: the outcome of anti-viral therapy in HCV genotype 2 and genotype 3 infected patients with chronic hepatitis. Aliment Pharmacol Ther 2008;28(4):397-404.

140. Slavenburg S, Weggelaar I, van Oijen MGH, Drenth JPH. Optimal length of antiviral therapy in patients with hepatitis C virus genotypes 2 and 3: a meta-analysis. Antivir Ther 2009;14(8):1139-48.

141. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. PEG-IFNa-2b and RBV for 12 vs 24 weeks in HCV G2/3. N Engl J Med 2005;352(25):2609-17.

142. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129(2):522-7.

143. D'Heygere F, George C, Van VH, Decaestecker J, Nakad A, Adler M, et al. Efficacy of interferon-based antiviral therapy in patients with chronic hepatitis C infected with genotype 5: a meta-analysis of two large prospective clinical trials. J Med Virol 2011;83(5):815-9.

144. Chao DT, Abe K, Nguyen MH. Systematic review: Epidemiology of hepatitis C genotype 6 and its management. Aliment Pharmacol Ther 2011;34(3):286-96.

145. Wright M, Forton D, Main J, Goldin R, Torok E, Tedder R, et al. Treatment of histologically mild hepatitis C virus infection with interferon and ribavirin: A multicentre randomized controlled trial. J Viral Hepat 2005;12(1):58-66.

146. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, et al. Peginterferon alfa-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology 2004;127(6):1724-32.

147. Chung RT, Andersen J, Volberding P, Robbins GK, Liu T, Sherman KE, et al. Peginterferon Alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. N Engl J Med 2004;351(5):451-9.

148. Carrat F, Bani-Sadr F, Pol S, Rosenthal E, Lunel-Fabiani F, Benzekri A, et al. Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients: A randomized controlled trial. JAMA 2004;292(23):2839-48.

149. Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-García J, Lazzarin A, et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. N Engl J Med 2004;351(5):438-50.

150. Tural C, Galeras JA, Planas R, Coll S, Sirera G, Gimenez D, et al. Differences in virological response to pegylated interferon and ribavirin between hepatitis C virus (HCV)-monoinfected and HCV-HIV-coinfected patients. Antiviral therapy 2008;13(8):1047-55.

151. Nunez M, Miralles C, Berdun MA, Losada E, Aguirrebengoa K, Ocampo A, et al. Role of weight-based ribavirin dosing and extended duration of therapy in chronic hepatitis C in HIV-infected patients: the PRESCO trial. AIDS Res Hum Retroviruses 2007;23(8):972-82.

152. Crespo M, Sauleda S, Esteban JI, Juarez A, Ribera E, Andreu AL, et al. Peginterferon alpha-2b plus ribavirin vs interferon alpha-2b plus ribavirin for chronic hepatitis C in HIV-coinfected patients. Journal of Viral Hepatitis 2007;14(4):228-38.

153. Chuang WL, Dai CY, Chang WY, Lee LP, Lin ZY, Chen SC, et al. Viral interaction and responses in chronic hepatitis C and B coinfected patients with interferon-alpha plus ribavirin combination therapy. Antivir Ther 2005;10(1):125-33.

154. Liu CJ, Chen PJ, Lai MY, Kao JH, Jeng YM, Chen DS. Ribavirin and interferon is effective for hepatitis C virus clearance in hepatitis B and C dually infected patients. Hepatology 2003;37(3):568-76.

155. Mauss S, Berger F, Goelz J, Jacob B, Schmutz G. A prospective controlled study of interferon-based therapy of chronic hepatitis C in patients on methadone maintenance. Hepatology 2004;40(1):120-4.

156. Bruggmann P, Falcato L, Dober S, Helbling B, Keiser O, Negro F, et al. Active intravenous drug use during chronic hepatitis C therapy does not reduce sustained virological response rates in adherent patients. Journal of Viral Hepatitis 2008;15:6.

157. Jack K, Willott S, Manners J, Varnam MA, Thomson BJ. Clinical trial: a primary-care-based model for the delivery of anti-viral treatment to injecting drug users infected with hepatitis C. Alimentary Pharmacology & Therapeutics 2009;29(1):38-45.

158. Melin P, Chousterman M, Fontanges T, Ouzan D, Rotily M, Lang J-P, et al. Effectiveness of chronic hepatitis C treatment in drug users in routine clinical practice: results of a prospective cohort study. European Journal of Gastroenterology & Hepatology 2010;22(9):1050-7 10.97/MEG.0b013e328338d9aa.

159. Hepburn MJ, Hepburn LM, Cantu NS, Lapeer MG, Lawitz EJ. Differences in treatment outcome for hepatitis C among ethnic groups. Am J Med 2004;117(3):163-8.

160. Loguercio C, Di Pierro M, Di Marino MP, Federico A, Disalvo D, Crafa E, et al. Drinking habits of subjects with hepatitis C virus-related chronic liver disease: prevalence and effect on clinical, virological and pathological aspects. Alcohol Alcohol 2000;35(3):296-301.

161. Mochida S, Ohnishi K, Matsuo S, Kakihara K, Fujiwara K. Effect of alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. Alcohol Clin Exp Res 1996;20(9 Suppl):371A-7A.

162. Tabone M, Sidoli L, Laudi C, Pellegrino S, Rocca G, Della Monica P, et al. Alcohol abstinence does not offset the strong negative effect of lifetime alcohol consumption on the outcome of interferon therapy. J Viral Hepat 2002;9(4):288-94.

163. Electronic Medicines Compendium. Copegus 200mg SPC. 2006.

164. Glue P, Fang JW, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, et al. Pegylated interferon-alpha2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. Clin Pharmacol Ther 2000;68(5):556-67.

165. Meyers CM, Seeff LB, Stehman-Breen CO, Hoofnagle JH. Hepatitis C and renal disease: an update. Am J Kidney Dis 2003;42(4):631-7.

166. Kraus MR, Schäfer A, Faller H, Csef H, Scheurlen M. Paroxetine for the treatment of interferon alpha induced depression in chronic hepatitis C. Aliment Pharmacol Ther 2002;16(6):1091-9.

167. Schaefer M, Schmidt F, Folwaczny C, Lorenz R, Martin G, Schindlbeck N, et al. Adherence and mental side effects during hepatitis C treatment with interferon alfa and ribavirin in psychiatric risk groups. Hepatology 2003;37(2):443-51.

168. Kiser JJ, Burton JR, Anderson PL, Everson GT. Review and management of drug interactions with boceprevir and telaprevir. Hepatology 2012;55(5):1620-8.

169. University of Liverpool. Hepatitis C drug interactions web site. [cited 20 May]. Available from url: http://www.hep-druginteractions.org/

170. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. Gastroenterology. 2003;124(6):1711-9.

171. Aspinall R, Pockros PJ. The management of side-effects during therapy for hepatitis C. Aliment Pharmacol Ther 2004;20(9):917-29.

172. Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients: A prospective, double-blind, randomized controlled study. Gastroenterology 2004;126(5):1302-11.

173. Dieterich DT, Wasserman R, Brau N, Hassanein TI, Bini EJ, Bowers PJ, et al. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. Am J Gastroenterol 2003;98(11):2491-9.

174. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339(21):1485-92.

175. Lawitz E, Zeuzem S, Nyberg LM, Nelson DR, Rossaro L, Balart LA, et al. Boceprevir (BOC) combined with peginterferon alfa-2b/ribavirin (p/rbv) in treatment-naive chronic HCV genotype 1 patients with compensated cirrhosis: sustained virologic response (SVR) and safety subanalyses from the Anemia Management Study. (Conference abstract). Hepatology 2012;56(Suppl S1):216A.

176. Sulkowski M, Roberts S, Afdhal NH, Andreone P, Diago M, Pol S, et al. Ribavirin dose modification in treatment-naive and previously treated patients who received telaprevir combination treatment: No impact on sustained virologic response in Phase 3 studies. (Conference abstract). J Hepatol 2012;56(Suppl 2):S459-S60.

177. Gopal DV, Rabkin JM, Berk BS, Corless CL, Chou S, Olyaei A, et al. Treatment of progressive hepatitis C recurrence after liver transplantation with combination interferon plus ribavirin. Liver Transpl 2001;7(3):181-90.

178. Patten SB, Barbui C. Drug-induced depression: A systematic review to inform clinical practice. Psychother Psychsom 2004;73(4):207-15.

179. Kraus MR, Schafer A, Csef H, Scheurlen M. Psychiatric side effects of pegylated interferon alfa-2b as compared to conventional interferon alfa-2b in patients with chronic hepatitis C. World J Gastroenterol 2005;11(12):1769-74.

180. Dereure O, Raison-Peyron N, Larrey D, Blanc F, Guilhou JJ. Diffuse inflammatory lesions in patients treated with interferon alfa and ribavirin for hepatitis C: a series of 20 patients. Br J Dermatol 2002;147(6):1142-6.

181. Gaeta GB, Precone DF, Felaco FM, Bruno R, Spadaro A, Stornaiuolo G, et al. Premature discontinuation of interferon plus ribavirin for adverse effects: A multicentre survey in 'real world' patients with chronic hepatitis C. Aliment Pharmacol Ther 2002;16(9):1633-9.

182. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hapatitis C virus infection. N Engl J Med 2011;364(25):2405-16.

183. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection N Engl J Med 2011;364(25):2417-28.

184. Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, et al. Response-guided telaprevir combination treatment for hepatitis C virus Infection. N Engl J Med 2011;365(11):1014-24.

185. Dalgard O, Bjoro K, Hellum K, Myrvang B, Bjoro T, Haug E, et al. Thyroid dysfunction during treatment of chronic hepatitis C with interferon alpha: no association with either interferon dosage or efficacy of therapy. J Intern Med 2002;251(5):400-6.

186. Murdolo G, Francisci D, Forini F, Baldelli F, Angeletti G, Stagni G, et al. Expression of endocrine autoantibodies in chronic hepatitis C, before and after interferon-a therapy. J Endocrinol Invest 2002;25(11):938-46.

187. Doi F, Kakizaki S, Takagi H, Murakami M, Sohara N, Otsuka T, Abe T, Mori M. Long-term outcome of interferon-alpha-induced autoimmune thyroid disorders in chronic hepatitis C. Liver Int. 2005;25(2):242-6.

188. Piche T, Schneider SM, Tran A, Benzaken S, Rampal P, Hebuterne X. Resting energy expenditure in chronic hepatitis C. J Hepatol 2000;33(4):623-7.

189. Seyam M, Freshwater DA, O'Donnell K, Mutimer DJ. Weight loss during pegylated interferon and ribavirin treatment of chronic hepatitis C*. J Viral Hepat 2005;12(5):531-5.

190. Sulkowski MS, Wasserman R, Brooks L, Ball L, Gish R. Changes in haemoglobin during interferon alpha-2b plus ribavirin combination therapy for chronic hepatitis C virus infection. J Viral Hepat 2004;11(3):243-50.

191. Nishiguchi S, Shiomi S, Enomoto M, Lee C, Jomura H, Tamori A, et al. Does ascorbic acid prevent retinopathy during interferon therapy in patients with chronic hepatitis C? J Gastroenterol 2001;36(7):486-91.

192. Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, et al. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. Gastroenterology 2004;126(4):1015-23.

193. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection. N Engl J Med 2011;364(25):2417-28.

194. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011;364(13):1207-17.

195. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.

196. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343(23):1673-80.

197. Singal AK, Singh A, Jaganmohan S, Guturu P, Mummadi R, Kuo Y, et al. Antiviral Therapy Reduces Risk of Hepatocellular Carcinoma in Patients With Hepatitis C Virus-Related Cirrhosis. Clinical Gastroenterology and Hepatology 2010;8(2):192-9.

198. Everson GT, Shiffman ML, Hoefs JC, Morgan TR, Sterling RK, Wagner DA, et al. Quantitative liver function tests improve the prediction of clinical outcomes in chronic hepatitis C: results from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis Trial. Hepatology 2012;55(4):1019-29.

199. Qu L, Chen H, Kuai X, Xu Z, Jin F, Zhou G. Effects of interferon therapy on development of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis: A meta-analysis of randomized controlled trials. Hepatology Research 2012(8):782-9 183.

200. Tanwar S, Wright M, Foster GR, Ryder SD, Mills PR, Cramp ME, et al. Randomized clinical trial: a pilot study investigating the safety and effectiveness of an escalating dose of peginterferon alpha-2a monotherapy for 48 weeks compared with standard clinical care in patients with hepatitis C cirrhosis. European Journal of Gastroenterology & Hepatology 2012;24(5):543-50.

201. Bruix J, Poynard T, Colombo M, Schiff E, Burak K, Heathcote EJL, et al. Maintenance therapy with peginterferon alfa-2b does not prevent hepatocellular carcinoma in cirrhotic patients with chronic hepatitis C. Gastroenterology 2011;140(7):1990-9.

202. Suarez F, Otero A, Gonzalez B, Gomez-Gutierrez M, Arnal F, Vazquez JL. Retransplantation for hepatitis C-related cirrhosis under long-term pegylated interferon therapy. Transplant Proc 2004;36(3):775-7.

203. Chalasani N, Manzarbeitia C, Ferenci P, Vogel W, Fontana RJ, Voigt M, et al. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. Hepatology 2005;41(2):289-98.

204. Giostra E, Kullak-Ublick GA, Keller W, Fried R, Vanlemmens C, Kraehenbuhl S, et al. Ribavirin/interferon-alpha sequential treatment of recurrent hepatitis C after liver transplantation. Transpl Int 2004;17(4):169-76.

205. Ghobrial RM, Steadman R, Gornbein J, Lassman C, Holt CD, Chen P, et al. A 10-year experience of liver transplantation for hepatitis C: analysis of factors determining outcome in over 500 patients. Ann Surg 2001;234(3):384-93.

206. Velidedeoglu E, Mange KC, Frank A, Abt P, Desai NM, Markmann JW, Reddy R,, JF. M. Factors differentially correlated with the outcome of liver transplantation in hcv+ and HCV- recipients. Transplantation 2004;77(12):1834-42.

207. Charlton M, Ruppert K, Belle SH, Bass N, Schafer D, Wiesner RH, et al. Long-term results and modeling to predict outcomes in recipients with HCV infection: Results of the NIDDK liver transplantation database. Liver Transpl 2004;10(9):1120-30.

208. Feurer ID, Wright JK, Payne JL, Kain AC, Wise PE, Hale P, et al. Effects of hepatitis C virus infection and its recurrence after liver transplantation on functional performance and health-related quality of life. J Gastrointest Surg 2002;6(1):108-15.

209. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C: a systematic review and critical analysis. Ann Intern Med 2003;139(1):46-50.

210. Tradati F, Colombo M, Mannucci PM, Rumi MG, De Fazio C, Gamba G, et al. A prospective multicenter study of hepatocellular carcinoma in Italian hemophiliacs with chronic hepatitis C. The Study Group of the Association of Italian Hemophilia Centers. Blood 1998;91(4):1173-7.

211. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. Hepatology 1997;25(3):754-8.

212. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol 2001;34(4):570-5.

213. Dieticians of Canada. Hepatitis C: nutrition care Canadian guidelines for health care providers. Can J Diet Pract Res 2003;64(3):139-41.

214. Als-Nielsen B, Koretz RL, Kjaergard LL, Gluud C. Branched-chain amino acids for hepatic encephalopathy. Cochrane Database of Systematic Reviews 2004, Issue 3.

215. Habu D, Nishiguchi S, Nakatani S, Kawamura E, Lee C, Enomoto M, et al. Effect of oral supplementation with branched-chain amino acid granules on serum albumin level in the early stage of cirrhosis: A randomized pilot trial. Hepatol Res 2003;25(3):312-8.

216. Nishiguchi S, Habu D. Effect of oral supplementation with branched-chain amino acid granules in the early stage of cirrhosis. Hepatol Res 2004;30S:36-41.

217. Gelatti U, Covolo L, Franceschini M, Pirali F, Tagger A, Ribero ML, et al. Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: A case-control study. J Hepatol 2005;42(4):528-34.

218. Inoue M, Yoshimi I, Sobue T, Tsugane S, JPHC Study Group. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. J Natl Cancer Inst 2005;97(4):293-300.

219. Takagi H, Nagamine T, Abe T, Takayama H, Sato K, Otsuka T, et al. Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. J Viral Hepat 2001;8(5):367-71.

220. Habu D, Shiomi S, Tamori A, Takeda T, Tanaka T, Kubo S, et al. Role of vitamin K2 in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. JAMA 2004;292(3):358-61.

221. Saeian K, Bajaj JS, Franco J, Knox JF, Daniel J, Peine C, et al. High-dose vitamin E supplementation does not diminish ribavirin-associated haemolysis in hepatitis C treatment with combination standard alpha-interferon and ribavirin. Aliment Pharmacol Ther 2004;20(10):1189-93.

222. Iwasa M, Kaitom M, Ikoma J, Kobayashi Y, Tanaka Y, Higuchi K, et al. Dietary iron restriction improves aminotransferase levels in chronic hepatitis C patients. Hepatogastroenterology 2002;49(44):529-31.

223. Department of Health. Dietary reference values of food energy and nutrients for the United Kingdom (Report on Health and Social Subjects 41). London: HMSO; 1991. (DoH report on Health and social Subjects 41)

224. Hu KQ, Kyulo NL, Esrailian E, Thompson K, Chase R, Hillebrand DJ, et al. Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States. J Hepatol 2004;40(1):147-54.

225. Hickman IJ, Clouston AD, Macdonald GA, Purdie DM, Prins JB, Ash S, et al. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. Gut 2002;51(1):89-94.

226. Strinko JM, Di Bisceglie AM, Hoffmann JA. A descriptive study of the relationship between mood disorders and hepatitis C treatment compliance: does nursing play a role? Issues Ment Health Nurs 2004;25(7):715-22.

227. Ahern M, Imperial J, Lam S. Impact of a designated hepatology nurse on the clinical course and quality of life of patients treated with rebetron therapy for chronic hepatitis C. Gastroenterol Nurs 2004;27(4):149-55.

228. Coughlan B, Sheehan J, Carr A, Cockram A, Crowe J. Evaluation of a brief group based psychological/educational treatment programme for women with an iatrogenic chronic hepatitis C virus infection. J Clin Psychol Med Settings 2004;11(4):303-14.

229. Tucker T, Fry CL, Lintzeris N, Baldwin S, Ritter A, Donath S, et al. Randomized controlled trial of a brief behavioural intervention for reducing hepatitis C virus risk practices among injecting drug users. Addiction 2004;99(9):1157-66.

230. Takase B, Uehata A, Fujioka T, Kondo T, Nishioka T, Isojima K, et al. Endothelial dysfunction and decreased exercise tolerance in interferon-alpha therapy in chronic hepatitis C: relation between exercise hyperemia and endothelial function. Clin Cardiol 2001;24(4):286-90.

231. Coon JT, Ernst E. Complementary and alternative therapies in the treatment of chronic hepatitis C: A systematic review. J Hepatol 2004;40(3):491-500.

232. Liu JP, Manheimer E, Tsutani K, Gluud C. Medicinal herbs for hepatitis C virus infection. Cochrane Database of Systematic Reviews 2004, Issue 2.

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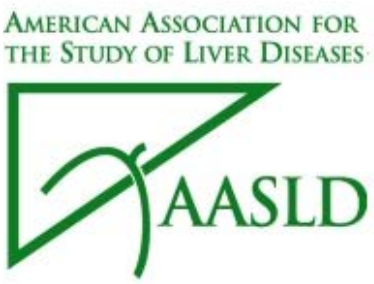

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INTRODUCTION

NOTICE: Guidance for hepatitis C treatment is changing constantly with the advent of new therapies and other developments. A static version of this guidance, such as printout of this website material, booklet, slides, and other materials, may be outdated by the time you read this. We urge you to review this guidance on this website (www.hcvguidelines.org) for the latest recommendations.

The landscape of treatment for hepatitis C virus (HCV) infection has evolved substantially since the introduction of highly effective HCV protease inhibitor therapies in 2011. The pace of change is expected to increase rapidly, as numerous new drugs with different mechanisms of action will likely become available over the next few years. To provide healthcare professionals with timely guidance as new therapies are available and integrated into HCV regimens, the Infectious Diseases Society of America (IDSA) and American Association for the Study of Liver Diseases (AASLD) have developed a web-based process for the rapid formulation and dissemination of evidence-based, expert-developed recommendations for hepatitis C management. The International Antiviral Society–USA (IAS–USA) provides the structure and assistance to sustain the process that represents the work of leading authorities in hepatitis C prevention, diagnosis, and treatment.

The AASLD/IDSA/IAS–USA hepatitis C Guidance addresses management issues ranging from testing and linkage to care, the crucial first steps toward improving health outcomes for HCV-infected persons, to the optimal treatment regimen in particular patient situations. Recommendations are based on evidence and are rapidly updated as new data from peer-reviewed evidence become available. For each treatment option, recommendations reflect the best possible management for a given patient and a given point of disease progression. Recommendations are graded with regard to the level of the evidence and strength of the recommendation. The AASLD/IDSA/IAS–USA hepatitis C Guidance is supported by the membership-based societies and not by pharmaceutical companies or other commercial interests. The Boards of Directors of AASLD and IDSA have appointed an oversight panel of 5 co-chairs and have selected panel members from the 2 societies based on their expertise in hepatitis C research and care**.** Likewise, the Guidance development process is generally consistent with that used by the IAS-USA (https://www.iasusa.org/about/program-development-policy).

This Guidance should be considered a "living document" in that new sections will be added (eg, Who and When to Initiate Treatment, and Monitoring Patients Who are On or Have Completed Therapy are coming soon) and the Guidance will be updated frequently as new information and treatments become available. This continually evolving report provides guidance on FDA-approved regimens. At times, it may also recommend off-label use of certain drugs or tests or provide guidance for regimens not yet approved by FDA. Readers should consult prescribing information and other resources for further information. Of note, the choice of treatment may, in the future, be further guided by data from cost-effectiveness studies.

Changes made on this page on March 12, 2014.

METHODS

The Guidance was developed by a panel of HCV experts in the fields of hepatology and infectious diseases, using an evidence-based review of information that is largely available to healthcare practitioners. The process and detailed methods for developing the Guidance are detailed in **Methods Table 1**. Recommendations were graded according to the strength of the recommendation and quality of the supporting evidence (see **Methods Table 2**). Commonly used abbreviations are expanded in **Methods Table 3**.

Methods Table 1. Summary of the Process and Methods for the Guidance Development

Topic	Description
Statement of Need	The introduction of direct-acting agents against HCV in 2011 has rapidly changed the treatment of HCV and the timely diagnosis of infection remains essential. This ever increasing pace of change anticipates numerous additional therapies in the next few years, requiring timely guidance on how each new development changes practice for health care professionals.
Goal of the Guidance	The goal of the Guidance is to provide up-to-date recommendations to health care practitioners on the optimal screening, management, and treatment for adults with HCV infection in the United States, considering the best available evidence. The Guidance will be updated regularly, as new data, information, and tools and treatments become available. The initial recommendations address 4 areas of priority: screening, testing, and linkage to care; initial treatment regimens in persons for whom the decision to treat has been made; retreatment regimens and considerations for persons for whom the decision to treat has been made; and treatment in unique patient populations.
Panel members	The Panel members were chosen because of their expertise in the diagnosis, management, and treatment of HCV infection in terms of research and patient care. Members from the fields of hepatology and infectious diseases are included. Members were appointed by the respective Sponsor Societies after vetting by an appointed Sponsor Society committee. At least 1 representative from the hepatitis C community serves on the Panel. The Panel chairs were appointed by the Society boards, 2 each from the Sponsor Societies and 1 representing the Collaborating Partner. All Panel chairs and members serve as volunteers (not compensated) for defined terms (3 years), which may be renewed.
Conflict of interest management	The panel was established with the goal of having no personal (ie, direct payment to the individual) financial conflicts among its chairs and among fewer than half of its members. All potential panel members were asked to disclose any relationship with a pharmaceutical, biotechnology, medical device, or health-related company or venture that may result in financial benefit. Disclosures were obtained prior to the panel member appointments and prior to the initiation of the work of the panel (October 2013). Individuals were asked to report activities and personal financial relationships/investments that were current or planned and for the preceding year (prior to October 2013). Individuals were also asked to disclose commercial funding of research activities to their institutions or organizations. Disclosures were reviewed by the HCV Guidance Chairs , which made assessments based on the conflict of interest policies of the sponsoring organizations (AASLD and IDSA) and the collaborative partner (IAS-USA). Personal and institutional financial relationships with commercial entities that

	have products in the field of hepatitis C were assessed. The following relationships were prohibited: ▪ Employment with an affected company ▪ An ownership interest in an affected company ▪ Participation in/payment for promotional or marketing activities including non-CME or speakers bureaus and lectures for affected companies The following relationships/activities were reportable but were not deemed to merit exclusion: ▪ Commercial support of Research. Due to the rapidly evolving nature of the subject matter, having individuals with expertise in the particular clinical topic was critical to developing the highest-quality and most-informed recommendations. To that end, research was not considered an unresolvable conflict if the funding of the research was paid to the institution, as opposed to the individual. In the instance of someone conducting clinical research in a community practice, research funds to the group practice were acceptable. ▪ Participation on commercial company Scientific advisory boards. Participation in advisory boards or in consultancies sponsored by the research arm of a company (eg, study design or data safety monitoring board) was considered a potential personal conflict but was not considered a criterion for exclusion. The HCV Guidance Chairs achieved a majority of panel members with no personal financial interests. The Panel met face-to-face in October 2013 and meets by conference call regularly to develop and update the Guidance. Panel members were asked to inform the group of any changes to their disclosure status and were given the opportunity to recuse themselves (or be recused) from the discussion where a perceived conflict of interest that could not be resolved existed. Financial disclosures for each Panel member can be accessed here.
Intended Audience	Medical practitioners especially those who provide care to or manage patients with hepatitis C.
Sponsors, funding, and collaborating partner	The American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) are the Sponsors of the Guidance and provide financial support. The International Antiviral Society-USA (IAS-USA) is the Collaborating Partner responsible for providing expertise and managing the Panel and the Guidance development process. Centers for Disease Control and Prevention (CDC) provided financial support for the gathering and review of evidence related to hepatitis C screening and testing recommendations and interventions to implement HCV screening in clinical settings.

Evidence identification and collection	The Guidance was developed using an evidence-based review of information that is largely available to health care practitioners. Data from the following sources are considered by Panel members when making recommendations: research published in the peer-reviewed literature or presented at major national or international scientific conferences, safety warnings from FDA or other regulatory agencies or from manufacturers, drug interaction data, prescribing information from FDA-approved products, and registration data for new products under FDA review. Unpublished or presented reports, data on file, and personal communications are generally not considered. Panel members were appointed based on their collective broad knowledge of available data and current research in the field. These experts were responsible for initially identifying and discussing relevant data, including recent reports from scientific conferences. An initial literature search was conducted on November 4, 2013, to ensure that the Panel addressed all relevant published data. A total of 3939 unique citations were retrieved. Medical subject headings and free text terms were combined to maximize retrieval of relevant citations from the PubMed, Scopus, EMBASE, and Web of Science databases. To be considered for inclusion, articles were required to have been published in English from 2010 to the present. Review articles, studies using mice or rats, and in vitro studies were excluded from consideration. The Panel members regularly monitor the field for new evidence, and the literature search is updated as needed.
Grading of the evidence and RECOMMENDATIONS	The Guidance is presented in the form of RECOMMENDATIONS. Each RECOMMENDATION is graded in terms of the level of the evidence and strength of the recommendation, using a scale adapted from the American College of Cardiology and the American Heart Association Practice Guidelines. (American Heart Association, 2014); (Shiffman, 2003) A summary of the supporting (and conflicting) evidence follows each RECOMMENDATION or set of RECOMMENDATIONS.
Data review and synthesis and preparation of RECOMMENDATIONS and supporting information	The Guidance was initially divided into 3 subsections: 1) Testing and Linkage to Care; 2) Choice of Regimen in Treatment-Naive Patients For Whom the Decision to Treat Has Been Made, and 3) Retreatment for Patients in Whom the decision to treat has been made. It was later decided to make treatment for unique patient populations a separate section. Subgroups of the panel were assigned to collect, review, and prepare initial draft RECOMMENDATIONS. Draft RECOMMENDATIONS were reviewed at the first full Panel meeting in October 2013. Subgroups of the Panel then met regularly by conference call and presented their updated RECOMMENDATIONS and supporting evidence at each of 3 full-Panel conference calls. Final approval of all RECOMMENDATIONS was made by full-Panel, general consensus. Initial recommendations and their grades were individually subject to Panel survey; panelists were given the opportunity to agree,

	disagree, and provide comment. This procedure helped identify any disagreement or inconsistency between Panel members for each recommendation. Sponsor Societies have final review and approval of each recommendation prior to release of the Guidance on the website, www.hcvguidelines.org.
Update Process	The Guidance will be expanded to cover more management issues as needed, and will be updated on an ongoing basis. Panel members will regularly monitor the field for data that may warrant modification of the Guidance. Updates may be prompted by new publications or presentations at major national or international scientific conferences, new drug approvals (or new indications, dosing formulations, or frequency of dosing), new safety warnings, or other information that may have a substantial impact on the clinical care of patients. Updated RECOMMENDATIONS and ratings, once agreed on by the full Panel and approved by the Sponsor Societies, are posted on the Guidance website.
Abbreviations	Commonly used abbreviations in the text with their expansions are listed in Methods Table 3.
Opportunity for Comments	Evidence-based comments may be submitted to the Panel by email hcvguidelines@iasusa.org, or clicking on the “Send a comment to the Panel” button on www.hcvguidelines.org/contact-us. The Panel considers evidence-based comments about the RECOMMENDATIONS, grades, and evidence summary, but should not be contacted for individual patient management questions.

Methods Table 2. Grading System Used to Rate the Level of the Evidence and Strength of the Recommendation for Each Recommendation

Recommendations are based on scientific evidence and expert opinion. Each recommended statement includes a Roman numeral (**I**, **II**, or **III**) that represents the level of the evidence that supports the recommendation, and a letter (**A**, **B**, or **C**) that represents the strength of the recommendation.

Classification	Description
Class I	Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation, procedure, or treatment is beneficial, useful, and effective
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness and efficacy of a diagnostic evaluation, procedure, or treatment
Class IIa	Weight of evidence and/or opinion is in favor of usefulness and efficacy
Class IIb	Usefulness and efficacy are less well established by evidence and/or opinion
Class III	Conditions for which there is evidence and/or general agreement that a diagnostic evaluation, procedure, or treatment is not useful and effective or if it in some cases may be harmful
Level of Evidence	**Description**
Level A*	Data derived from multiple randomized clinical trials, meta-analyses, or equivalent
Level B*	Data derived from a single randomized trial, nonrandomized studies, or equivalent
Level C	Consensus opinion of experts, case studies, or standard of care

Adapted from the American College of Cardiology and the American Heart Association Practice Guidelines. (American Heart Association, 2011); (Shiffman, 2003)

*In some situations, such as for IFN-sparing HCV treatments, randomized clinical trials with an existing standard-of-care arm cannot ethically or practicably be conducted. The US Food and Drug Administration (FDA) has suggested alternative study designs, including historical controls or immediate versus deferred, placebo-controlled trials. For additional examples and definitions see FDA link: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM225333.pdf. In those instances for which there was a single pre-determined, FDA-approved equivalency established, panel members considered the evidence as equivalent to a randomized controlled trial for levels A or B.

Methods Table 3. Commonly Used Abbreviations and Their Expansions

Abbreviation	Expansion or Notes
These terms are not expanded in text	
HCV	hepatitis C virus. In this Guidance "hepatitis C virus" and HCV refer to the virus. Hepatitis C and HCV infection or HCV disease refer to the resulting disease.
IFN	interferon alfa
PEG	peginterferon alfa
These terms are expanded at first mention in text	
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BOC	boceprevir
CBC	complete blood cell (eg, complete blood cell count)
CrCl	creatinine clearance
CTP	Child Turcotte Pugh (see below)
DAA	direct-acting antiviral
ESRD	end-stage renal disease
GFR	glomerular filtration rate
HBsAg	hepatitis B virus surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
IDU	injection drug use or user
INR	international normalized ratio
MELD	model for end-stage liver disease
MSM	men who have sex with men
OATP	organic anion-transporting polypeptide
P-gp	p-glycoprotein
RAV	resistance-associated variant
RBC	red blood cell (eg, red blood cell count)
RBV	ribavirin
RGT	response-guided therapy
RVR	rapid virologic response
sAg	surface antigen
SMV	simeprevir; used for the treatment of those with genotype 1 of hepatitis C virus (HCV) who have compensated liver disease, including cirrhosis
SOF	sofosbuvir; a nucleoside analogue used in combination with other drugs for the treatment of HCV infection
SVR12 (or 24 or 48, etc)	sustained virologic response at 12 weeks (or at 24 weeks, or at 48 weeks, etc)
TSH	thyroid-stimulating hormone
TVR	telaprevir; an antiviral agent to treat hepatitis C
US FDA	US Food and Drug Administration

Definition of Terms			
Child Turcotte Pugh	**Class A**	**Class B**	**Class C**

(CTP) classification of the severity of cirrhosis	Total points	5–6	7–9	10–15
	Factor	**1 Point**	**2 Points**	**3 Points**
	Total bilirubin (µmol/L)	<34	34–50	>50
	Serum albumin (g/L)	>35	28–35	<28
	Prothrombin time/international normalized ratio	<1.7	1.71–2.30	>2.30
	Ascites	None	Mild	Moderate to Severe
	Hepatic encephalopathy	None	Grade I–II (or suppressed with medication)	Grade III–IV (or refractory)
IFN ineligible	IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/µL, a baseline platelet count below 90,000/µL or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease			
Relapser	a person who has achieved an undetectable level of virus during a prior treatment course of PEG/RBV and relapsed after treatment was stopped			

HCV TESTING AND LINKAGE TO CARE

Expansions and notes for abbreviations used in this section can be found in Methods Table 3.

A summary of recommendations for Testing and Linkage to Care is found in the BOX.

HCV testing is recommended at least once for persons born between 1945 and 1965.

Rating: Class I, Level B

Other persons should be screened for risk factors for HCV infection, and one-time testing should be performed for all persons with behaviors, exposures, and conditions associated with an increased risk of HCV infection.

1. *Risk behaviors*

Injection-drug use (current or ever, including those who injected once)

Intranasal illicit drug use

2. *Risk exposures*

Long-term hemodialysis (ever)

Getting a tattoo in an unregulated setting

Healthcare, emergency medical, and public safety workers after needle sticks, sharps, or mucosal exposures to HCV-infected blood

Children born to HCV-infected women

Prior recipients of transfusions or organ transplants, including persons who:

- were notified that they received blood from a donor who later tested positive for HCV infection
- received a transfusion of blood or blood components, or underwent an organ transplant before July 1992
- received clotting factor concentrates produced before 1987

Persons who were ever incarcerated

3. *Other medical conditions*

HIV infection

Unexplained chronic liver disease and chronic hepatitis including elevated alanine aminotransferase levels

Rating: Class I, Level B

Of the estimated 2.7 million to 3.9 million persons (1999 to 2008 National Health and Nutrition Examination Survey data [Armstrong, 2006]) chronically infected with HCV in the United States, 45% to 85% are unaware that they are infected. (Smith, 2012) Identification of those with active infection is the first step toward improving health outcomes among persons with HCV infection and preventing transmission. (Smith, 2012); (US Preventive Services Task Force, 2013); (Centers for Disease Control and Prevention, 1998)

HCV testing is recommended in select populations based on demography, prior exposures, high-risk behaviors, and medical conditions. Recommendations for testing are based on HCV prevalence in these populations, proven benefits of care and treatment in reducing the risk of hepatocellular carcinoma and all-cause mortality, and the potential public health benefit of reducing transmission through early treatment, viral clearance, and reduced risk behaviors. (Smith, 2012); (US Preventive Services Task Force, 2013); (Centers for Disease Control and Prevention, 1998)

HCV is primarily transmitted through percutaneous exposure to blood. Other modes of transmission include mother-to-infant and contaminated devices shared for non-injection drug use; sexual transmission also occurs but generally seems to be inefficient except among HIV-infected men who have unprotected sex with men. (Schmidt, 2014) The most important risk for HCV infection is injection-drug use, accounting for at least 60% of acute HCV infections in the United States. Health-care exposures are important sources of transmission, including the receipt of blood products before 1992 (after which routine screening of blood supply was implemented), receipt of clotting factor concentrates before 1987, long-term hemodialysis, needle-stick injuries among healthcare workers, and patient-to-patient transmission resulting from poor infection control practices. Other risk factors include having been born to an HCV-infected mother, having been incarcerated, and having received a tattoo in an unregulated setting. The importance of these risk factors might differ based on geographic location and population. (US Preventive Services Task Force, 2013); (Centers for Disease Control and Prevention, 1998). An estimated 29% of incarcerated persons in North America are anti-HCV positive, supporting the recommendation to test this population for HCV. (Larney, 2013) Because of shared transmission modes, persons with HIV infection are at risk for HCV; sexual transmission is a particular risk for HIV-infected men who have unprotected sex with men. (Hosein, 2013); (van de Laar, 2010) Recent data also support testing in all cadaveric and living solid-organ donors because of the risk of HCV infection posed to the recipient. (Seem, 2013); (Lai, 2013)

In 2012, CDC expanded its guidelines originally issued in 1998 (Centers for Disease Control and Prevention, 1998) for risk-based HCV testing with a recommendation to offer a 1-time HCV test to all

persons born between 1945 and 1965 without prior ascertainment of HCV risk-factors. This recommendation was supported by evidence demonstrating that a risk-based strategy alone failed to identify more than 50% of HCV infections in part due to patient underreporting of their risk and provider limitations in ascertaining risk-factor information. Furthermore, persons in the 1945 to 1965 birth cohort accounted for nearly three-fourths of all HCV infections, with a 5-times higher prevalence (3.25%) than other persons, reflecting a higher incidence of HCV infections in the 1970s and 1980s (peaking at 230,000 versus 15,000 in 2009). A recent retrospective review showed that 68% of persons with HCV infection would have been identified through a birth-cohort testing strategy, whereas only 27% would have been screened with the risk-based approach. (Mahajan, 2013) The cost-effectiveness of 1-time birth cohort testing is comparable to that of current risk-based screening strategies. (Smith, 2012)

CDC and the US Preventive Services Task Force (USPSTF) both recommend a 1-time HCV test in asymptomatic persons belonging to the 1945 to 1965 birth cohort and other persons based on exposures, behaviors, and conditions that increase risk for HCV infection.

Annual HCV testing is recommended for persons who inject drugs and for HIV-seropositive men who have unprotected sex with men. Periodic testing should be offered to other persons with ongoing risk factors for exposure to HCV.

Rating: Class IIA, Level C

Evidence regarding the frequency of testing in persons at risk for ongoing exposure to HCV is lacking; therefore, clinicians should determine the periodicity of testing based on the risk of reinfection. Because of the high incidence of HCV infection among persons who inject drugs and among HIV-infected MSM who have unprotected sex (Aberg, 2013); (Linas, 2012); (Wandeler, 2012); (Witt, 2013); (Bravo, 2012); (Williams, 2011), at least annual HCV testing is recommended in these subgroups.

An anti-HCV test is recommended for HCV testing, and if the result is positive, current infection should be confirmed by a sensitive RNA test.

Rating: Class I, Level A

Among persons with a negative anti-HCV test who are suspected of having liver disease, testing for HCV RNA or follow-up testing for HCV antibody is recommended if exposure to HCV occurred within the past 6 months; testing for HCV RNA can also be considered in persons who are immunocompromised.

Rating: Class I, Level C

Among persons suspected of reinfection after previous spontaneous or treatment-related viral clearance, initial HCV-RNA testing is recommended because an anti-HCV test is expected to be positive.

Rating: Class I, Level C

Quantitative HCV RNA testing is recommended prior to the initiation of antiviral therapy to document the baseline level of viremia (ie, baseline viral load).

Rating: Class I, Level A

Testing for HCV genotype is recommended to guide selection of the most appropriate antiviral regimen.

Rating: Class I, Level A

If found to have positive results for anti-HCV test and negative results for HCV RNA by PCR, persons should be informed that they do not have evidence of current (active) HCV infection.

Rating: Class I, Level A

All persons recommended for HCV testing should first be tested for HCV antibody (anti-HCV) (Centers for Disease Control and Prevention [CDC], 2013); (Alter, 2003) using an FDA-approved test. FDA-approved tests include laboratory-based assays and a point-of-care assay (ie, OraQuick HCV Rapid Antibody Test [OraSure Technologies]). (Lee, 2011) The latter is an indirect immunoassay with a sensitivity and specificity similar to those of FDA-approved laboratory-based HCV antibody assays.

A positive test result for anti-HCV indicates either current (active) HCV infection (acute or chronic), past infection that has resolved, or a false-positive test result. (Pawlotsky, 2002) Therefore, an HCV nucleic acid test (NAT) to detect viremia is necessary to confirm current (active) HCV infection and guide clinical management, including initiation of HCV treatment. HCV RNA testing should also be performed in persons with a negative anti-HCV test who are either immunocompromised (eg, persons receiving chronic hemodialysis) (KDIGO, 2008) or who might have been exposed to HCV within the last 6 months (including those who are possibly reinfected after previous spontaneous or treatment-related viral clearance) because these persons may be anti-HCV negative. An FDA-approved quantitative or qualitative NAT with a detection level of 25 IU/mL or lower should be used to detect HCV RNA. **Testing and Linkage to Care Table 1** lists FDA-approved, commercially available anti-HCV screening assays. **Testing and Linkage to Care Figure 1** shows the CDC-recommended testing algorithm.

Prior to the initiation of HCV therapy, quantitative HCV RNA testing is necessary to document the baseline level of viremia (ie, viral load), because the degree of initial viral decline is a crucial marker of the effectiveness of treatment. Testing for HCV genotype helps to guide selection of the most appropriate treatment regimen. Persons who have positive results for an anti-HCV test and negative results for HCV RNA by PCR should be informed that they do not have laboratory evidence of current (active) HCV infection. Additional HCV testing is typically unnecessary. However, some practitioners or persons may seek additional testing to learn if the HCV antibody test represents a remote HCV infection that has

resolved or a false-positive result. For patients with no apparent risk for HCV infection, the likelihood of a false-positive HCV antibody test is directly related to the HCV prevalence in the tested population; false-positive test results for anti-HCV are most common for populations with a low prevalence of HCV infection. (Alter, 2003) If further testing is desired to distinguish between true positivity and biologic false positivity for HCV antibody, testing may be done with a second FDA-approved HCV antibody assay that is different from the assay used for initial antibody testing. A biologic false result should not occur with 2 different tests. (Vermeersch, 2008); (Centers for Disease Control and Prevention [CDC]), 2013) The HCV RNA test can be repeated when there is a high index of suspicion of infection or in patients with prior or ongoing risk factors for HCV infection.

Persons with current (active) HCV infection should receive education and interventions aimed at reducing progression of liver disease and preventing transmission of HCV.

Rating: Class IIa, Level B

1. *Abstinence from alcohol and, when appropriate, interventions to facilitate cessation of alcohol consumption should be advised for all persons with HCV infection.*

   Rating: Class IIa, level B

2. *Evaluation for other conditions that may accelerate liver fibrosis, including HBV and HIV infections, is recommended for all persons with HCV infection.*

   Rating: Class IIb, level B

3. *Evaluation for advanced fibrosis, using liver biopsy, imaging, or non-invasive markers, is recommended in all persons with HCV infection to facilitate an appropriate decision regarding HCV treatment strategy and determine the need for initiating additional screening measures (eg, hepatocellular carcinoma [HCC] screening).*

   Rating: Class I, Level B

4. *Vaccination against hepatitis A and hepatitis B is recommended for all persons with HCV infection who are susceptible to these types of viral hepatitis.*

   Rating: Class IIa, Level C

5. *All persons with HCV infection should be provided education on how to avoid HCV transmission to others.*

   Rating: Class I, level C

In addition to receiving therapy, HCV-infected persons should be educated about how to prevent further damage to their liver. Most important is prevention of the potential deleterious effect of alcohol. Numerous studies have found a strong association between the use of excess alcohol and the development or progression of liver fibrosis and even the development of HCC. (Poynard, 1997); (Harris, 2001); (Wiley, 1998); (Corrao, 1998); (Bellentani, 1999); (Noda, 1996); (Safdar, 2004)

Excess alcohol intake may also cause steatohepatitis. The daily consumption of more than 50 grams of alcohol has a high likelihood of worsening fibrosis. Some studies indicate that daily consumption of smaller amounts of alcohol also have a deleterious effect on the liver; however, these data are controversial. (Westin, 2002) Alcohol screening and brief interventions such as those outlined by the National Institute of Alcohol Abuse and Alcoholism (http://pubs.niaaa.nih.gov/publications/Practitioner/CliniciansGuide2005/clinicians_guide.htm) have been demonstrated to reduce alcohol consumption and episodes of binge drinking in the general population and among HCV-infected persons who consume alcohol heavily.(Whitlock, 2004); (Dieperink, 2010); (Proeschold-Bell, 2012) Persons identified as abusing alcohol and having alcohol dependence require treatment and consideration for referral to an addiction specialist.

HBV and HIV coinfection have been associated with poorer prognosis of HCV in cohort studies. (Thein, 2008); (Zarski, 1998) Due to overlapping risk factors for these infections and additional benefits of their identification and treatment, persons with HCV should be tested for HIV antibody and HBsAg using standard assays for screening (Moyer, 2013); (Centers for Disease Control and Prevention, 2008) (http://www.aafp.org/afp/2008/0315/p819.html and http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5708a1.htm) and counseled how to reduce their risk of acquiring these infections, including through HBV vaccination (see below).

Patients with obesity and metabolic syndrome having underlying insulin resistance are more prone to have nonalcoholic fatty liver disease, which is a risk factor for fibrosis progression in HCV-infected persons. (Hourigan, 1999); (Ortiz, 2002) Therefore, HCV-infected persons who are overweight or obese (defined by a body mass index 25 kg/m^2 or higher or 30 kg/m^2 or higher, respectively) should be counseled regarding strategies to reduce weight and improve insulin resistance via diet, exercise, and medical therapies. (Musso, 2010); (Shaw, 2006) Patients with HCV infection and hyperlipidemia or cardiovascular comorbidities may also benefit from various hypolipidemic drugs. Prospective studies have demonstrated the safety and efficacy of statins in patients with chronic HCV and others with compensated chronic liver disease. (Lewis, 2007) Therefore, these agents should not be withheld in HCV-infected patients.

The severity of liver disease associated with chronic HCV infection is a key factor in determining the initial and follow-up evaluation of patients. Although patients with more advanced disease generally have a lower response to HCV therapy, they are also most likely to derive the greatest survival benefit. (Ghany, 2011) A liver biopsy can provide objective, semi-quantitative information regarding the amount and pattern of collagen or scar tissue in the liver, which can assist with treatment and monitoring plans. The Metavir fibrosis score (0-4) and Ishak fibrosis score (0-6) are commonly used to score the amount of hepatic collagen. A liver biopsy can also help assess the severity of liver inflammation, or of hepatic steatosis, and help exclude competing causes of liver injury. (Kleiner, 2005) However, the procedure has a low but real risk of complications, and sampling artifact makes its serial use in most patients less desirable. (Regev, 2002) Non-invasive methods frequently used to estimate liver disease severity include a liver-directed physical exam (normal in most patients), routine blood tests (eg, serum alanine transaminase, albumin, bilirubin, international normalized ratio levels, and complete cell blood counts with platelets), serum fibrosis

marker panels, liver imaging (eg, ultrasound, computed tomography scan), and liver elastography. Simple blood tests (eg, serum aspartate aminotransferase/platelet ratio index) (Wai, 2003) (http://www.hepatitisc.uw.edu/page/clinical-calculators/apri) and assessment of liver surface nodularity and spleen size by liver ultrasound or other cross-sectional imaging modalities can help determine if patients with HCV have occult portal hypertension, which is associated with a greater likelihood of developing future hepatic complications in untreated patients. (Chou, 2013); (Rockey, 2006) Liver elastography can provide instant information regarding liver stiffness at the point-of-care but can only reliably distinguish cirrhosis from non-cirrhosis. (Castera, 2012) Since persons with known or suspected bridging fibrosis and cirrhosis are at increased risk of developing complications of advanced liver disease, they require more frequent follow up; these persons also should avoid ulcerogenic drugs and receive ongoing imaging surveillance for liver cancer and varices. (Sangiovanni, 2006); (Fontana, 2010)

Exposure to infected blood is the primary mode of HCV transmission. HCV-infected persons must be informed of the precautions needed to avoid exposing others to infected blood. This is particularly important for persons who use injection drugs, given that HCV transmission in this population primarily results from the sharing of needles and other infected implements. Recently, epidemics of acute HCV due to sexual transmission in HIV-infected men who have sex with men have also been described. (van de Laar, 2009); (Urbanus, 2009); (Fierer, 2008) **Testing and Linkage Table 2** outlines measures to avoid HCV transmission. HCV is not spread by sneezing, hugging, holding hands, coughing, or sharing eating utensils or drinking glasses, nor is it transmitted through food or water.

Evaluation by a practitioner who is prepared to provide comprehensive management, including consideration of antiviral therapy, is recommended for all persons with current (active) HCV infection.

Rating: Class IIa, level C

The definition of evaluation is: *Patient has attended a medical care visit with a practitioner able to complete a full assessment, the pros and cons of antiviral therapy have been discussed, and the patient has been transitioned into treatment, if appropriate.*

Improvement in identification of current (active) HCV infection and advances in treatment regimens will have limited impact on HCV-related morbidity and mortality without concomitant improvement in linkage to care. All patients with current HCV infection and a positive HCV RNA test result should be evaluated by a practitioner with expertise in assessment of liver disease severity and HCV treatment. Subspecialty care is required for persons with HCV infection who have advanced fibrosis/cirrhosis (stage III or above on METAVIR scale), including possible referral for consideration of liver transplantation. In the United States, only an estimated 13% to 18% of persons chronically infected with HCV receive treatment. (Holmberg, 2013) Lack of appropriate practitioner assessment and delays in linkage to care can result in negative health outcomes. Further, patients who are lost to follow-up fail to benefit from evolving evaluation and treatment options.

Commonly cited patient-related barriers to treatment initiation include contraindications to treatment (eg, medical or psychiatric comorbidities), lack of acceptance of treatment (eg, asymptomatic nature of disease, competing priorities, low treatment efficacy, and long treatment duration and adverse effects), and lack of

access to treatment (eg, cost and distance to specialist). (Khokhar, 2007); (Arora, 2011); (Clark, 2012) Common practitioner–related barriers include perceived patient-related barriers (eg, fear of adverse effects, treatment duration, cost, and effectiveness), lack of expertise in HCV treatment, lack of specialty referral resources, resistance to treating persons currently using illicit drugs or alcohol, and concern about cost of HCV treatment. (Morrill, 2005); (Reilley, 2013); (McGowan, 2013) Some possible strategies to address these barriers are listed in **Testing and Linkage to Care Table 3**. One strategy that addresses several barriers is co-localization of HCV screening, evaluation, and treatment with other medical or social services. Co-localization has already been applied to settings with a high prevalence of HCV infection (eg, correctional facilities and programs providing needle exchange, substance abuse treatment, and methadone maintenance) but is not uniformly available. (Islam, 2012); (Stein, 2012); (Bruggmann, 2013)

A strategy that addresses lack of access to specialists (a primary barrier to hepatitis C care) is participation in models involving close collaboration between primary-care practitioners and subspecialists. (Arora, 2011); (Rossaro, 2013); (Miller, 2012) Such collaborations have used telemedicine and knowledge networks to overcome geographic distances to specialists. (Arora, 2011); (Rossaro, 2013) For example, Project ECHO (Extension for Community Healthcare Outcomes [http://www.echohcvexperts.com]) uses videoconferencing to enhance primary care practitioner capacity in rendering HCV care and treatment to New Mexico's large rural and underserved population. (Arora, 2011) Through case-based learning and real-time feedback from a multidisciplinary team of specialists (ie, gastroenterology, infectious diseases, pharmacology, and psychiatry practitioners), Project ECHO has expanded access to HCV infection treatment in populations that might have otherwise remained untreated.

Additional strategies of enhancing linkage to care could be adapted from other fields, such as tuberculosis and HIV, but remain to be evaluated for HCV infection. For example, use of directly observed therapy has enhanced adherence to TB treatment, and use of case managers and patient navigators has reduced loss of follow-up in HIV care. (Govindasamy, 2012) An assessment of efficacy and comparative effectiveness of these strategies is a crucial area of future research for patients with HCV infection. Replication and expansion of best practices and new models for linkage to HCV care will also be crucial to maximize the public health impact of newer treatment paradigms.

Testing and Linkage to Care Box: Summary of Recommendations for Testing and Linkage to Care

Testing and Linkage To Care Box. Summary of Recommendations for Testing and Linkage to Care

HCV testing is recommended at least once for persons born between 1945 and 1965.

Rating: Class I, Level B

Other persons should be screened for risk factors for HCV infection, and one-time testing should be performed for all persons with behaviors, exposures, and conditions associated with an increased risk of HCV infection.

1. *Risk behaviors*

    Injection drug use (current or ever, including those who injected once)

    Intranasal illicit drug use

2. *Risk exposures*

    Long-term hemodialysis (ever)

    Getting a tattoo in an unregulated setting

    Healthcare, emergency medical, and public safety workers after needle sticks, sharps, or mucosal exposures to HCV-infected blood

    Children born to HCV-infected women

    Prior recipients of transfusions or organ transplants, including persons who:

    - were notified that they received blood from a donor who later tested positive for HCV infection
    - received a transfusion of blood or blood components, or underwent an organ transplant before July 1992

- received clotting factor concentrates produced before 1987

Persons who were ever incarcerated

3. *Other medical conditions*

HIV infection

Unexplained chronic liver disease and chronic hepatitis including elevated alanine aminotransferase levels

Rating: Class I, Level B

Annual HCV testing is recommended for persons who inject drugs and for HIV-seropositive men who have unprotected sex with men. Periodic testing should be offered to other persons with ongoing risk factors for exposure to HCV.

Rating: Class IIA, Level C

An anti-HCV test is recommended for HCV testing, and if the result is positive, current infection should be confirmed by a sensitive RNA test.

Rating: Class I, Level A

Among persons with a negative anti-HCV test who are suspected of having liver disease, testing for HCV RNA or follow-up testing for HCV antibody is recommended if exposure to HCV occurred within the past 6 months; testing for HCV RNA can also be considered in persons who are immunocompromised.

Rating: Class I, Level C

Among persons suspected of reinfection after previous spontaneous or treatment-related viral clearance, initial HCV-RNA testing is recommended because an anti-HCV test is expected to be positive.

Rating: Class I, Level C

Quantitative HCV RNA testing is recommended prior to the initiation of antiviral therapy to document the baseline level of viremia (ie, baseline viral load).

Rating: Class I, Level A

Testing for HCV genotype is recommended to guide selection of the most appropriate antiviral regimen.

Rating: Class I, Level A

If found to have positive results for anti-HCV test and negative results for HCV RNA by PCR, persons should be informed that they do not have evidence of current (active) HCV infection.

Rating: Class I, Level A

Persons with current (active) HCV infection should receive education and interventions aimed at reducing progression of liver disease and preventing transmission of HCV.

Rating: Class IIa, Level B

1. *Abstinence from alcohol and, when appropriate, interventions to facilitate cessation of alcohol consumption should be advised for all persons with HCV infection.*

   Rating: Class IIa, level B

2. *Evaluation for other conditions that may accelerate liver fibrosis, including HBV and HIV infections, is recommended for all persons with HCV infection.*

   Rating: Class IIb, level B

3. *Evaluation for advanced fibrosis is recommended using liver biopsy, imaging, or non-invasive markers in all persons with HCV infection to facilitate an appropriate decision regarding HCV treatment strategy and to determine the need for initiating additional screening measures (eg, hepatocellular carcinoma [HCC] screening).*

   Rating: Class I, Level B

4. *Vaccination against hepatitis A and hepatitis B is recommended for all persons with HCV infection who are susceptible to these types of viral hepatitis.*

   Rating: Class IIa, Level C

5. *All persons with HCV infection should be provided education on how to avoid HCV transmission to others.*

Rating: Class I, level C

Evaluation by a practitioner who is prepared to provide comprehensive management, including consideration of antiviral therapy, is recommended for all persons with current (active) HCV infection.

Rating: Class IIa, level C

Testing and Linkage to Care Table 1. FDA-approved, Commercially Available Anti-HCV Screening Assays

Assay	Manufacturer	Format
Abbott HCV EIA 2.0	Abbott	EIA (Manual)
Advia Centaur HCV	Siemens	CIA (Automated)
ARCHITECT Anti-HCV	Abbott	CMIA (Automated)
AxSYM Anti-HCV	Abbott	MEIA (Automated)
OraQuick HCV Rapid Antibody Test	OraSure	Immunochromatographic (Manual)
Ortho HCV Version 3.0 EIA	Ortho	EIA (Manual)
VITROS Anti-HCV	Ortho	CIA (Automated)

Anti-HCV = HCV antibody; EIA = enzyme immunoassay; CIA = chemiluminescent immunoassay; MEIA = microparticle enzyme immunoassay; CMIA = chemiluminescent microparticle immunoassay

Table prepared by Saleem Kamili, PhD, Centers for Disease Control and Prevention.

Testing and Linkage to Care Table 2. Measures to Prevent Transmission of HCV

Persons with HCV infection should be counseled to avoid sharing toothbrushes and dental or shaving equipment, and be cautioned to cover any bleeding wound to prevent the possibility of others coming into contact with their blood.

Persons should be counseled to stop using illicit drugs and enter substance abuse treatment. Those who continue to inject drugs should be counseled to avoid reusing or sharing syringes, needles, water, cotton, and other drug preparation equipment; use new sterile syringes and filters and disinfected cookers; clean the injection site with a new alcohol swab; and dispose of syringes and needles after one use in a safe, puncture-proof container.

Persons with HCV infection should be advised not to donate blood and to discuss HCV serostatus prior to donation of body organs, other tissue, or semen.

Persons with HIV infection and those with multiple sexual partners or sexually transmitted infections should be encouraged to use barrier precautions to prevent sexual transmission. Other persons with HCV infection should be counseled that the risk of sexual transmission is low and may not warrant barrier protection.

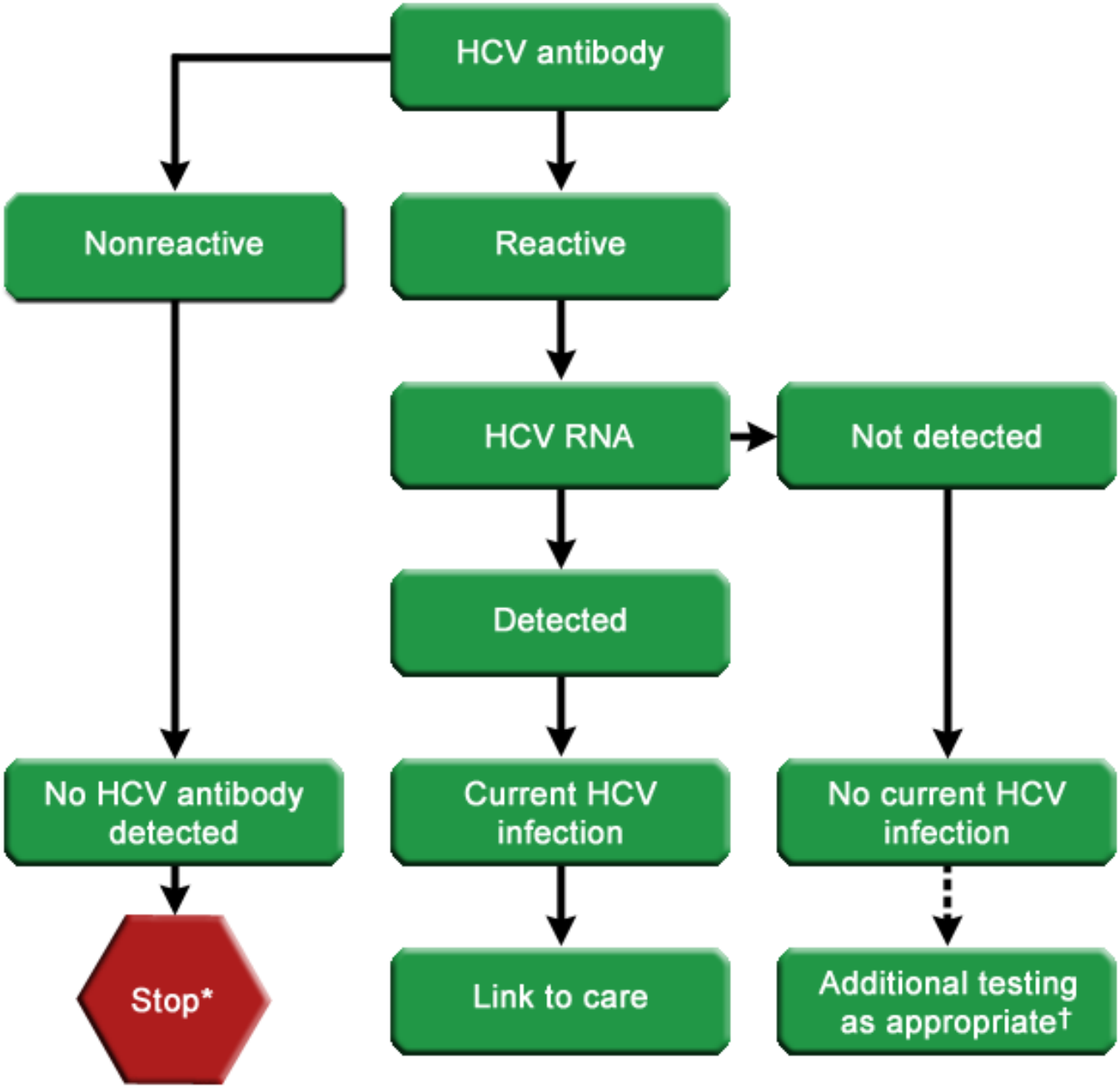
Household surfaces and implements contaminated with visible blood from an HCV-infected person should be cleaned using a dilution of 1 part household bleach to 9 parts water. Gloves should be worn when cleaning up blood spills.

Testing and Linkage to Care Table 3: Common Barriers to HCV Treatment and Potential Strategies

Barrier	Strategy
Contraindications to treatment (eg, comorbidities, substance abuse, and psychiatric disorders)	Counseling and education Referral to services (eg, psychiatry and opioid substitution therapy) Optimize treatment with simpler and less toxic regimens
Competing priority and loss to follow-up	Conduct counseling and education Engage case managers and patient navigators (HIV model) Co-localize services (eg, primary care, medical homes, and drug treatment)
Long treatment duration and adverse effects	Optimize treatment with simpler and better tolerated regimens Education and monitoring Directly observed therapy (tuberculosis model)
Lack of access to treatment (high cost, lack of insurance, geographic distance, and lack of availability of specialists)	Leverage expansion of coverage through the Patient Protection and Affordable Care Act Participate in models of care involving close collaboration between primary care practitioners and specialists

	Pharmaceutical patient assistance programs Co-localize services (primary care, medical homes, drug treatment)
Lack of practitioner expertise	Collaboration with specialists (eg, via Project ECHO-like models and telemedicine) Develop accessible and clear HCV treatment guidelines Develop electronic health record performance measures and clinical decision support tools (eg, pop-up reminders and standing orders)

Testing and Linkage to Care Figure 1. CDC Recommended Testing Sequence for Identifying Current HCV Infection



* For persons who might have been exposed to HCV within the past 6 months, testing for HCV RNA or follow-up testing for HCV antibody should be performed. For persons who are immunocompromised, testing for HCV RNA should be performed.

† To differentiate past, resolved HCV infection from biologic false positivity for HCV antibody, testing with another HCV antibody assay can be considered. Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

Adapted from Centers for Disease Control and Prevention (CDC), 2013. (Centers for Disease Control and Prevention [CDC], 2013)

WHEN AND IN WHOM TO INITIATE HCV THERAPY

Successful hepatitis C treatment results in sustained virologic response (SVR), which is tantamount to virologic cure; virologic cure is expected to benefit chronically infected persons. Limitations of workforce and societal resources may limit the feasibility of treating all patients within a short period of time. Therefore, when such limitations exist, initiation of therapy should be prioritized first to those specific populations that will derive the most benefit or have the greatest impact on further HCV transmission. Others should be treated as resources allow.

Expansions and notes for abbreviations used in this section can be found in Methods Table 3.

A summary of recommendations for When and in Whom to Initiate HCV Therapy is found in the BOX.

Goal of treatment

The goal of treatment of HCV-infected persons is to reduce all-cause mortality and liver-related health adverse consequences, including end-stage liver disease and hepatocellular carcinoma, by the achievement of virologic cure as evidenced by an SVR.

Rating: Class I, Level A

Clinical Benefit of Cure

The proximate goal of HCV therapy is SVR (virologic cure), defined as the continued absence of detectable HCV RNA at least 12 weeks after completion of therapy. SVR is a marker for cure of HCV infection and has been shown to be durable in large prospective studies in more than 99% of patients followed up for 5 years or more. (Swain, 2010); (Manns, 2013) Patients in whom an SVR is achieved have HCV antibodies, but no longer have detectable HCV RNA serum, liver tissue, or mononuclear cells, and achieve substantial liver histology improvement. (Marcellin, 1997); (Coppola, 2013); (Garcia-Bengoechea, 1999) Assessment of viral response, including documentation of SVR, requires use of US Food and Drug Administration (FDA)-approved quantitative or qualitative nucleic acid test (NAT) with a detection level of 25 IU/mL or lower.

Patients who are cured of their HCV infection experience numerous health benefits, including a decrease in liver inflammation as reflected by improved aminotransferase (ie, alanine aminotransferase [ALT], aspartate aminotransferase[AST]) levels and a reduction in the rate of progression of liver fibrosis. (Poynard, 2002b) Of 3010 treatment-naive HCV-infected patients with pretreatment and posttreatment biopsies from 4 randomized trials of 10 different IFN-based regimens (biopsies separated by a mean of 20 months), 39% to 73% of patients achieving an SVR had improvement in liver fibrosis and necrosis (Poynard, 2002b) and cirrhosis resolved in half of the cases. Portal hypertension, splenomegaly, and other clinical manifestations of advanced liver disease also improved. Among HCV-infected persons, SVR is

associated with a more than 70% reduction in the risk of liver cancer (hepatocellular carcinoma) and a 90% reduction in the risk of liver-related mortality and liver transplantation. (Morgan, 2013); (van der Meer, 2012); (Veldt, 2007)

Cure of HCV infection also reduces symptoms and mortality from severe extrahepatic manifestations, including cryoglobulinemic vasculitis, a condition affecting 10% to 15% of HCV-infected patients. (Fabrizi, 2013); (Landau, 2010) HCV-infected persons with non-Hodgkin lymphoma and other lymphoproliferative disorders achieve complete or partial remission in up to 75% of cases following successful antiviral therapy for HCV infection. (Gisbert, 2005); (Takahashi, 2012); (Svoboda, 2005); (Mazzaro, 2002); (Hermine, 2002) These reductions in disease severity contribute to dramatic reductions in all-cause mortality. (van der Meer, 2012); (Backus, 2011) Lastly, patients achieving SVR have substantially improved quality of life, which includes physical, emotional, and social health. (Neary, 1999); (Younossi, 2013) Because of the myriad benefits associated with successful HCV treatment, clinicians should treat HCV-infected patients with antiviral therapy with the goal of achieving an SVR, preferably early in the course of their chronic HCV infection before the development of severe liver disease and other complications.

Recommendations for when and in whom to initiate treatment

Treatment is recommended for patients with chronic HCV infection.

Rating: Class I, Level A

Treatment is assigned the highest priority for those patients with advanced fibrosis (Metavir F3), those with compensated cirrhosis (Metavir F4), liver transplant recipients, and patients with severe extrahepatic hepatitis C (Table 1).

Based on available resources, treatment should be prioritized as necessary so that patients at high risk for liver-related complications and severe extrahepatic hepatitis C complications are given high priority (Table 1).

Ratings: See tables

The most immediate and high-impact benefits of SVR will be realized by populations that are at the highest risk for liver-related complications due to progressive liver disease (Metavir F3 or F4) and transplant recipients or those with clinically severe extrahepatic manifestations **(**Table 1**)**.

Other populations at high risk for liver disease progression (Metavir F2) or with substantial extrahepatic manifestations **(**Table 1**)** are also expected to garner appreciable benefits, although the time course for realizing these benefits may be more protracted.

SVR will also remove the risk of further transmission. Treatment of individuals at high risk to transmit HCV to others **(**Table 2**)** may yield long-term future benefits from decreased transmission and a potential decrease in HCV disease prevalence.

When and in Whom to Initiate HCV Therapy Table 1. Settings of Liver-Related Complications and Extrahepatic Disease in Which HCV Treatment is Most Likely to Provide the Most Immediate and Impactful Benefits

Highest Priority for Treatment Owing to Highest Risk for Severe Complications

Advanced fibrosis (Metavir F3) or compensated cirrhosis (Metavir F4)

Rating: Class I, Level A

Organ transplant

Rating: Class I, Level B

Type 2 or 3 essential mixed cryoglobulinemia with end-organ manifestations (eg, vasculitis)

Rating: Class I, Level B

Proteinuria, nephrotic syndrome, or membranoproliferative glomerulonephritis

Rating: Class IIa, Level B

High Priority for Treatment Owing to High Risk for Complications

Fibrosis (Metavir F2)

Rating: Class I, level B

HIV-1 coinfection

Rating: Class I, Level B

Hepatitis B virus (HBV) coinfection

Rating: Class IIa, Level C

Other coexistent liver disease (eg, [NASH])

Rating: Class IIa, Level C

Debilitating fatigue

Rating: Class IIa, Level B

Type 2 Diabetes mellitus (insulin resistant)

Rating: Class IIa, Level B

Porphyria cutanea tarda

Rating: Class IIb, Level C

Ratings refer to the strength and level of evidence with regard to benefits of treatment in these settings.

When and in Whom to Initiate HCV Therapy Table 2. Persons Whose Risk of HCV Transmission is High and in Whom HCV Treatment May Yield Transmission Reduction Benefits

High HCV Transmission Risk*

Men who have sex with men (MSM) with high-risk sexual practices

Active injection drug users

Incarcerated persons

Persons on long-term hemodialysis

Rating: Class IIa, Level C

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*Patients at high risk of transmitting HCV should be counseled on ways to decrease transmission and minimize the risk of reinfection.

Persons with Advanced Liver Disease

For persons with advanced liver disease (Metavir stage F3 or F4), the risk of developing complications of liver disease such as hepatic decompensation (child Turcotte Pugh [CTP] Class B or C [Methods Table 3]) or hepatocellular carcinoma is substantial and may occur in a relatively short timeframe. A large prospective study of patients with cirrhosis resulting from HCV infection examined the risk of decompensation, including hepatocellular carcinoma, ascites, jaundice, bleeding, and encephalopathy, and found that the overall annual incidence rate was 3.9%. (Sangiovanni, 2006) The National Institutes of Health (NIH)-sponsored HALT–C study included a group of 220 patients with cirrhosis resulting from HCV infection who were observed for approximately 8 years. A primary outcome of death, hepatic decompensation, hepatocellular carcinoma, or advance in CTP score of 2 or higher occurred at a rate of 7.5% per year. (Everson, 2006); (Di Bisceglie, 2008) Patients with a CTP score of 7 or higher experienced a death rate of 10% per year.

Numerous studies have demonstrated that hepatitis C therapy and the achievement of an SVR in this population results in dramatic decreases in hepatic decompensation events, hepatocellular carcinoma, and liver-related mortality. (Morgan, 2013); (van der Meer, 2012); (Backus, 2011); (Dienstag, 2011); (Berenguer, 2009); (Mira, 2013) In the HALT-C study, patients with advanced fibrosis secondary to HCV infection who achieved an SVR, compared with patients with similarly advanced liver fibrosis who did not achieve an SVR, had a decreased need for liver transplantation (hazard ratio [HR], .17, 95% confidence interval [CI], .06–.46), development of liver-related morbidity and mortality (HR, .15, 95% CI, .06–.38) and hepatocellular carcinoma (HR, .19, 95% CI, .04–.80). (Dienstag, 2011) Based on these considerations, prompt HCV treatment is recommended for persons with advanced liver disease unless contraindicated (eg, hypersensitivity) or substantial nonhepatic life-limiting comorbidities are present. Importantly, persons

with advanced liver disease also require long-term follow-up and hepatocellular carcinoma (HCC) surveillance regardless of treatment outcome (see **Monitoring Section**).

Given the clinical complexity and the need for close monitoring, patients with advanced liver disease that has already decompensated (CTP Class B or C [Methods Table 3]) should, in general, be treated by physicians with experience in treating HCV in conjunction with a liver transplantation center if possible.

Persons Who Have Undergone Liver Transplantation

In HCV-infected individuals, HCV infection of the allograft occurs universally in patients in whom viral replication is ongoing at the time of transplantation. Histologic features of hepatitis develop in about 75% of recipients in the first 6 months following liver transplantation. (Neumann, 2004) By the fifth postoperative year, untreated, up to 30% have progressed to cirrhosis. (Neumann, 2004); (Charlton, 1998) A small proportion of patients (4%-7%) develop an accelerated course of liver injury (cholestatic hepatitis C, associated with very high levels of viremia) with subsequent rapid allograft failure. Recurrence of HCV infection post- transplantation has led to shorter period of graft survival for recipients with HCV infection than for recipients who undergo liver transplantation for other indications. (Forman, 2002)

Effective antiviral therapy pre-transplantation resulting in a SVR (virologic cure) prevents HCV recurrence post-transplantation. (Everson, 2003) In addition, complete HCV viral suppression prior to transplantation prevents recurrent HCV infection of the graft in the majority of cases. (Forns, 2004); (Everson, 2005) Preliminary data from a study of patients with complications of cirrhosis secondary to HCV infection who are wait-listed for liver transplantation that included patients with MELD scores up to 14 and CPT scores up to 8 found that treatment with sofosbuvir and weight-based ribavirin (RBV) for up to 48 weeks was well tolerated and was associated with an overall post-transplant SVR of 69%. (Curry, 2013b) Post-transplant SVR was near ubiquitous among patients who had undetectable HCV RNA for 28 days or longer prior to transplantation.

Treatment of established HCV infection post-transplantation also yields substantial improvements in patient and graft survival. (Berenguer, 2008); (Picciotto, 2007) The availability of effective IFN-free HCV treatment regimens has addressed the major hurdles to treating HCV recurrence post-transplantation: poor tolerability and efficacy. In a multicenter, open-label study evaluating the ability of sofosbuvir plus RBV to induce virologic suppression in 40 post–liver-transplant patients with compensated recurrence of HCV infection, daily sofosbuvir and RBV for 24 weeks achieved an SVR at 12 weeks (SVR12) in 70%. (Charlton, 2013) No deaths, graft losses, or episodes of rejection occurred. Six patients had serious adverse events, all of which were considered unrelated to study treatment. There were no drug interactions with sofosbuvir and any of the concomitant immunosuppressive agents reported. In contrast, treatment with sofosbuvir plus RBV with or without PEG in 64 patients with severe, decompensated cirrhosis resulting from recurrence of HCV infection following liver transplantation was associated with an overall SVR12 of 57% and a mortality rate of 25%. (Forns, 2013c) On an intent-to-treat basis, treatment was associated with clinical improvement in 64% and stable disease in 11% of patients.

Persons at Greater Risk of Rapidly Progressive Fibrosis and Cirrhosis

Fibrosis progression is variable across different patient populations as well as within the same individual over time. Many of the components that determine fibrosis progression and development of cirrhosis in an individual are unknown. However, certain factors, such as coinfection with HIV or hepatitis B virus (HBV) and prevalent coexistent liver diseases (eg, nonalcoholic steatohepatitis [NASH]), are well-recognized contributors to accelerated fibrosis progression. Patients with these conditions should be prioritized for hepatitis C therapy.

HIV coinfection. HIV coinfection accelerates fibrosis progression among HCV-infected persons, (Benhamou, 1999); (Macias, 2009); (Konerman, 2014) although control of HIV replication and restoration of CD4+ cell counts may mitigate this to some extent. (Benhamou, 2001); (Bräu, 2006) In the largest paired biopsy study, 282 HIV/HCV-coinfected patients with 435 paired biopsies were prospectively evaluated; (Konerman, 2014) one-third of patients showed fibrosis progression of at least 1 Metavir stage at a median of 2.5 years. Importantly, 45% of patients with no fibrosis on initial biopsy had progression. Finally, a more rapid progression to death following decompensation, combined with a lack of widespread access to liver transplantation and poor outcomes following transplantation, argue for treatment prioritization in this population, regardless of the current fibrosis stage. (Pineda, 2005); (Merchante, 2006); (Terrault, 2012)

HBV coinfection and other coexistent liver diseases. The prevalence of HBV/HCV coinfection is estimated at 1.4% in the United States and 5% to 10% globally. (Tyson, 2013); (Chu, 2008) Persons with HBV/HCV coinfection and detectable viremia of both viruses are at increased risk for disease progression, decompensated liver disease, and the development of hepatocellular carcinoma.

HBV/HCV coinfected individuals are susceptible to a process called viral interference wherein one virus may interfere with the replication of the other virus. Thus, when treating one or both viruses with antiviral drugs, it is prudent to periodically retest HBV DNA and HCV RNA levels during and after therapy, particularly if only one of the viruses is being treated at a time. Treatment of HCV in such cases utilizes the same genotype-specific regimens as are recommended for HCV monoinfection **(see** Treatment Section**)**. HBV infections in such cases should be treated as recommended for HBV monoinfection. (Lok, 2009)

Persons with other chronic liver diseases who have coincident chronic HCV infection should be considered for hepatitis C therapy, given the potential for rapid progression of liver disease. An IFN-free regimen is generally preferred for immune-mediated liver diseases such as autoimmune hepatitis because of the potential for IFN to exacerbate them.

Persons with Severe Extrahepatic Manifestations of Chronic HCV Infection

Chronic hepatitis C is associated with a syndrome of cryoglobulinemia and an immune complex and lymphoproliferative disorder that produces arthralgias, fatigue, palpable purpura, renal disease (eg, membranoproliferative glomerulonephritis), neurologic disease (eg, peripheral neuropathy, central nervous system vasculitis), and reduced complement levels. (Agnello, 1992) Because patients with chronic hepatitis C frequently have laboratory evidence of cryoglobulins (more than 50% in some series), antiviral treatment should be prioritized for those with the syndrome of cryoglobulinemia and symptoms or objective evidence of end-organ manifestations. IFN-based regimens can produce clinical remission; however, the adverse effects of IFN may mimic manifestations of cryoglobulinemia. (Saadoun, 2014) Although clinical data are not yet available, the use of IFN-free direct-acting antiviral (DAA) regimens is an attractive alternative for these patients. Organ-threatening disease (eg, severe neuropathy, renal failure, digital ischemia), in addition to the antiviral HCV therapy, should also be treated more acutely with immunosuppressive agents or plasmapheresis to clear immune complexes.

Glomerular disease results from deposition of HCV-related immune complexes in the glomeruli. (Johnson, 1993) Successful treatment of HCV using IFN-based regimens can reverse proteinuria and the nephrotic syndrome, but usually does not fully ameliorate azotemia. (Johnson, 1994) No clinical trial data are yet available using IFN-free regimens, but the high rates of SVR (virologic cure) using antiviral therapy support their use in management of hepatitis C-related renal disease and cryoglobulinemia.

The relationship between chronic hepatitis C and diabetes (most notably type 2 diabetes and insulin

resistance) is complex and incompletely understood. The prevalence and incidence of diabetes is increased in the context of hepatitis C. (White, 2008) In the United States, type 2 diabetes occurs more frequently in HCV-infected patients with a more than 3-fold greater risk in persons over 40 years of age. (Mehta, 2000) The positive correlation between quantity of plasma HCV RNA and established markers of insulin resistance confirms this relationship. (Yoneda, 2007) Insulin resistance and type 2 diabetes are independent predictors of a more rapid progression of liver fibrosis and impaired response to IFN-based therapy. (Petta, 2008) Patients with type 2 diabetes and insulin resistance also are at increased risk for hepatocellular carcinoma. (Hung, 2010)

Successful antiviral treatment has been associated with improved markers of insulin resistance and greatly reduced incidence of new onset of type 2 diabetes and insulin resistance in HCV-infected patients. (Arase, 2009) Most recently, antiviral therapy for HCV infection has been shown to improve clinical outcomes related to diabetes. In a large prospective cohort from Taiwan, the incidence of ESRD, ischemic stroke, and acute coronary syndrome were greatly reduced in HCV-infected patients with diabetes who received antiviral therapy compared with untreated, matched controls. (Hsu, 2014) Therefore, antiviral therapy in patients with pre-diabetes who have hepatitis C may prevent progression to diabetes and reduce renal and cardiovascular complications in hepatitis C patients with established diabetes.

Persons with Other Extrahepatic Manifestations of HCV Infection

In patients with chronic hepatitis C, fatigue is the most frequently reported symptom and has a major effect on quality of life and activity level evidenced by numerous measures of impaired quality of life. (Foster, 1998) The presence and severity of fatigue appears to correlate poorly with disease activity, although it may be more common and severe in HCV-infected individuals with cirrhosis. (Poynard, 2002a) Despite difficulties in separating fatigue symptoms associated with hepatitis C from those associated with other concurrent conditions (eg, anemia, depression), numerous studies have reported a reduction in fatigue after cure of HCV. (Bonkovsky, 2007) In the Virahep-C study, 401 HCV patients were evaluated for fatigue prior to treatment and after therapy using validated scales assessing presence and severity of fatigue. (Sarkar, 2012) At baseline, 52% of patients reported having fatigue, which was more frequent and severe in patients with cirrhosis than in those without cirrhosis. Achieving an SVR was associated with a substantial decrease in frequency and severity of fatigue. A recent analysis of 413 patients who achieved an SVR12 from the NEUTRINO and FUSION trials treated with a sofosbuvir-containing regimen demonstrated improvement in patient fatigue (present in 12%) from the pretreatment level. (Younossi, 2014) After achieving an SVR12, participants had marked improvement in fatigue over their pretreatment scores using 3 separate validated questionnaires.

The reported prevalence of HCV infection in patients with porphyria cutanea tarda approximates 50% and occurs disproportionately in those with cirrhosis. (Gisbert, 2003) The treatment of choice for active porphyria cutanea tarda is iron reduction by phlebotomy and maintenance of a mildly iron-reduced state without anemia. However, although improvement of porphyria cutanea tarda during HCV treatment with IFN has frequently been described (Takikawa, 1995), there are currently insufficient data to determine whether treating HCV infection with DAAs and achieving an SVR improves porphyria cutanea tarda.

Lichen planus is characterized by pruritic papules involving mucous membranes, hair, and nails. Antibodies to HCV are present in 10% to 40% of patients with lichen planus, but the causal link with chronic infection is not established. Resolution of lichen planus has been reported with IFN-based regimens, but there have also been reports of exacerbation of lichen planus with these treatments. Although it is unknown whether DAAs will have more success against lichen planus, treatment with IFN-free regimens would appear to be a more advisable approach to addressing this disorder. (Gumber, 1995)

Persons at High Risk of Transmitting HCV

Persons who have successfully achieved an SVR (virologic cure) no longer transmit the virus to others. As such, successful treatment benefits public health. Several health models have shown that even modest increases in successful treatment of HCV infection among persons who inject drugs can decrease prevalence and incidence. (Martin, 2013a); (Durier, 2012); (Martin, 2013b); (Hellard, 2012) Models developed to estimate the impact of HCV testing and treatment on the burden of hepatitis C at a country level reveal that large decreases in HCV prevalence and incidence are possible as more persons are successfully treated. (Wedemeyer, 2014) There are also benefits between couples and among families to eradicating HCV infection and thus eliminating the perception that an individual might be contagious. The safety and efficacy of treating pregnant women to prevent transmission to the fetus have not yet been established.

Successful treatment of HCV-infected persons at greatest risk for transmission represents a formidable tool to help stop HCV transmission in those who continue to engage in high-risk behaviors. To guide implementation of hepatitis C treatment as a prevention strategy, studies are needed to define the best candidates for treatment to stop transmission; the additional interventions needed to maximize the benefits of HCV treatment (eg, preventing reinfection), and the cost effectiveness of the strategies when used in the target populations.

Persons who inject drugs. Injection drug use is the most common risk factor for HCV infection in the United States and Europe with an HCV seroprevalence from 10% to 70%; (Amon, 2008); (Nelson, 2011) injection drug use also accounts for the majority of new infections (approximately 70%) and is the key driving force in perpetuation of the epidemic. Given these facts, and the absence of an effective vaccine against HCV, testing and linkage to care combined with treatment of HCV infection using potent IFN-free regimens has the potential to dramatically decrease HCV incidence and prevalence. (Martin, 2013b) However, treatment-based strategies to prevent HCV transmission have yet to be studied, including how to integrate hepatitis C treatment with other risk reduction strategies including opiate substitution therapy and needle and syringe exchange programs. (Martin, 2013a)

Studies of IFN-containing treatments in persons who inject drugs have shown comparable adherence and efficacy rates to patients who do not use injection drugs. A recent meta-analysis of treatment in active or recent injection drug users with PEG with or without RBV showed SVR rates of 37% and 67% for genotype 1 or 4 and 2 or 3, respectively. (Aspinall, 2013) As shorter, better-tolerated, more-efficacious IFN-free therapies are introduced, these SVR rates are expected to improve. Importantly, the rate of reinfection in this population is lower (2.4/100 person years of observation) than incident infection in the injection drug user population in general (6.1-27.2/100 person years); though reinfection increases with active or ongoing injection drug use (6.44/100 person years) and available data are limited in follow-up duration. (Aspinall, 2013); (Grady, 2013)

Ideally, treatment of HCV-infected persons who inject drugs should be delivered in a multidisciplinary care setting with services to reduce the risk of reinfection and for management of the common social and psychiatric comorbidities in this population. Regardless of the treatment setting, recent and active injection drug use should not be seen as an absolute contraindication to HCV therapy. Scale up of HCV treatment in persons who inject drugs is necessary to positively impact the HCV epidemic in the United States and globally.

HIV-infected men who have sex with men (MSM) with high-risk sexual practices. Over the past decade a dramatic increase in incident HCV infections has been demonstrated in several US cities among

HIV-infected MSM who did not report injection drug use as a risk factor. (van de Laar, 2010) Recognition and treatment of HCV (including acute infection) in this population may represent an important step in preventing subsequent infections. As with persons who inject drugs, HIV-infected MSM with ongoing high-risk sexual practices should be treated for their HCV infection in conjunction with continued education on risk reduction strategies. In particular, safer sex strategies should be emphasized given the high rates of reinfection after SVR, which may approach 30% over 2 years, in HIV-infected MSM with acute HCV infection. (Lambers, 2011)

Incarcerated persons. Among incarcerated individuals, the HCV seroprevalence ranges from 30% to 60% (Post, 2013) and an acute infection rate of about 1%. (Larney, 2013) Screening for HCV is relatively uncommon in state prison systems. Treatment uptake has been limited in part because of the toxic effects and long treatment duration of older IFN-based therapies as well as concerns about cost. (Spaulding, 2006) In particular, truncation of treatment owing to release from prison during therapy has been cited as a major limitation to widespread, effective HCV treatment in correctional facilities. (Post, 2013); (Chew, 2009) Shorter (12-week to 24-week) HCV therapies reduce duration of stay-related barriers to HCV treatment in prisons. Likewise, the improved safety of newer, all-oral regimens diminishes toxicity concerns. Coordinated treatment efforts within prison systems would likely rapidly decrease the prevalence of HCV infection in this at-risk population, although research is needed in this area.

Persons on hemodialysis. The prevalence of HCV infection is markedly elevated in persons on hemodialysis and ranged from 2.6 to 22.9% in a large multinational study. (Fissell, 2004) Studies in the United States found a similarly elevated prevalence of 7.8% to 8.9%. (Centers for Disease Control and Prevention, 2001); (Finelli, 2005) Importantly, the seroprevalence of HCV was found to increase with time on dialysis suggesting that nosocomial transmission, among other risk factors, plays a role in HCV acquisition in these patients. (Fissell, 2004) Improved education and strict adherence to universal precautions can drastically reduce nosocomial HCV transmission risks for persons on hemodialysis, (Jadoul, 1998) but clearance of HCV viremia through treatment-induced SVR eliminates the potential for transmission.

HCV-infected persons on hemodialysis have a decreased quality of life and increased mortality compared with persons on hemodialysis without HCV infection. (Fabrizi, 2002); (Fabrizi, 2007); (Fabrizi, 2009) HCV infection in this population also has a deleterious impact on kidney transplantation outcomes with decreased patient and graft survival. (Fabrizi, 2014) The increased risk for nosocomial transmission combined with the substantial clinical impact of HCV infection in those on hemodialysis suggest that this group should also be prioritized for HCV therapy as effective antiviral regimens that can be used in advanced renal failure become available.

For all these populations, the decision to treat should be based on a favorable risk-benefit ratio taking into account the anticipated reduction in transmission(s) versus the likelihood of reinfection.

Populations Unlikely to Benefit from HCV Treatment

Patients with limited life expectancy for whom HCV therapy would not improve symptoms or prognosis do not require treatment. Chronic hepatitis C is associated with a wide range of comorbid conditions. (Butt, 2011); (Louie, 2012) Little evidence exists to support initiation of HCV treatment in patients with limited life expectancy (less than 12 months) due to non–liver-related comorbid conditions. For these patients, the benefits of HCV treatment are unlikely to be realized, and palliative care strategies should take precedence. (Holmes, 2006); (Maddison, 2011)

Recommendations for pretreatment assessment

An assessment of the degree of hepatic fibrosis, using noninvasive testing or liver biopsy, is recommended.

Rating: Class I, Level A

An accurate assessment of fibrosis is vital in assessing the urgency for treatment. The degree of hepatic fibrosis is one of the most robust prognostic factors used to predict disease progression and clinical outcomes. (Everhart, 2010) Those with substantial fibrosis (defined as Metavir F2 or higher) should be given priority for therapy in an effort to decrease the risk of clinical consequences such as cirrhosis, liver failure, and hepatocellular cancer. However, those with severe fibrosis (Metavir stage F3 and F4) are most in need of immediate therapy. In addition to urgency for antiviral therapy, individuals with severe fibrosis require surveillance monitoring for liver cancer, esophageal varices, and hepatic function. (Garcia-Tsao, 2007); (Bruix, 2011)

Although liver biopsy is the diagnostic standard, sampling error and observer variability limit test performance, particularly when inadequate sampling occurs. Up to one-third of bilobar biopsies had a difference of at least 1 stage between the lobes. (Bedossa, 2003) In addition, the test is invasive and minor complications are common, limiting patient and practitioner acceptance. Serious complications such as bleeding, although rare, are well recognized.

Noninvasive tests to stage the degree of fibrosis in patients with chronic HCV infection include models incorporating indirect serum biomarkers (routine tests), direct serum biomarkers (components of the extracellular matrix produced by activated hepatic stellate cells), and vibration-controlled transient liver elastography. No single method is recognized to have high accuracy alone and each test must be interpreted carefully. A recent publication of the Agency for Healthcare Research and Quality found evidence in support of a number of blood tests; however, at best they are only moderately useful for identifying clinically significant fibrosis or cirrhosis. (Selph, 2014)

Vibration-controlled transient liver elastography is a noninvasive way to measure liver stiffness and correlates well with measurement of substantial fibrosis or cirrhosis in patients with chronic HCV infection. A cutoff value of 8.7 kPa correlates with Metavir F2 or higher fibrosis stage; greater than 9.5 kPa with F3; and 14.5 or higher kPa with F4 or cirrhosis. The measurement range does overlap between stages. (Ziol, 2005)

The most efficient approach to fibrosis assessment is to combine direct biomarkers and vibration-controlled transient liver elastography. (Boursier, 2012) A biopsy should be considered for any patient who has discordant results between the 2 modalities that would affect clinical decision making. For example, 1 shows cirrhosis and the other does not. The need for liver biopsy with this approach is markedly reduced.

Alternatively, if direct biomarkers or vibration-controlled transient liver elastography are not available, the aspartate aminotransferase-to-platelet ratio index (APRI) or fibrosis-4 index (FIB-4) can help identify those most likely to have F3 or F4 fibrosis stage. (Sebastiani, 2009); (Castera, 2010); (Chou, 2013b) An APRI above 2.0 or FIB-4 above 3.25 has a high specificity for advanced fibrosis or cirrhosis, although neither test

is sensitive enough to rule out substantial fibrosis if values are below these thresholds. (Chou, 2013b) Biopsy should be considered in those in whom more accurate fibrosis staging would impact treatment decisions.

Individuals with clinically evident cirrhosis do not require additional staging (biopsy or noninvasive assessment).

Recommendation for repeat liver disease assessment

Ongoing assessment of liver disease is recommended for persons in whom therapy is deferred.

Rating: Class I, Level C

When therapy is deferred, it is especially important to monitor liver disease in these patients. Among individuals with less-advanced stages of fibrosis, fibrosis progression over time will help determine the urgency of subsequent antiviral therapy. Fibrosis progression varies markedly between individuals based on host, environmental, and viral factors (**Table 3**). (Feld, 2006) Fibrosis may not progress linearly. Some individuals (often those who are aged 50 years or older) may progress slowly for many years followed by an acceleration of fibrosis progression. Others may never develop substantial liver fibrosis despite longstanding infection. The presence of existing fibrosis is a strong risk factor for future fibrosis progression. Fibrosis results from chronic hepatic necroinflammation and thus a higher activity grade on liver biopsy and higher serum transaminase values is associated with more rapid fibrosis progression. (Ghany, 2003) However, even patients with normal alanine aminotransferase (ALT) levels may develop substantial liver fibrosis over time. (Pradat, 2002); (Nutt, 2000)

Host factors associated with more rapid fibrosis progression include male sex, longer duration of infection, and older age at the time of infection. (Poynard, 2001) Many patients have concomitant nonalcoholic fatty liver disease, and the presence of hepatic steatosis with or without steatohepatitis on liver biopsy as well as elevated body mass index and insulin resistance are associated with fibrosis progression, as is iron overload. (Konerman, 2014); (Everhart, 2009) Chronic alcohol use is an important risk factor because alcohol consumption has been associated with more rapid fibrosis progression. (Feld, 2006) A safe amount of alcohol consumption has not been established. Cigarette smoking may also lead to more rapid fibrosis progression.

Immunosuppression leads to more rapid fibrosis progression, particularly HIV coinfection and solid organ transplantation. (Macias, 2009); (Konerman, 2014); (Berenguer, 2013) Therefore, immunocompromised patients should be prioritized for antiviral therapy even if they have mild liver fibrosis at presentation.

The level of virus in the serum (HCV RNA) is not highly correlated with the stage of disease (degree of inflammation or fibrosis). Available data suggest that fibrosis progression occurs most rapidly in patients with genotype 3 HCV infection. (Kanwal, 2014) (Bochud, 2009) Aside from coinfections with HBV or HIV, no other viral factors are consistently associated with disease progression.

Although an ideal interval for assessment has not been established, annual evaluation is appropriate to

discuss modifiable risk factors and update testing for hepatic function and markers for disease progression. For all individuals with advanced fibrosis, liver cancer screening dictates a minimum of every 6 months evaluation.

When and in Whom to Initiate HCV Therapy Table 3. Factors Associated with Accelerated Fibrosis Progression

Host	Viral
Non-Modifiable Fibrosis stage Inflammation grade Older age at time of infection Male sex Organ transplant **Modifiable** Alcohol consumption Nonalcoholic fatty liver disease Obesity Insulin resistance	Genotype 3 Coinfection with hepatisis B virus (HBV) or HIV

When and in Whom to Initiate HCV Therapy Box. Summary of Recommendations for When and in Whom to Initiate HCV Therapy

When and in Whom to Initiate HCV Therapy Box. Summary of Recommendations for When and in Whom to Initiate HCV Therapy

Goal of treatment

The goal of treatment of HCV-infected persons is to reduce all-cause mortality and liver-related health adverse consequences, including end-stage liver disease and hepatocellular carcinoma, by the achievement of virologic cure as evidenced by an SVR.

Rating: Class I, Level A

Recommendations for when and in whom to initiate treatment

Treatment is recommended for patients with chronic HCV infection.

Rating: Class I, Level A

Treatment is assigned the highest priority for those patients with advanced fibrosis (Metavir F3), those with compensated cirrhosis (Metavir F4), liver transplant recipients, and patients with severe extrahepatic hepatitis C (Table 1).

Based on available resources, treatment should be prioritized as necessary so that patients at high risk for liver-related complications and severe extrahepatic hepatitis C complications are given high priority (Table 1).

Ratings: See tables

Recommendations for pretreatment assessment

An assessment of the degree of hepatic fibrosis, using noninvasive testing or liver

biopsy, is recommended.

Rating: Class I, Level A

Recommendation for repeat liver disease assessment

Ongoing assessment of liver disease is recommended for persons in whom therapy is deferred.

Rating: Class I, Level C

Table 1. Settings of Liver-Related Complications and Extrahepatic Disease in Which HCV Treatment is Most Likely to Provide the Most Immediate and Impactful Benefits

Highest Priority for Treatment Owing to Highest Risk for Severe Complications
Advanced fibrosis (Metavir F3) or compensated cirrhosis (Metavir F4) **Rating:** Class I, Level A
Organ transplant **Rating:** Class I, Level B
Type 2 or 3 essential mixed cryoglobulinemia with end-organ manifestations (eg, vasculitis) **Rating:** Class I, Level B
Proteinuria, nephrotic syndrome, or membranoproliferative glomerulonephritis **Rating:** Class IIa, Level B

High Priority for Treatment Owing to High Risk for Complications
Fibrosis (Metavir F2) **Rating:** Class I, level B **HIV-1 coinfection** **Rating:** Class I, Level B **HBV coinfection** **Rating:** Class IIa, Level C **Other coexistent liver disease (eg, NASH)** **Rating:** Class IIa, Level C **Debilitating fatigue** **Rating:** Class IIa, Level B **Type 2 Diabetes mellitus (insulin resistant)** **Rating:** Class IIa, Level B **Porphyria cutanea tarda** **Rating:** Class IIb, Level C

Ratings refer to the strength and level of evidence with regard to benefits of treatment in these settings.

Table 2. Persons Whose Risk of HCV Transmission is High and in Whom HCV Treatment May Yield Transmission Reduction Benefits

High HCV Transmission Risk*

MSM with high-risk sexual practices

Active injection drug users

Incarcerated persons

Persons on long-term hemodialysis

Rating: Class IIa, Level C

*Patients at high risk of transmitting HCV should be counseled on ways to decrease transmission and minimize the risk of reinfection.

Table 3. Factors Associated with Accelerated Fibrosis Progression

Host	Viral
Non-Modifiable	Genotype 3
Fibrosis stage	Coinfection with HBV or HIV
Inflammation grade	
Older age at time of infection	
Male sex	
Organ transplant	
Modifiable	
Alcohol consumption	
Nonalcoholic fatty liver disease	
Obesity	
Insulin resistance	

INITIAL TREATMENT OF HCV INFECTION IN PATIENTS STARTING TREATMENT

*/

Expansions and notes for abbreviations used in this section can be found in Methods Table 3.

A summary of recommendations for initial treatment is found in the BOX***.***

This section provides guidance on the recommended initial treatments for persons with chronic HCV infection who are naive to HCV treatment or who have achieved an undetectable level of virus during a prior treatment course of PEG/RBV and relapsed (relapsers). Although PEG/RBV relapsers are being retreated, their treatment recommendations are presently the same as for persons being treated for the first time as described below. This section assumes that ***a decision to treat has been made*** and provides guidance regarding optimal treatment. In many instances, however, it may be advisable to delay treatment for some patients with documented early fibrosis stage (F 0-2), because waiting for future highly effective, pangenotypic, DAA combinations in IFN-free regimens may be prudent. Potential advantages of waiting to begin treatment will be provided in a future update to this guidance.

The level of evidence available to inform the best treatment decisions for each patient varies, as does the strength of the recommendation, and is graded accordingly (see **Methods Table 2**). In addition, when treatment differs for a particular group, such as those infected with specific HCV genotypes, specific recommendations are given. A regimen is classified as either "Recommended" when it is favored for most patients or "Alternative" when optimal in a particular subset of patients in that category. When a treatment is clearly inferior or is deemed harmful, it is classified as "Not Recommended." Unless otherwise indicated, such regimens should not be administered to patients with HCV infection. Specific considerations of persons with HIV/HCV coinfection, compensated and decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C), post-liver transplant HCV, and those with severe renal impairment or ESRD are addressed in other sections of the document.

As always, patients receiving antiviral therapy require careful pretreatment assessment for comborbidities that may influence treatment response. All patients should have careful monitoring during treatment, particularly for anemia if ribavirin is included in the regimen.

I. Genotype 1

Recommended regimen for treatment-naive patients with HCV genotype 1 who are eligible to receive IFN.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is recommended for IFN-eligible persons with HCV genotype 1 infection, regardless of subtype.

Rating: Class I, Level A

Sofosbuvir is a prodrug of a nucleotide analogue inhibitor of the HCV NS5B RNA-dependent RNA polymerase. The phase 3 NEUTRINO trial evaluated sofosbuvir (400 mg daily) in combination with PEG (2a) (180 ?g by subcutaneous injection weekly) and weight-based RBV (1000 mg to 1200 mg daily) for 12 weeks in 291 treatment-naive patients with chronic HCV genotype 1 infection. (Lawitz, 2013b) The SVR12 for patients with genotype 1 infection was 89%. SVR12 did not differ substantially by baseline characteristic but was lower in patients with cirrhosis (80%) than in those without cirrhosis (92%). (Lawitz, 2013b)

Recommended regimen for treatment-naive patients with HCV genotype 1 who are not eligible to receive IFN.

Daily sofosbuvir (400 mg) plus simeprevir (150 mg), with or without weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] for 12 weeks is recommended for IFN-ineligible IFN ineligible is defined as one or more of the below:
• Intolerance to IFN
• Autoimmune hepatitis and other autoimmune disorders
• Hypersensitivity to PEG or any of its components
• Decompensated hepatic disease
• Major uncontrolled depressive illness
• A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL
• A history of preexisting cardiac disease **patients with HCV genotype 1 infection, regardless of subtype.**

Rating: Class I, Level B

COSMOS is an ongoing phase 2 clinical trial of sofosbuvir (400 mg daily) plus simeprevir (150 mg daily), a specific inhibitor of the HCV NS3/4A serine protease, with or without RBV for 12 or 24 weeks. (Jacobson, 2013b) The study enrolled 2 cohorts: cohort 1 included patients with a prior null response to PEG/RBV with Metavir fibrosis stage of 0 or 2 (n=80); Cohort 2 included patients who were either treatment-naive or had a prior null response with Metavir fibrosis stage of 3 or 4 (n=87). In cohort 1, the 12-week treatment groups, SVR12 was 96% and 93% in patients treated with or without RBV, respectively. The 24-week treatment groups had SVR12 of 79.3% and 93% in patients treated with or without RBV, respectively. No viral breakthrough was observed in cohort 1 during treatment, and 3 patients experienced viral relapse after stopping therapy. All 3 patients with viral relapse were infected with HCV genotype 1a and had the Q80K polymorphism.

Preliminary SVR4 results are available for cohort 2. The 12-week treatment duration group had 100% SVR in treatment-naive patients treated with or without RBV, and 100% and 93.3% in prior null responder patients treated with or without RBV, respectively. No viral breakthrough was observed during treatment; 1 patient infected with HCV genotype 1a/Q80K experienced viral relapse after stopping therapy. No SVR

data are yet available from cohort 2, which received 24 weeks of treatment.

Among patients who had viral relapse, simeprevir (protease) resistance-associated variants have been observed; sofosbuvir (polymerase) resistance-associated variants have not been detected. Safety data have been presented for all 167 patients treated. The combination was well tolerated, with only 2.4% of patients prematurely discontinuing therapy due to adverse events. Data on the use of simeprevir in patients with hepatic impairment are not available at this time.

For patients infected with genotype 1a HCV, baseline resistance testing for the Q80K polymorphism may be considered. However, in contrast to using simeprevir to treat a genotype 1a HCV patient with PEG/RBV when the mutation markedly alters the probability of an SVR, the finding of the Q80K polymorphism does not preclude treatment with simeprevir and sofosbuvir, because the SVR rate was high in patients with genotype 1a/Q80K infection (SVR12 rate for cohort 1 was 86% [24 of 28 patients]; SVR4 rate for cohort 2 was 90% [10 of 11 patients]). To date, virologic failure has not been observed in patients in either cohort infected with HCV genotype 1b and with HCV genotype 1a in the absence of the Q80K polymorphism. Thus Q80K testing can be considered but is not strongly recommended.

This regimen should be considered only in those patients who require immediate treatment, because it is anticipated that safer and more effective IFN-free regimens will be available by 2015.

Alternative regimens for treatment-naive patients with HCV genotype 1 who are eligible to receive IFN.

Daily simeprevir (150 mg) for 12 weeks and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 24 weeks is an acceptable regimen for IFN-eligible persons with either

1. *HCV genotype 1b or*
2. *HCV genotype 1a infection in whom the Q80K polymorphism is not detected prior to treatment.*

Rating: Class IIa, Level A

Two randomized, placebo-controlled phase 3 trials evaluated the efficacy and safety of simeprevir (150 mg once daily) for 12 weeks plus PEG and weight-based RBV for a total of 24 weeks (RGT design found no advantage to extending PEG/RBV to 48 weeks). (Jacobson, 2013a); (Poordad, 2013)

In both studies, SVR24 rates were significantly higher among the simeprevir-containing arms (80% to 81%) than in the non-simeprevir-containing arms (50%). If the HCV RNA at week 4 of treatment is less than 25 IU/mL, therapy should be continued to week 24. If the HCV RNA is greater than 25 IU/mL at treatment week 4 or any treatment week thereafter, the regimen should be discontinued. In patients with HCV genotype 1a infection, the presence of a naturally occurring NS3-4A protease polymorphism (Q80K) prior

to treatment was associated with a substantial reduction in SVR among patients treated with simeprevir. A statistically significant difference in SVR12 rates exists between simeprevir-treated persons who are infected with HCV genotype 1a but do not have the Q80K polymorphism and placebo-treated patients who likewise have no such polymorphism. This difference was noted in both the pooled treatment-naive studies and the relapser study (SVR rates of 84% versus 43%, respectively [treatment-naive study] and 78% versus 24%, respectively [relapse study]). The overall SVR in the subgroup of patients with baseline Q80K polymorphism was no better than that in the placebo group. In the United States, persons with genotype 1a HCV infection have a high prevalence of Q80K polymorphism. Because these persons may require alternative therapy, baseline testing for Q80K is recommended for all patients before treatment with the simeprevir plus PEG/RBV regimen is initiated.

For the simeprevir plus PEG/RBV treatment regimen, if the HCV RNA at week 4 of treatment is less than 25 IU/mL, therapy should be continued to week 24. If the HCV RNA is greater than 25 IU/mL at treatment week 4 or any treatment week thereafter, the regimen should be discontinued.

Alternative regimens for treatment-naive patients with HCV genotype 1 who are not eligible to receive IFN.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is an acceptable regimen for IFN-ineligible IFN ineligible is defined as one or more of the below:

- Intolerance to IFN
- Autoimmune hepatitis and other autoimmune disorders
- Hypersensitivity to PEG or any of its components
- Decompensated hepatic disease
- Major uncontrolled depressive illness
- A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL
- A history of preexisting cardiac disease **persons with HCV genotype 1 infection, regardless of subtype; however, preliminary data suggest that this regimen may be less effective than daily sofosbuvir (400 mg) plus simeprevir (150 mg), particularly among patients with cirrhosis.**

Rating: Class IIb, Level B

Sofosbuvir plus RBV was evaluated in 60 treatment-naive patients with HCV genotype 1 with unfavorable treatment characteristics (eg, African American race and advanced fibrosis). (Osinusi, 2013) In part 1 of the study, 10 participants with early to moderate liver fibrosis were treated with sofosbuvir (400 mg daily) plus weight-based RBV for 24 weeks. Nine participants (90%) achieved SVR24. In part 2, 50 participants with any stage of liver fibrosis were randomized 1:1 to receive 400 mg sofosbuvir with RBV either weight-based or low-dose (600 mg daily) for 24 weeks; SVR24 was 68% (17/25) in the weight-based group and 48% (12/25) in the low-dose group. The regimens used in part 2 of this study were well tolerated, with no

discontinuations due to adverse events. Seven of the 13 participants (54%) with advanced liver fibrosis treated in this study relapsed, including all 4 with cirrhosis.

Several additional studies have evaluated the effectiveness of sofosbuvir in persons with HCV genotype 1. In the QUANTUM trial, 38 treatment-naive patients with HCV genotype 1 who did not have cirrhosis were assigned either 12 (n=19) or 24 (n=19) weeks of sofosbuvir (400 mg daily) and weight-based RBV. (Lalezari, 2013) Ten of 19 (53%) in the 12-week arm and 9 of 19 (47%) subjects in the 24-week arm achieved SVR12 (overall 50%). In the ELECTRON trial, 25 treatment-naive subjects with HCV genotype 1 who did not have cirrhosis received sofosbuvir plus RBV for 12 weeks. Twenty-one (84%) achieved SVR12. (Gane, 2013b) In the PHOTON-1 trial, 86 of 113 (76%) treatment-naive subjects with genotype 1 HCV/HIV coinfection achieved SVR12 with sofosbuvir plus RBV for 24 weeks. (Sulkowski, 2013c) Taken together, in a total of 211 subjects, the range of SVR for regimens incorporating sofosbuvir plus daily weight-based RBV (1000 mg to 1200 mg) for up to 24 weeks in treatment-naive persons with HCV genotype 1 was 50% to 84%, with an overall SVR of 72%. Sofosbuvir resistance-associated amino acid variants have not been detected among those patients treated with this combination who did not achieve SVR.

This regimen should be considered only in those patients who require immediate treatment. It is estimated that the FDA will approve safer and more effective IFN-free regimens by 2015.

The following regimens are NOT recommended for treatment-naive patients with HCV genotype 1.

PEG/RBV with or without telaprevir or boceprevir for 24 to 48 weeks

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Although regimens of PEG/RBV plus telaprevir or boceprevir for 24 to 48 weeks using RGT are also FDA approved, they are markedly inferior to the preferred and alternative regimens. These regimens are associated with their higher rates of serious adverse events (eg, anemia and rash), longer treatment duration, high pill burden, numerous drug-drug interactions, frequency of dosing, intensity of monitoring for continuation and stopping of therapy, and the requirement to be taken with food or with high-fat meals.

PEG/RBV for 48 weeks for treatment-naive subjects with HCV genotype 1 has been superseded by treatments incorporating DAAs and should not be used.

II. Genotype 2

Recommended regimen for treatment-naive patients with HCV genotype 2, regardless of eligibility for IFN therapy:

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 12 weeks is recommended for treatment-naive patients with HCV genotype 2 infection.

Rating: Class I, Level A

Sofosbuvir (400 mg daily) was combined with weight-based RBV (1000 mg to 1200 mg) to treat HCV genotype 2 treatment-naive patients across 3 clinical trials: FISSION, POSITRON, and VALENCE. (Lawitz, 2013b); (Jacobson, 2013c); (Zeuzem, 2013b) The FISSION study randomized patients to daily PEG/RBV (800 mg) for 24 weeks or sofosbuvir plus daily weight-based RBV (1000 mg to 1200 mg). (Lawitz, 2013b) The SVR was higher (94%) in patients who received sofosbuvir plus RBV compared with those who received PEG/RBV (78%) (52/67). Across all 3 trials, 201 of 214 (94%) patients with HCV genotype 2 achieved SVR with sofosbuvir plus RBV. Among patients who did not achieve SVR, sofosbuvir resistance-associated amino acid variants were not detected. (US FDA, 2013a)

Alternative Regimens for treatment-naive patients with genotype 2:

None

The following regimens are NOT recommended for treatment-naive patients with HCV genotype 2.

PEG/RBV for 24 weeks

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir-, boceprevir-, or simeprevir-based regimens

Rating: Class III, Level A

PEG (2a) (180 µg weekly) or PEG (2b) (1.5 µg/kg weekly) plus RBV (800 mg daily) for 24 weeks was directly compared with sofosbuvir (400 mg daily) plus weight-based RBV (1000 mg to 1200 mg daily) in the

FISSION trial. (Lawitz, 2013b) The SVR12 achieved with PEG/RBV was lower than that achieved with sofosbuvir/RBV overall (78% and 95%, respectively) and in the subgroups of patients with or without cirrhosis. Safety and tolerability of PEG/RBV was inferior to the profile observed with sofosbuvir and RBV, with greater frequency of reported adverse events and laboratory abnormalities as well as a higher rate of treatment due to adverse events. Further, the duration of therapy with PEG/RBV is 12 weeks longer than that of sofosbuvir plus RBV.

Due to their poor in vitro and in vivo activity, boceprevir and simeprevir should not be used as therapy for patients with HCV genotype 2 infection. Although telaprevir combined with PEG/RBV has antiviral activity against HCV genotype 2, (Foster, 2011) the additional side effects and longer duration of therapy do not support use of this regimen.

III. Genotype 3

Recommended regimen for treatment-naive patients with HCV genotype 3, regardless of eligibility for IFN therapy:

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is recommended for treatment-naive patients with HCV genotype 3 infection.

Rating: Class I, Level B

The VALENCE study assessed the efficacy and safety of sofosbuvir (400 mg daily) plus RBV for 24 weeks in 250 treatment-naive (42%) and treatment-experienced (58%) subjects with HCV genotype 3 infection. The overall SVR12 was 84% and was higher among treatment-naive than treatment-experienced patients (93% versus 77%, respectively). These results suggest higher response rates can be achieved with a 24-week duration of sofosbuvir plus RBV than those reported for the 12- or 16-week durations studied in the FISSION (Lawitz, 2013b) (12 weeks, SVR12: 63%), POSITRON, (Jacobson, 2013c) (12 weeks, SVR 12: 61%) and FUSION (12 weeks, SVR12: 30%, 16 weeks, SVR12: 62%) trials. The primary reason for the higher SVR with extended therapy among treatment-naive patients was a reduction in the relapse rate from 40% to 5%. In sub-analysis, response rates were similarly high among those with (n=45) and without (n=100) cirrhosis (92% and 93%, respectively).

Alternative regimens for treatment-naive patients with genotype 3 who are eligible to receive IFN.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is an acceptable regimen for IFN-eligible persons with HCV genotype 3.

Rating: Class IIa, Level A

The combination of sofosbuvir plus PEG/RBV has been evaluated in patients with genotype 3 infection. In 2 phase 2 clinical trials, PROTON and ELECTRON, 38 of 39 (97%) treatment-naive patients with genotype 3 infection achieved SVR with sofosbuvir plus PEG (4 to 12 weeks of therapy)/RBV. (Gane, 2013b) For many patients with genotype 3, the adverse effects and increased monitoring requirements of PEG make this less acceptable than the recommended regimen of sofosbuvir plus weight-based RBV.

The following regimens are NOT recommended for treatment-naive patients with HCV genotype 3.

PEG/RBV for 24 to 48 weeks

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir-, boceprevir-, or simeprevir-based regimens should not be used for patients with genotype 3 HCV infection.

Rating: Class III, Level A

Although the combination of PEG/RBV is an FDA-approved regimen for HCV genotype 3, its less acceptable adverse effect profile, requirement for more intensive monitoring, and overall lower efficacy make it less desirable than the recommended regimen.

Because of their limited in vitro and in vivo activity against genotype 3, boceprevir, telaprevir, and simeprevir should not be used as therapy for patients with HCV genotype 3 infection.

IV. Genotype 4

Few data are available to help guide decision-making in patients infected with HCV genotype 4. Nonetheless, for those patients for whom immediate treatment is required, the following recommendations have been drawn from available data.

Recommended regimen for treatment-naive patients with HCV genotype 4 who are eligible to receive IFN.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is recommended for IFN-eligible persons

with HCV genotype 4 infection.

Rating: Class IIa, Level B

In the Phase 3 NEUTRINO trial, (Lawitz, 2013b) 28 treatment-naive patients with HCV genotype 4 infection were treated with sofosbuvir (400 mg daily) plus PEG (2a) (180 µg weekly) and weight-based RBV (1000 mg 1200 mg once daily) for 12 weeks. Of the 28 patients with genotype 4, 27 (96%) achieved SVR12. The one patient who did not achieve SVR had cirrhosis and relapsed after therapy. The adverse event profile was similar to that seen with PEG/RBV therapy.

Recommended regimen for treatment-naive patients with genotype 4 who are not eligible to receive IFN.

Daily sofosbuvir (400 mg) plus weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is recommended for IFN-ineligible IFN ineligible is defined as one or more of the below:
• Intolerance to IFN
• Autoimmune hepatitis and other autoimmune disorders
• Hypersensitivity to PEG or any of its components
• Decompensated hepatic disease
• Major uncontrolled depressive illness
• A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL
• A history of preexisting cardiac disease **patients with HCV genotype 4 infection.**

Rating: Class IIb, Level B

In a small study of Egyptian patients in the United States treated with sofosbuvir plus weight-based RBV (1000 mg to 1200 mg), SVR12 was achieved in 11/14 (79%) and 14/14 (100%) in treatment-naive patients treated for 12 weeks and 24 weeks, respectively. In treatment-experienced patients treated for 12 weeks and 24 weeks, SVR12 was achieved in 10/17 (59%) and 13/15 (87%), respectively. (Ruane, 2014)

Alternative regimens for treatment-naive patients with HCV genotype 4 who are eligible to receive IFN.

Daily simeprevir (150 mg) for 12 weeks and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 24 to 48 weeks is an alternative regimen for IFN-eligible persons with HCV genotype 4 infection.

Rating: Class IIb, Level B

A Phase 3 trial in patients with HCV genotype 4 is currently under way. This trial compares PEG and weight-based RBV (1000 mg to 1200 mg) for 48 weeks with a 12-week regimen of simeprevir 150 mg once daily plus PEG and weight-based RBV (1000 mg to 1200 mg) followed by an additional 12 or 36 weeks of PEG/RBV alone. (Moreno, 2013) In another study, the RESTORE trial, an RGT approach is used in place of the simeprevir arm. Patients who have HCV RNA below 25 IU/mL at week 4 and undetectable HCV RNA by week 12 continue PEG/RBV for an additional 12 weeks, and those who do not achieve this response continue PEG/RBV for an additional 36 weeks (total 48 weeks of therapy). The study has enrolled 107 patients, of whom 35 are treatment-naive, including 2 with cirrhosis. To date, 10 of 11 patients (91%) who met criteria for shortened therapy have achieved SVR4, and 3 of 3 have achieved SVR12. To date, therapy has failed in 4 patients: 3 had detectable virus at the end of treatment and 1 experienced virologic relapse. Anemia was reported in 8.4% and hyperbilirubinemia in 1.9% of all study participants (n=107) (including treatment-experienced patients). Four serious adverse events were attributed to simeprevir. No episodes of rash were reported. (Moreno, 2013)

The following regimens are NOT recommended for treatment-naive patients with HCV genotype 4.

PEG/RBV for 48 weeks

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir- or boceprevir-based regimens

Rating: Class III, Level A

PEG/RBV for 48 weeks was the previously recommended regimen for patients with HCV genotype 4. The addition of sofosbuvir (400 mg daily) to PEG/RBV increases response rates and markedly shortens therapy with no apparent additional adverse effects. The addition of simeprevir to PEG/RBV increases response rates with a minimal increase in adverse events and can shorten therapy to 24 weeks.

Because of their limited in vitro and in vivo activity against genotype 4, boceprevir or telaprevir should not be used as therapy for patients with HCV genotype 4 infection.

V. Genotype 5 or 6

Few data are available to help guide decision-making in patients infected with HCV genotype 5 or 6. Nonetheless, for those patients for whom immediate treatment is required, the following recommendations have been drawn from available data. No data are available to support the use of a non-PEG containing regimen for patients with HCV genotype 5 or 6 infection.

Recommended regimen for treatment-naive patients with HCV genotype 5 or 6.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is recommended for IFN-eligible persons with HCV genotype 5 or 6 infection.

Rating: Class IIa, Level B

In the Phase 3 NEUTRINO trial (Lawitz, 2013b), treatment-naive patients with genotypes 1 (n=291), 4 (n=28), 5 (n=1), and 6 (n=6) were treated with sofosbuvir (400 mg daily) plus PEG (2a) (180 µg per week) and weight-based RBV (1000 mg 1200 mg once daily) for 12 weeks. All 6 patients with HCV genotype 6 and the 1 patient with genotype 5 achieved SVR12. The adverse event profile in these patients and in the larger study population was similar to that seen with PEG/RBV therapy.

Alternative regimens for treatment-naive patients with HCV genotype 5 or 6.

Daily weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 48 weeks is an acceptable regimen for persons infected with HCV genotype 5 or 6.

Rating: Class IIb, Level A

PEG/RBV for 48 weeks was the previously recommended regimen for patients infected with HCV genotype 5 or 6. Sofosbuvir has activity against genotypes 5 and 6, and when combined with PEG/RBV for 12 weeks led to SVR in the 6 patients in whom it was studied. (Lawitz, 2013b) The addition of sofosbuvir (400 mg daily) to PEG/RBV shortens duration of therapy with no apparent additional adverse effects and likely substantially increases response rates.

The following regimens are NOT recommended for treatment-naive patients with genotype 5 or 6 HCV.

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir- or boceprevir-based regimens

Rating: Class III, Level A

Because of their limited activity in vitro and in vivo against genotypes 5 and 6, boceprevir or telaprevir should not be used as therapy for patients with genotype 5 or 6 HCV infection.

Initial Treatment Box. Summary of Recommendations for Patients Who are Initiating Therapy for HCV Infection or Who Experienced Relapse after Prior PEG/RBV Therapy, by HCV Genotype

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Initial Treatment Box. Summary of Recommendations for Patients Who are Initiating Therapy for HCV Infection or Who Experienced Relapse after Prior PEG/RBV Therapy, by HCV Genotype

Genotype	Recommended	Alternative	NOT Recommended
1	**IFN eligible:** SOF + PEG/RBV x 12 weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: SOF + SMV ± RBV x 12 weeks	**IFN eligible:** SMV x 12 weeks + PEG/RBV x 24 weeks* IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: SOF + RBV x 24 weeks	TVR + PEG/RBV x 24 or 48 weeks (RGT) BOC + PEG/RBV x 28 or 48 weeks (RGT) PEG/RBV x 48 weeks Monotherapy with PEG, RBV, or a DAA Do not treat decompensated cirrhosis with PEG or SMV
2	SOF + RBV x 12 weeks	None	PEG/RBV x 24 weeks

			Monotherapy with PEG, RBV, or a DAA Any regimen with TVR, BOC, or SMV
3	SOF + RBV x 24 weeks	SOF + PEG/RBV x 12 weeks	PEG/RBV x 24-48 weeks Monotherapy with PEG, RBV, or a DAA Any regimen with TVR, BOC, or SMV
4	**IFN eligible:** SOF + PEG/RBV x 12 weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: SOF + RBV x 24 weeks	SMV x 12 weeks + PEG/RBV x 24-48 weeks	PEG/RBV x 48 weeks Monotherapy with PEG, RBV, or a DAA Any regimen with TVR or BOC
5 or 6	SOF + PEG/RBV x 12 weeks	PEG/RBV x 48 weeks	Monotherapy with PEG, RBV, or a DAA Any regimen with TVR or BOC

For genotype 1a, baseline resistance testing for Q80K should be performed and alternative treatments considered if this mutation is present.

RETREATMENT OF PERSONS IN WHOM PRIOR THERAPY HAS FAILED

Expansions and notes for abbreviations used in this section can be found in Methods Table 3.

A summary of recommendations for retreatment is found in the* BOX*.

This section provides guidance on the retreatment of a person with chronic HCV infection in whom prior therapy has failed. In general, treatment responses of patients achieving an undetectable level of virus during a prior treatment course who relapse following cessation of therapy (**relapser**) are similar to those of treatment-naive persons (see Initial Treatment). Treatment responses are generally lower in prior **non-responders**, which includes null responders (those in whom serum HCV RNA levels declined less than 2 $\log_{10}$ IU/mL by week 12 during a prior treatment course) and partial responders (those with a $\geq$ 2 $\log_{10}$ IU/mL response whose virus remained detectable up to 24 weeks or the end of treatment). This section assumes that a ***decision to treat has been made*** and advises on the optimal treatment. In many instances, however, it may be advisable to delay treatment for some patients with documented early fibrosis stage (F 0-2), because waiting for future highly effective, pangenotypic, combinations in IFN-free regimens may be prudent. Potential advantages of waiting to begin to treatment will be provided in a future update to this guidance.

The level of the evidence supporting the best treatment for each patient and the corresponding confidence in the recommendation varies as does the strength of the recommendation, and is graded in the same manner as the section on initial treatment of treatment-naive patients (Methods Table 2). In addition, when treatment differs for a particular group (eg, those infected with various genotypes) specific recommendations are given. Regimens are classified as "Recommended" when it is favored for most patients or "Alternative" when it might be optimal in a particular subset of patients in that category. When a treatment is clearly inferior or should not be used, it is classified as "Not Recommended."

As always, patients receiving antiviral therapy require careful pretreatment assessment for comorbidities that may influence treatment response. All patients should have careful monitoring during treatment, particularly for anemia if ribavirin is included in the regimen.

I. Genotype 1

Recommended regimen for HCV genotype 1 PEG/RBV (without an HCV protease inhibitor) nonresponder patients:

Daily sofosbuvir (400 mg) plus simeprevir (150 mg), with or without weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 12 weeks is recommended for retreatment of HCV genotype 1 infection, regardless of subtype or IFN eligibility.

Rating: Class IIa, Level B

Recommended regimen for HCV genotype 1 PEG/RBV (with an HCV protease inhibitor) nonresponder patients:

Daily sofosbuvir (400 mg) for 12 weeks plus weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) and weekly PEG for 12 to 24 weeks is recommended for retreatment of HCV genotype 1 infection, regardless of subtype.

Rating: Class IIb, Level C

COSMOS is a phase 2a randomized trial in which participants received sofosbuvir (400 mg once daily) plus simeprevir (150 mg once daily) with or without weight-based RBV (1000 mg to 1200 mg daily) for 12 or 24 weeks (Jacobson, 2013b). Of the 80 null responders with a Metavir fibrosis stage of 2 or less included in this trial, 79% to 96% achieved SVR (79%-96% in RBV-containing arms and 93% in both RBV-free arms). Among those null responders with a Metavir fibrosis stage of 3 or 4 (n=47) who received 12 weeks of sofosbuvir and simeprevir, SVR4 was observed in 14 (93%) of 15 patients in the ribavirin-containing arm and 100% (all 7 participants) in the RBV-free arm. Although benefit from RBV is not apparent from these preliminary results, it cannot be excluded before availability of SVR12 data. Post-treatment results are not yet available for the 24-week arms. Excluding nonvirologic failures, patients with HCV genotype 1a with Q80K mutations had slightly lower numeric response rates (fibrosis stage 0-2: SVR12=89% [n=27]; fibrosis stage 3 or 4: SVR4=91% [n=11]) than genotype 1a patients without Q80K and genotype 1b (fibrosis stage 2: SVR12 100%, n=47; fibrosis stage 3 or 4: SVR4=100% [n=29]). However, because the study was not powered to assess this comparison, insufficient evidence exists on the role of testing for the Q80K mutation at this time. These regimens were well tolerated, although adverse events (eg, anemia and hyperbilirubinemia) were seen more often in patients on RBV-containing regimens. (Jacobson, 2013b)

The safety and efficacy of simeprevir have not been studied in HCV-infected patients with moderate or severe hepatic impairment (Child-Pugh Class B or C). The uncertain impact of cholestasis and the occasional association of SMV with elevated transaminases create potential for drug accumulation or impaired hepatic function during SMV use. Clinical trials with SMV have been limited to patients with compensated disease who have CTP class A, total bilirubin of 1.5 x ULN or lower, and transaminases 10 x ULN or lower. For these reasons, simeprevir use should be limited to patients with compensated liver disease. Use of simeprevir is not recommended in patients with moderate to severe hepatic impairment. The combination of PEG/RBV is contraindicated in patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C).

Alternative regimen for PEG/RBV (with or without an HCV protease inhibitor) nonresponder patients with HCV genotype 1.

Eligible to receive IFN:

Daily sofosbuvir (400 mg) for 12 weeks and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 to 24 weeks is an alternative for retreatment of IFN-eligible persons with HCV genotype 1 infection, regardless of subtype.

Rating: Class IIb, Level C

Ineligible to receive IFN:

Daily sofosbuvir (400 mg) for 24 weeks and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is an alternative for retreatment of IFN-ineligible persons with HCV genotype 1 infection, regardless of subtype.

Rating: Class IIb, Level C

NEUTRINO is an open-label, single-arm trial that evaluated 12 weeks of sofosbuvir plus PEG/RBV in treatment-naive subjects with HCV genotypes 1, 4, 5, or 6; 89% had HCV genotype 1, and 17% had cirrhosis. The SVR was 89% (261 of 292) and was somewhat lower in patients with genotype 1b than 1a (82% and 92%, respectively) and those with cirrhosis versus those without (80% versus 92%, respectively). (Lawitz, 2013a) Treatment-experienced subjects who did not respond to PEG/RBV with or without an HCV protease inhibitor were not included in this study. There are no data available to estimate the response in patients who have been previously treated with a protease inhibitor. However, among patients who were previously treated with PEG/RBV, the FDA estimates that the response rate in such patients would approximate the observed response rate in those NEUTRINO subjects with baseline factors traditionally associated with a lower response to IFN-based treatment. (US FDA, 2013a) In the NEUTRINO trial, SVR rate was 71% among participants with HCV genotype 1 with IL28B non-C/C alleles, high HCV RNA levels, and METAVIR 1 fibrosis stage F3 or F4 (37 of 52 patients). (Gilead Sciences, 2013; Sovaldi package insert)

In a prospective, multicenter trial of sofosbuvir (400 mg/day) plus RBV (ascending dose of 400 mg/day, escalating based on hemoglobin level) including treatment-experienced patients with recurrent HCV infection after liver transplantation, SVR12 was 70% in this population. (Charlton, 2013a)

Alternative regimen for PEG/RBV (without an HCV protease inhibitor) nonresponder patients with HCV genotype 1 who are eligible to receive IFN.

Daily simeprevir (150 mg) for 12 weeks plus weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) and weekly PEG for 48 weeks is an alternative for IFN-eligible persons with HCV genotype 1 infection. (All patients with cirrhosis who are receiving simeprevir should have well compensated liver disease.)

Rating: Class IIa, Level A

Simeprevir was combined with PEG/RBV in patients who had previously failed to respond to PEG/RBV dual therapy in the Phase 2b ASPIRE trial. (Zeuzem, 2013a); (Janssen Therapeutics, 2013) (www.fda.gov; package insert). SVR24 after 48 weeks of triple therapy in the simeprevir 150 mg/day arm was 65% in patients with a previous partial response (n=23) and 53% in patients with a prior null response (n=17). Patients with HCV genotype 1a infection had inferior response rates compared with those with genotype 1b (SVR24: 47% vs 77% in patients with a partial response and 41% vs 47% in patients with a null response, respectively). Despite lower SVR in patients with HCV genotype 1a infection, SVR rates were similar with and without the presence of the Q80K mutations at baseline. SVR rates in patients with advanced fibrosis (METAVIR stage F3 or F4) treated with simeprevir (150 mg daily) plus PEG/RBV for 48 weeks were 59% in patients with a partial response (n=33) and 35% in patients with a null response (n=34). Safety in patients exposed to simeprevir was similar to that of persons in the placebo arms; however, there was a higher incidence of hyperbilirubinemia (8%) and photosensitivity/rash (5%). (Zeuzem, 2013a)

The safety and efficacy of simeprevir have not been studied in HCV-infected patients with moderate or severe hepatic impairment (Child-Pugh Class B or C). The uncertain impact of cholestasis and the occasional association of simeprevir with elevated transaminases pose potential for impaired hepatic function during simeprevir use. Clinical trials with simeprevir have been limited to patients with compensated disease who have CTP class A, total bilirubin level of 1.5 x ULN or lower, and transaminase level of 10 x ULN or lower. For these reasons, simeprevir use should be limited to patients with compensated liver disease. Use of simeprevir is not recommended in patients with moderate to severe hepatic impairment. Use of the drug in this population is not recommended at this time. The combination of PEG/RBV is contraindicated in patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C).

The following regimens are NOT recommended for PEG/RBV (with or without an HCV protease inhibitor) nonresponder patients with HCV genotype 1:

PEG/RBV with or without telaprevir or boceprevir

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

For nonresponder patients with genotype 1 and a history of decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C), treatment is not indicated because of the risks of PEG and boceprevir and telaprevir in this population.

Triple therapy with boceprevir plus PEG/RBV for 48 weeks may result in SVR for up to 52% of PEG/RBV partial responders (RESPOND 2; (Bacon, 2011)) and 38% of null responders (PROVIDE; (Di Bisceglie, 2013)). Similarly, telaprevir plus PEG/RBV resulted in SVR24 of 54% to 59% among partial responders and an SVR24 of 29% to 33% among null responders (REALIZE; (Zeuzem, 2011)). Due to the relatively poor efficacy, prolonged duration of therapy (48 weeks), and poor tolerability, these regimens are no longer recommended.

Monotherapy with PEG, RBV, or any of the available DAAs is ineffective; further, DAA monotherapy leads to rapid selection of resistant variants.

Patients with advanced liver disease are at increased risk for sepsis, worsening decompensation, and death when treated with dual or triple IFN-based therapy. (Crippin, 2002); (Coilly, 2014) Simeprevir is primarily metabolized by the liver and should not be used in patients with advanced cirrhosis (CTP B or C), as the AUC is increased 2.4- to 5.2-fold. (Janssen Therapeutics, 2013) (Olysio package insert, Janssen).

II. Genotype 2

Recommended regimen for genotype 2 PEG/RBV nonresponders.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 12 weeks is recommended for retreatment of HCV genotype 2 infection. (Patients with cirrhosis may benefit by extension of treatment to 16 weeks.)

Rating: Class I, Level A

High SVR12 rates have been demonstrated in non-cirrhotic genotype 2 treatment-experienced patients who received 12 weeks of sofosbuvir plus RBV. Limited data are available in cirrhotic genotype 2 treatment experienced patients; however, in the FUSION study, numerically higher SVR12 rates were seen with extension of therapy from 12 weeks (60%) to 16 weeks (78%). (Jacobson, 2013b) In contrast, the VALENCE trial found high SVR12 rates among HCV genotype 2-infected persons with cirrhosis after only 12 weeks of sofosbuvir plus RBV (88%). (Zeuzem, 2013b) Thus, at this time definitive recommendations on the appropriate duration of sofosbuvir and RBV for treatment-experienced, HCV genotype 2-infected persons with cirrhosis cannot be made. The decision to extend therapy to 16 weeks should be made on a case-by-case basis.

Alternative regimen for PEG/RBV nonresponder patients with HCV genotype 2 infection who are eligible to receive IFN.

Retreatment with daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75

kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is an alternative for IFN-eligible persons with HCV genotype 2 infection.

Rating: Class IIa Level B

Recognizing the potential limitations of sofosbuvir plus RBV in harder-to-treat genotype 2 nonresponders, particularly those with cirrhosis, combination therapy with PEG has been studied. The LONESTAR-2 trial (an open-label, single site, single-arm phase 2 trial) evaluated PEG (180 ?g weekly), sofosbuvir (400 mg daily), and weight-based RBV (1000 mg to 1200 mg daily in 2 divided doses for 12 weeks) in treatment-experienced patients with HCV genotype 2 or 3. Cirrhosis was present at baseline in 61% of patients. SVR12 was achieved in 22 (96%) of 23 persons with genotype 2 HCV infection. For patients with and without cirrhosis, SVR occurred in 13 (93%) of 14 and 9 (100%) of 9, respectively. Despite the limitations of this small study (and accounting for the potential challenges inherent with IFN therapy), combination PEG plus sofosbuvir and RBV is an alternative 12-week regimen for genotype 2-infected patients with cirrhosis.

The following regimens are NOT recommended for nonresponder patients with HCV genotype 2.

PEG/RBV with or without telaprevir, boceprevir or simeprevir

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

No HCV protease inhibitors have been approved or are indicated for the treatment of genotype 2 infection. Although PEG/RBV has been the mainstay of treatment of genotype 2, it requires a longer duration of therapy, is less efficacious, and has more adverse effects than the regimen recommended above.

III. Genotype 3

Recommended regimen for HCV genotype 3 PEG/RBV nonresponders.

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is recommended for retreatment of HCV genotype 3 infection.

Rating: Class IIa, Level A

The phase 3 FUSION trial compared 12 weeks (n=103) with 16 weeks (n=98) of daily sofosbuvir (400 mg) and weight-based RBV (1000 mg to 1200 mg) in genotype 2 or 3 HCV-infected patients in whom previous PEG/RBV therapy had failed. Of patients, 63% had genotype 3; 34% of all patients had cirrhosis. Because persons who had experienced prior relapses to IFN-based therapy accounted for 75% of patients, the number of patients with a prior nonresponse in the study was limited. The SVR rate for genotype 3 patients in the 12-week arm was 30% (19% among patients with cirrhosis and 37% among those without cirrhosis). Extending therapy to 16 weeks increased the SVR rate among genotype 3 patients to 62% (61% among patients with and 63% in those without cirrhosis).

Based on results from FUSION, the phase 3 multicenter, randomized placebo-controlled VALENCE trial was amended to evaluate the effect of extending sofosbuvir plus RBV therapy to 24 weeks in all patients with HCV genotype 3. As with the FUSION study, most (65%) treatment-experienced patients had relapsed. The SVR12 rates after 24 weeks of therapy for treatment-experienced patients with genotype 3 was 79% (60% among patients with and 87% in those without cirrhosis). The increased efficacy with 24 weeks of sofosbuvir plus RBV therapy across all fibrosis stages combined with a favorable safety and tolerability profile supports the recommendation to use 24 weeks of sofosbuvir plus RBV in all genotype 3 patients despite the minimal number of patients studied to date. The response rate for HCV genotype 3-infected patients with cirrhosis treated for 24 weeks in the VALENCE trial (60%) was similar to that observed after 16 weeks of treatment in the FUSION trial (61%). Although longer treatment duration with a well-tolerated regimen may potentially be more successful in these more difficult-to-treat patients, data remain limited. Either duration of treatment is considered acceptable at this time (see below).

Alternate regimen for HCV genotype 3 PEG/RBV nonresponder patients who are eligible to receive IFN.

Retreatment with daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is an alternative for IFN-eligible persons with HCV genotype 3 infection.

Rating: Class IIa Level B

Choice of specific regimen may be influenced by previous or anticipated tolerance to PEG or the presence of advanced fibrosis or cirrhosis. For most patients, the ease of administration and tolerability of sofosbuvir plus RBV will outweigh any potential benefit associated with the addition of PEG. However, for HCV genotype 3-infected patients who have cirrhosis, responses to sofosbuvir and RBV alone for 24 weeks were suboptimal.

In the LONESTAR-2 study, adding 12 weeks of PEG to the sofosbuvir and RBV regimen resulted in numerically higher response rates among persons with HCV genotype 3 than those obtained with sofosbuvir and RBV for 24 weeks. Of HCV genotype 3-infected patients with and without cirrhosis, 10 (83%) of 12 achieved SVR. Given the limited number of patients in this demographic in both the VALENCE and LONESTAR-2 studies, these differences in response rates should be interpreted with caution.

The following regimens are NOT recommended for nonresponder patients with HCV genotype 3 infection.

PEG/RBV with or without telaprevir, boceprevir or simeprevir

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

No HCV protease inhibitors have been approved or are indicated for the treatment of genotype 3 HCV infection. Although PEG/RBV has been the mainstay of treatment of genotype 3 HCV, it is less efficacious and has more adverse effects than the recommended regimens.

IV. Genotypes 4, 5, and 6

***Recommended regimen for HCV genotype 4, PEG/RBV nonresponder patients*.**

Daily sofosbuvir (400 mg) for 12 weeks and daily weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is recommended for retreatment of IFN-eligible persons with HCV genotype 4 infection

Rating: Class IIa, Level C

***Alternate regimen for HCV genotype 4, PEG/RBV nonresponder patients*.**

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is recommended for retreatment of HCV genotype 4 infection.

Rating: Class IIa, Level B

The following regimens are NOT recommended for nonresponder patients with genotype 4

HCV infection.

PEG/RBV with or without telaprevir or boceprevir

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Recommended regimen for HCV genotype 5 or 6, PEG/RBV nonresponder patients.

Daily sofosbuvir (400 mg) for 12 weeks and daily weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG for 12 weeks is recommended for retreatment of IFN-eligible persons with HCV genotype 5 or 6 infection.

Rating: Class IIa, Level C

Alternate regimen for PEG/RBV nonresponder patients with HCV genotype 5 or 6.

None

The following regimens are NOT recommended for nonresponder patients with HCV genotype 5 or 6.

PEG/RBV with or without telaprevir or boceprevir

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

In the NEUTRINO trial, high SVR rates were seen in small numbers of treatment-naive patients with HCV genotypes 4, 5, and 6 treated with sofosbuvir plus PEG/RBV for 12 weeks (genotype 4: n=28, SVR=96%; genotype 5: n=1, SVR=100%; and genotype 6: n=6, SVR=100%). (Lawitz, 2013a) In a pilot study of treatment-experienced HCV genotype 4 patients of Egyptian ancestry, SVR12 was 59% in patients treated with sofosbuvir plus RBV for 12 weeks; SVR4 was 93% in patients treated for 24 weeks. In this cohort, 24% to 27% of patients had cirrhosis. (Ruane, 2013) The only available data with simeprevir for treatment-experienced patients with genotype 4 come from the ongoing RESTORE trial, in which patients (n=50) are receiving treatment with daily simeprevir 150 mg for 12 weeks plus PEG/RBV for a total of 48 weeks (10 prior partial responders, 40 prior null responders). Interim analysis revealed a 40% to 49% RVR rate using this regimen. Final SVR results are pending. (Moreno, 2013) Given the relative paucity of data, expert consultation is needed to determine optimal duration of therapy in patients with genotype 4, 5, or 6 treated with sofosbuvir.

Explanations of highlighted changes made on March 21, 2014 are available here.

Retreatment Box. Summary of Recommendations for Patients in Whom Previous Treatment Has Failed

Retreatment Box. Summary of Recommendations for Patients in Whom Previous Treatment Has Failed[†]

Genotype	Recommended	Alternative	NOT Recommended
Patients in whom previous PEG/RBV has failed*			
1	SOF + SMV ± RBV x 12 weeks	SOF x 12 weeks + PEG/RBV x 12-24 weeks SOF + RBV x 24 weeks SMV x 12 weeks + PEG/RBV x 48 weeks**	PEG/RBV ± telaprevir or boceprevir Monotherapy with PEG, RBV, or a DAA Do not treat decompensated cirrhosis with PEG or SMV
2	SOF + RBV x 12 weeks	SOF + PEG/RBV x 12 weeks	PEG/RBV ± telaprevir or boceprevir Monotherapy with PEG, RBV, or a direct-acting antiviral agent Do not treat decompensated cirrhosis with PEG
3	SOF + RBV x 24 weeks	SOF + PEG/RBV x 12 weeks	PEG/RBV ± any current protease inhibitor Monotherapy with PEG, RBV, or a DAA Do not treat decompensated cirrhosis with PEG
4	SOF + PEG/RBV x 12 weeks	SOF + RBV x 24 weeks	PEG/RBV ± any current HCV protease inhibitor Monotherapy with PEG, RBV, or a

			DAA Do not treat decompensated cirrhosis with PEG
5 or 6	SOF x 12 weeks + PEG/RBV 12 weeks		PEG/RBV ± any current HCV protease inhibitor Monotherapy with PEG, RBV, or a DAA Do not treat decompensated cirrhosis with PEG
Patients in whom previous treatment with PEG/RBV plus either telaprevir or boceprevir* has failed †† †††			
1	SOF x 12 weeks + PEG/RBV x 12-24 weeks	SOF + RBV x 24 weeks‡	PEG/RBV ± telaprevir or boceprevir or SMV
		SOF + PEG/RBV x 24 weeks‡‡	Monotherapy with PEG, RBV, or a DAA Do not treat decompensated cirrhosis with PEG or SMV

*Failure (non response) is defined as partial or null response to treatment with PEG/RBV. Relapse to prior therapy should be treated the same as treatment-naive (see Initial Treatment section)

**For genotype 1a, baseline resistance testing for Q80K should be performed and alternative treatments considered if this mutation is present

*** Failure (non response) is defined as partial or null response to treatment with PEG/RBV plus telaprevir or boceprevir. Relapse to prior therapy should be treated the same as treatment naive (see Initial Treatment section)

† Consideration should be given to postponing treatment, pending release of new drugs for patients with

limited (F 0-2) hepatic fibrosis

†† A recommendation for simeprevir use for patients with previous telaprevir or boceprevir exposure not provided due to potential risk of preexistant resistance to protease inhibitor treatment.

††† Given the lack of prior approval of protease inhibitor therapy for genotypes 2, 3, 4, 5, 6 and the lack of sufficient data, no recommendations are given for these genotypes at this time

‡IFN ineligible

‡‡IFN eligible

MONITORING PATIENTS WHO ARE STARTING HEPATITIS C TREATMENT, ARE ON TREATMENT, OR HAVE COMPLETED THERAPY

Expansions and notes for abbreviations used in this section can be found in Methods Table 3.

A summary of recommendations for monitoring is found in the BOX.

This section provides guidance on monitoring patients with chronic hepatitis C who are starting treatment, are on treatment, or have completed treatment, and is divided into 3 parts: pretreatment and on-treatment monitoring, post-treatment follow-up for persons in whom treatment has failed to clear virus, and post-treatment follow-up for those who achieved a sustained virologic response (SVR; virologic cure).

Recommended assessments prior to starting antiviral therapy

Assessment of potential drug-drug interactions with concomitant medications is recommended prior to starting antiviral therapy.

Rating: Class I, Level C

The following laboratory tests are recommended within 6 weeks prior to starting antiviral therapy:

- **Complete blood cell (CBC) count; international normalized ratio (INR)**
- **Hepatic function panel (albumin, total and direct bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels)**
- **Thyroid-stimulating hormone (TSH; if IFN is used)**
- **Calculated glomerular filtration rate (GFR)**

Rating: Class I, Level B

The following laboratory test is recommended within 12 weeks of starting antiviral therapy:

- **HCV genotype and quantitative HCV viral load**

Rating: Class I, Level B

Recommended monitoring during antiviral therapy

CBC count, creatinine level, calculated GFR, and hepatic function panel are recommended every 4 weeks during antiviral therapy. TSH is recommended every 12 weeks for patients on IFN. More frequent assessment for drug-related toxic effects (eg, CBC count for patients receiving RBV) is recommended as clinically indicated.

Rating: Class I, Level B

Quantitative HCV viral load testing is recommended after 4 weeks of therapy, at the end of treatment, and at 12 weeks following completion of therapy.

Rating: Class I, Level B

Quantitative HCV viral load monitoring at 4 weeks is recommended, but discontinuation of treatment because this test result is missing is NOT recommended.

Rating: Class III, Level C

Recommended monitoring for pregnancy-related issues prior to and during antiviral therapy that includes RBV

Women of childbearing age should be cautioned not to become pregnant while receiving RBV-containing antiviral regimens, and for up to 6 months after stopping.

Rating: Class I, Level C

Serum pregnancy testing is recommended for women of childbearing age prior to beginning treatment with a regimen that includes RBV.

Rating: Class I, Level C

Assessment of contraceptive use and of possible pregnancy is recommended at appropriate intervals during (and for 6 months after) RBV treatment for women of childbearing potential, and for female partners of men who receive RBV treatment.

Rating: Class I, Level C

Treatment with RBV is NOT recommended for pregnant women or for women who are unwilling to adhere to use of adequate contraception, who are either receiving RBV themselves or who are sexual partners of male patients who are receiving RBV.

Rating: Class III, Level C

Female patients and sexual partners of male patients who have received RBV should NOT become pregnant for at least 6 months after stopping RBV.

Rating: Class III, Level C

Pretreatment and On-Treatment Monitoring

The pretreatment testing described here assumes that a decision to treat with antiviral medications has already been made and that the testing involved in deciding to treat, including testing for HCV genotype and assessment of hepatic fibrosis, has already been completed [see **Whom and When to Treat**].

Individuals receiving antiviral therapy for chronic HCV infection should have careful monitoring for toxic effects, virologic response, and adherence to therapy. The assessment of HCV viral load at week 4 of therapy is useful to determine adherence or futility, and the end of treatment viral load will help to differentiate potential viral breakthrough versus relapse. Although HCV-RNA testing is recommended at week 4 of treatment, the absence of testing results at week 4 is not a reason to discontinue treatment. Some may choose to forego end of treatment viral load testing given the high rates of viral response with the newer regimens but to focus on the week 12 post-treatment viral load.

The availability of IFN-free treatment regimens has simplified hepatitis C therapy with shorter-duration, all-

oral therapy for many patients. However, PEG/RBV-based regimens are still recommended for some patients and these require specific monitoring for the toxic effects (eg, anemia or neutropenia) associated with PEG or RBV use. (RBV prescribing information, 2014); (PEG prescribing information, 2014) In patients without history of cardiovascular disease, RBV dose reduction to 600 mg per day is recommended for those with hemoglobin (Hgb) level below 10 g/dL and discontinuation for those with Hgb below 8.5 g/dL. In addition, although the newer all-oral regimens are generally well tolerated, adverse effects do occur. Expansion of therapy into a large population of patients may reveal toxic effects that are not apparent in registration trials. Furthermore, drug-drug interactions are possible. Thus, the pretreatment evaluation should explore potential drug-drug interactions (eg, http://www.hep-druginteractions.org/) for the selected antiviral medications, address potential drug adverse effects, and include laboratory testing. The purpose of on-treatment monitoring is to document viral response, evaluate for possible drug toxic effects, and assess adherence to the regimen.

RBV is reported to cause fetal death and fetal abnormalities in animals and thus it is imperative for persons of childbearing potential who receive the drug to use at least 2 reliable forms of effective contraception during treatment and for a period of 6 months thereafter. The safety of newer antivirals during pregnancy is unknown. The education of patients and caregivers about potential adverse effects and their management is an integral component of treatment and is important for a successful outcome.

Monitoring patients who have completed treatment

Patients who do not achieve an SVR, because of failure of the treatment to clear or maintain clearance of HCV infection with relapse after treatment completion, have ongoing HCV infection and the possibility of continued liver injury. Such patients should be monitored for progressive liver disease and considered for retreatment when alternative treatments are available. Patients who have undetectable HCV RNA in the serum, when assessed by a sensitive polymerase chain reaction (PCR) assay, 12 or more weeks after completing treatment, are deemed to have achieved an SVR. In these patients, hepatitis C-related liver injury stops, although the patients remain at risk for non-HCV related liver disease, such as fatty liver disease or alcoholic liver disease.

Recommended monitoring for patients in whom treatment failed to achieve an SVR

Disease progression assessment every 6 months to 12 months with a hepatic function panel, CBC count, and INR is recommended.

Rating: Class I, Level C

Surveillance for hepatocellular carcinoma with ultrasound testing every 6 months is recommended for patients with more advanced fibrosis (ie, Metavir F3 or F4).

Rating: Class I, Level C

Endoscopic surveillance for esophageal varices is recommended if cirrhosis is present.

Rating: Class I, Level A

Evaluation for retreatment is recommended as effective alternative treatments become available.

Rating: Class I, Level C

Monitoring for HCV drug resistance-associated variants (RAVs) on and after therapy is NOT recommended.

Rating: Class III, Level C

Patients in whom treatment failed to achieve an SVR remain at risk for ongoing liver injury and progression of liver fibrosis. (Dienstag, 2011) Thus, patients in whom treatment fails should be monitored for signs and symptoms of cirrhosis. There is currently no conclusive evidence to suggest that failure of antiviral treatment results in more severe liver injury or more rapidly progressive liver disease than would have occurred if the patient had not received treatment.

A small number of patients in whom an initial antiviral treatment failed have achieved SVR when treated with the same drugs for a longer duration, or when treated with alternative antiviral regimens. (Lawitz, 2014) Thus, patients in whom treatment has failed to achieve an SVR should be considered for treatment when alternative antiviral regimens are available. Advice from a physician experienced in HCV treatment may be beneficial when considering retreatment after antiviral therapy failure.

Patients in whom antiviral therapy failed to achieve an SVR may harbor viruses that are resistant to 1 or more of the antivirals at the time of virologic “breakthrough.” (Lawitz, 2014); (Schneider, 2014) However, there is no evidence to date that the presence of RAVs results in more progressive liver injury than would have occurred if the patient did not have resistant viruses. Furthermore, RAVs are often not detectable with routine (population sequencing) detection methods, nor with more sensitive tests of HCV variants, after patients are followed up for several months. (Schneider, 2014) Subsequent retreatment with combination antivirals, particularly regimens containing antiviral drugs that have a high barrier to resistance, such as nonstructural protein 5B nucleotide polymerase inhibitors (eg, sofosbuvir), may overcome the presence of resistance to 1 or more antivirals. Certain telaprevir- and boceprevir-associated resistant mutations may impact simeprevir activity in vitro. However, there are currently no data on SVR rates when patients are retreated with sofosbuvir plus simeprevir after emergence of telaprevir- or boceprevir-associated resistance mutations. With the exception of testing for Q80K polymorphism at baseline in patients with HCV genotype 1a before treatment with simeprevir plus PEG/RBV, testing for RAVs prior to repeat antiviral treatment is not recommended. If in doubt, consultation with an expert in the treatment of HCV infection may be useful.

Recommended follow-up for patients who achieve an SVR

For patients who do not have advanced fibrosis (ie, those with Metavir F0, F1, or F2), recommended follow-up is the same as if they were never infected with HCV.

Rating: Class I, Level B

Assessment for HCV recurrence or reinfection is recommended only if the patient has ongoing risk for HCV infection or otherwise unexplained hepatic dysfunction develops. In such cases, a quantitative HCV RNA assay rather than an anti-HCV serology test is recommended to test for HCV recurrence or reinfection.

Rating: Class I, Level A

Surveillance for hepatocellular carcinoma with twice yearly ultrasound testing is recommended for patients with advanced fibrosis (ie, Metavir F3 or F4), who achieve an SVR.

Rating: Class I, Level C

A baseline endoscopy is recommended to screen for varices if cirrhosis is present. Patients in whom varices are found should be treated and followed up as indicated.

Rating: Class I, Level C

Assessment of other causes of liver disease is recommended for patients who develop persistently abnormal liver tests after achieving an SVR.

Rating: Class I, Level C

Routine assessment for regression in liver fibrosis after achieving SVR is NOT recommended.

Rating: Class III, Level C

With the advent of highly effective HCV antiviral regimens, the likelihood of achieving an SVR among adherent, immunologically competent, treatment-naive patients with compensated liver disease generally

exceeds 90%. Of patients who achieved an SVR with PEG/RBV treatment, more than 99% have remained free of HCV infection when followed up for 5 years after completing treatment. (Manns, 2013) Thus, achieving an SVR is considered a virologic cure of HCV infection.

SVR typically aborts progression of liver injury with regression of liver fibrosis in most but not all treated patients. (Morisco, 2013); (Morgan, 2010); (George, 2009); (Morgan, 2013); (Singal, 2010) Because of lack of progression, patients without advanced liver fibrosis (ie, Metavir F0, F1, or F2) who achieve an SVR should receive standard medical care that is recommended for patients who were never infected with HCV.

Among patients with advanced liver fibrosis (ie, Metavir F3 or F4) who achieve an SVR, decompensated liver disease (with the exception of hepatocellular carcinoma) rarely develops during follow-up, and overall survival is prolonged. (Morisco, 2013); (Morgan, 2010); (George, 2009); (Morgan, 2013); (Singal, 2010) Patients who have advanced fibrosis or cirrhosis continue to be at risk for development of hepatocellular carcinoma after achieving an SVR, although the risk in these patients is lower than the risk in persistently viremic patients. (Morisco, 2013); (Morgan, 2010); (George, 2009); (Morgan, 2013); (Singal, 2010) Patients with cirrhosis who achieve SVR experience increased survival (compared with patients with cirrhosis who are untreated or fail to respond), but still may be at some risk of hepatocellular carcinoma; thus, they should continue to undergo regular surveillance for hepatocellular carcinoma despite the lowered risk that results after viral eradication. (Bruix, 2011) The risk of hepatocellular carcinoma among patients with advanced fibrosis prior to treatment but who have regression to minimal fibrosis after treatment is not known. In the absence of data to the contrary, such patients remain at some risk for hepatocellular carcinoma and should be monitored at least annually for hepatocellular carcinoma.

Liver fibrosis and liver function test results improve in most patients who achieve an SVR. (Morisco, 2013); (Morgan, 2010); (George, 2009); (Morgan, 2013); (Singal, 2010) Bleeding from esophageal varices is rare after an SVR. (Morisco, 2013); (Morgan, 2010); (George, 2009); (Morgan, 2013); (Singal, 2010) Patients with cirrhosis should receive routine surveillance endoscopy for detection of esophageal varices if not previously done and these should be treated or followed up as indicated. (Garcia-Tsao, 2007)

There is no evidence that monitoring for liver fibrosis regression after an SVR provides clinical benefit.

Patients in whom an SVR is achieved but who have another potential cause of liver disease (eg, excessive alcohol use, metabolic syndrome with or without proven fatty liver disease, or iron overload) remain at risk for progression of fibrosis. It is recommended that such patients be educated about the risk of liver disease and monitored for liver disease progression with periodic physical examinations, blood tests, and potentially, tests of liver fibrosis by a liver disease specialist.

Testing patients with ongoing risk for HCV infection (eg, illicit drug use, high-risk sexual exposure) periodically for HCV reinfection is recommended. Flares in liver enzyme test results should prompt evaluation of possible de novo re-infection with HCV through a new exposure (see **Management of Acute HCV Infection**). Antibody to HCV (anti-HCV) remains positive in most patients following an SVR. Thus, it is recommended that testing for reinfection with HCV be performed with an assay that detects HCV RNA (eg, a quantitative HCV RNA test).

Monitoring for HCV during chemotherapy and immunosuppression

Prospective monitoring for HCV recurrence among patients who achieved an SVR and who are receiving immunosuppressive treatment (eg, systemic

corticosteroids, antimetabolites, chemotherapy, etc.) is NOT routinely recommended.

Rating: Class III, Level C

In patients with inactive or past hepatitis B virus (HBV) infection, reactivation of infection and clinically apparent hepatitis during immunosuppressive treatment or chemotherapy can occur. Although some patients with active HCV infection, primarily those with hematologic malignancy, may have a flare in their liver enzymes during chemotherapy, this is unusual. (Mahale, 2012) Reactivation of past HCV infection, such as after SVR or spontaneous clearance, is not anticipated since there is no residual reservoir for the virus. Thus, routine testing of HCV RNA during immunosuppressive treatment, or prophylactic administration of antivirals during immunosuppressive treatment, is not routinely recommended. However, acute liver injury is common among patients receiving chemotherapy or potent immunosuppressive agents; quantitative HCV RNA testing should be included in the laboratory assessment of the cause of liver injury.

Monitoring Box. Summary of the Recommendations for Monitoring Patients Who Are Starting HCV Treatment, Are On Treatment, Or Have Completed Therapy

Recommended assessments prior to starting antiviral therapy

Assessment of potential drug-drug interactions with concomitant medications is recommended prior to starting antiviral therapy.

Rating: Class I, Level C

The following laboratory tests are recommended within 6 weeks prior to starting antiviral therapy:

- **Complete blood cell (CBC) count; international normalized ratio (INR)**
- **Hepatic function panel (albumin, total and direct bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels)**
- **Thyroid-stimulating hormone (TSH; if IFN is used)**
- **Calculated glomerular filtration rate (GFR)**

Rating: Class I, Level B

The following laboratory tests are recommended within 12 weeks prior to starting antiviral therapy:

HCV genotype and quantitative HCV viral load

Rating: Class I, Level B

Recommended monitoring during antiviral therapy

CBC count, creatinine level, calculated GFR, and hepatic function panel are recommended every 4 weeks during antiviral therapy. TSH is recommended every 12 weeks for patients on IFN. More frequent assessment for drug-related toxic effects (eg, CBC count for patients receiving RBV) is recommended, as clinically indicated.

Rating: Class I, Level B

Quantitative HCV viral load testing is recommended after 4 weeks of therapy, at the end of treatment, and at 12 weeks following completion of therapy.

Rating: Class I, Level B

Quantitative HCV viral load monitoring at 4 weeks is recommended, but discontinuation of treatment because this test result is missing is NOT recommended.

Rating: Class III, Level C

Recommended monitoring for pregnancy-related issues prior to and during antiviral therapy that includes RBV.

Women of child bearing age should be cautioned not to become pregnant while receiving RBV-containing antiviral regimens, and for up to 6 months after stopping.

Rating: Class I, Level C

Serum pregnancy testing is recommended for women of child bearing age prior to beginning treatment with a regimen that includes RBV.

Rating: Class I, Level C

Assessment of contraceptive use and of possible pregnancy is recommended at appropriate intervals during (and for 6 months after), RBV treatment for women of child bearing potential, and for female partners of men who receive RBV treatment.

Rating: Class I, Level C

Treatment with RBV is NOT recommended for pregnant women or for women who are unwilling to adhere to use of adequate contraception, who are either receiving RBV themselves or who are sexual partners of male patients who are receiving RBV.

Rating: Class III, Level C

Female patients and sexual partners of male patients who have received RBV should NOT become pregnant for at least 6 months after stopping RBV.

Rating: Class III, Level C

Recommended monitoring for patients in whom treatment failed to achieve an SVR

Disease progression assessment every 6 months to 12 months with a hepatic function panel, CBC count, and INR is recommended.

Rating: Class I, Level C

Surveillance for hepatocellular carcinoma with ultrasound testing every 6 months is recommended for patients with more advanced fibrosis (ie, Metavir F3 or F4).

Rating: Class I, Level C

Endoscopic surveillance for esophageal varices is recommended if cirrhosis is present.

Rating: Class I, Level A

Evaluation for retreatment is recommended as effective alternative treatments become available.

Rating: Class I, Level C

Monitoring for HCV drug resistance-associated variants (RAVs) on and after therapy is NOT recommended.

Rating: Class III, Level C

Recommended follow-up for patients who achieve an SVR

For patients who do not have advanced fibrosis (ie, those with Metavir F0, F1, or F2), recommended follow-up is the same as if they were never infected with HCV.

Rating: Class I, Level B

Assessment for HCV recurrence or reinfection is recommended only if the patient has ongoing risk for HCV infection or otherwise unexplained hepatic dysfunction develops. In such cases, a quantitative HCV RNA assay rather than an anti-HCV serology test is recommended to test for HCV recurrence or reinfection.

Rating: Class I, Level A

Surveillance for hepatocellular carcinoma with twice yearly ultrasound testing is recommended for patients with advanced fibrosis (ie, Metavir F3 or F4), who achieve an SVR.

Rating: Class I, Level C

A baseline endoscopy is recommended to screen for varices if cirrhosis is present. Patients in whom varices are found should be treated and followed up as indicated.

Rating: Class I, Level C

Assessment of other causes of liver disease is recommended for patients who develop persistently abnormal liver tests after achieving an SVR.

Rating: Class I, Level C

Routine assessment for regression in liver fibrosis after achieving SVR is NOT recommended.

Rating: Class III, Level C

Monitoring for HCV during chemotherapy and immunosuppression

Prospective monitoring for HCV recurrence among patients who achieved an SVR and who are receiving immunosuppressive treatment (eg, systemic corticosteroids, antimetabolites, chemotherapy, etc.) is NOT routinely recommended.

Rating: Class III, Level C

UNIQUE PATIENT POPULATIONS

Expansions and notes for abbreviations used in this section can be found in Methods Table 3.

1. Patients with HIV/HCV Coinfection

The summary of recommendations for HIV-coinfected patients is in the BOX.

Recommended regimen(s) for treatment-naive and prior relapser HIV/HCV-coinfected patients with genotype 1 infection who are eligible to receive IFN:

Sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) plus weekly PEG for 12 weeks is recommended for IFN-eligible persons with HCV genotype 1 infection, regardless of subtype.

Rating: Class I, Level B

Recommended regimen(s) for treatment-naive and prior relapser HIV/HCV-coinfected patients with genotype 1 who are ineligible or unwilling to receive IFN.

Sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) for 24 weeks is recommended for treatment-naive HIV/HCV-coinfected patients with HCV genotype 1 infection.

Rating: Class I, Level B

Sofosbuvir (400 mg once daily) plus simeprevir (150 mg once daily), with or without weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) for 12 weeks is recommended for treatment-naive and prior PEG/RBV relapser HIV/HCV-coinfected patients with genotype 1 infection. Simeprevir should only be used with antiretroviral drugs with which it does not have significant interactions: raltegravir, rilpivirine, maraviroc, enfuvirtide, tenofovir, emtricitabine, lamivudine, and abacavir.

Rating: Class IIa Level C

Recommended regimen(s) for treatment-experienced patients with HCV genotype 1 with a history of PEG/RBV nonresponse, regardless of IFN eligibility

Sofosbuvir (400 mg once daily) plus simeprevir (150 mg once daily) with or without weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) for 12 weeks is recommended for prior PEG/RBV nonresponder, HIV/HCV-coinfected patients with genotype 1 infection. Simeprevir should only be used with antiretroviral drugs with which it does not have significant interactions: raltegravir, rilpivirine, maraviroc, enfuvirtide, tenofovir, emtricitabine, lamivudine, and abacavir.

Rating: Class IIa, Level C

Recommended regimen(s) for treatment-experienced patients with HCV genotype 1 with a history of PEG/RBV plus telaprevir or boceprevir nonresponse

Treat as recommended for HCV-monoinfected individuals.

Recommended regimen(s) for treatment-naive and treatment-experienced HIV/HCV-coinfected patients with genotype 2 and 3 infection

Use the same regimens as is recommended for persons with HCV monoinfection; specifically:

For patients with genotype 2 infection: sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) for 12 weeks is recommended for treatment-naive and treatment-experienced HIV/HCV-coinfected patients. Patients who are prior nonresponders and have cirrhosis may benefit by extension of treatment to 16 weeks.

Rating: Class I, Level B

For patients with genotype 3 infection: sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) for 24 weeks is recommended for treatment-naive and treatment-experienced HIV/HCV-coinfected patients.

Rating: Class I, Level B

Recommended regimen(s) for treatment-naive and treatment-experienced HIV/HCV-coinfected patients with genotype 4, 5, or 6 HCV:

Treat as recommended for persons with HCV monoinfection.

HIV/HCV coinfection results in increased liver-related morbidity and mortality, non-hepatic organ dysfunction, and overall mortality. Even in the potent antiretroviral era, HIV infection remains independently associated with advanced liver fibrosis and cirrhosis in patients with HCV coinfection. (Thein, 2008); (de Ledinghen, 2008); (Fierer, 2013) Similar to HCV-monoinfected patients, HIV/HCV-coinfected patients cured with PEG/RBV have lower rates of hepatic decompensation, hepatocellular carcinoma, and liver related mortality. (Berenguer, 2009); (Limketkai, 2012); (Mira, 2013) Uptake of HCV therapy is limited in the HIV/HCV-coinfected population due to historically lower response rates, patient comorbidities, patient and practitioner perception, and the adverse events associated with IFN-based therapy. (Mehta, 2006); (Thomas, 2008) Due to the special population designation, the first 2 approved DAAs, telaprevir and boceprevir, remain off label for use in HIV/HCV-coinfected patients, further limiting access to treatment in this population. With the availability of the DAAs sofosbuvir and simeprevir, a milestone has been reached in HIV/HCV coinfected patients. Treatment of HIV/HCV-coinfected patients requires awareness and attention to the complex drug interactions that can occur between DAA and HIV antiretroviral medications.

Pharmacokinetics and Drug Interactions

Sofosbuvir is not metabolized by the hepatic P450 enzyme complex and is a substrate (but not an inhibitor) of drug transporters, p-glycoprotein (P-gp), and breast cancer resistance protein (BCRP). It is not a substrate of OATP. Drug interaction studies with antiretroviral drugs (ie, efavirenz, tenofovir, emtricitabine, rilpivirine, darunavir/ritonavir, and raltegravir) in non-infected persons identified no clinically significant interactions (Kirby, 2013) making sofosbuvir an ideal therapy for patients with HIV/HCV coinfection. Sofosbuvir is not recommended for use with tipranavir because of the potential of this antiretroviral drug to induce P-gp (see package insert).

Simeprevir is metabolized primarily by cytochrome P450 3A4 (CYP3A4) and therefore is susceptible to drug interactions with inhibitors and inducers of the enzyme. Simeprevir is also an inhibitor of the OATP and P-gp transporters leading to additional drug interaction concerns. Drug interaction studies with antiretroviral drugs in non-infected volunteers suggested no substantial interactions with tenofovir, rilpivirine, or raltegravir; however, simeprevir concentrations were substantially decreased when dosed with efavirenz and substantially increased when dosed with darunavir/ritonavir, resulting in their exclusion from the Phase III C212 clinical trial investigating simeprevir in combination with PEG/RBV in patients with HIV/HCV coinfection. (Ouwerkerk-Mahadevan, 2012)

Ribavirin has the potential for dangerous drug interactions with didanosine resulting in mitochondrial toxicity with hepatomegaly/steatosis, pancreatitis, and lactic acidosis; thus the concomitant administration of these 2 drugs is contraindicated. (Fleischer, 2004) The combined use of RBV and zidovudine has been reported to increase the rates of anemia and the need for RBV dose reduction, and thus zidovudine is not recommended for use with RBV. (Alvarez, 2006)

Sofosbuvir (400 mg once daily) as part of a triple-therapy regimen with PEG (180 ?g weekly) and weight-based RBV (1000 mg to 1200 mg daily given in divided doses) is safe and efficacious in patients with HCV monoinfection, with an overall SVR12 of 89% in HCV genotype 1 patients. The P7977-1910 study was a single-center, single-arm trial (N=23) investigating this same 12-week triple therapy regimen in HIV-infected patients coinfected with HCV genotypes 1, 2 3, or 4. (Rodriguez-Torres, 2013) Allowable

antiretrovirals included either efavirenz, atazanavir/ritonavir, darunavir/ritonavir, raltegravir, or rilpivirine in combination with tenofovir/emtricitabine. Of patients with HCV genotype 1 (N=19), 89% achieved SVR12; 2 patients discontinued the study early due to adverse events (ie, anemia and altered mood). This regimen is therefore recommended for persons with HIV/HCV genotype 1 coinfection who are eligible to receive IFN and are either treatment-naive or have had prior PEG/RBV relapse.

The Phase III PHOTON-1 study enrolled 182 treatment-naive patients with HIV/HCV coinfection (n=114 with genotype 1; n=26 with genotype 2; n=42 with genotype 3) in a single-arm clinical trial investigating sofosbuvir (400 mg once daily) plus weight-based RBV (1000 mg to 1200 mg daily given in divided doses) for 24 (genotype 1) or 12 (genotypes 2 and 3) weeks. (Sulkowski, 2013c) The population had well-controlled HIV with mean CD4 counts of 559 to 636 cells/?L. The same ARVs were allowed as those in the P7977-1910 study. Of participants, 90% completed treatment and 3% discontinued treatment due to adverse events. SVR12 was achieved in 76%, 88%, and 67% of participants with HCV genotypes 1, 2, and 3, respectively. For the combination of sofosbuvir plus RBV, genotype 1b subtype was a predictor of poorer response. Cirrhosis and African American race also exhibited trends toward lower SVR12. Based on the potential for lower response in HIV/HCV-coinfected patients with cirrhosis, the use of sofosbuvir plus PEG/RBV should be considered over sofosbuvir plus RBV. This regimen is otherwise recommended for HIV/HCV genotype 1-coinfected patients who are treatment naive or have relapsed after receipt of PEG/RBV and are ineligible for IFN.

The combination of simeprevir plus sofosbuvir with or without RBV has been studied in the phase II COSMOS trial in patients with HCV monoinfection. (Jacobson, 2013b) This study is the basis for the recommendation supporting the use of this all-oral combination as an alternative regimen for patients with HCV monoinfection who cannot tolerate the recommended regimens. Although sofosbuvir plus simeprevir has been used anecdotally in patients with HIV/HCV coinfection, this drug combination has never been studied in this population. Despite the absence of data, this regimen may be considered for the treatment of HCV genotype 1 infection in patients with HIV infection who are not eligible for IFN and who are receiving antiretroviral therapy that may be coadministered with simeprevir (ie, raltegravir, rilpivirine, maraviroc, enfuvirtide, tenofovir, emtricitabine, lamivudine, and abacavir).

Similarly, no data exist for the combination of sofosbuvir plus simeprevir for the (re)treatment of HCV infection in HIV-infected patients. However, preliminary results obtained in HCV-monoinfected patients, including those with prior treatment failure and advanced fibrosis, support the expectation that this regimen will be highly effective in coinfected patients receiving compatible antiretroviral therapy as described above (see Retreatment of HCV Monoinfected Patients). (Jacobson, 2013b) Given the lack of clinical data in this population, it may be prudent to reserve this regimen for the treatment of persons with advanced fibrosis in whom a delay of therapy may lead to adverse clinical outcomes.

No data with sofosbuvir currently exist to guide retreatment recommendations for coinfected patients with HCV genotype 2 or 3 HCV infection. The ongoing PHOTON-1 study enrolled 41 treatment-experienced patients coinfected with HCV genotype 2 or 3, receiving sofosbuvir (400 mg once daily) plus weight-based RBV (1000 mg to 1200 mg daily given in divided doses) for 24 weeks. (Sulkowski, 2013b) Results are expected in early 2014. In the absence of data, current recommendations for the retreatment of HIV patients coinfected with HCV genotype 2 or 3 are the same as those for HCV-monoinfected patients. Data also are lacking regarding use of sofosbuvir among patients coinfected with HCV genotype 4, 5, or 6 and HIV. Similarly, with no current data on the use of sofosbuvir in patients with genotype 4, 5, or 6 HCV and HIV coinfection, but given evidence of safety and efficacy of sofosbuvir-based regimens in this population, the recommended regimens for treatment in treatment-naive and treatment-experienced patients with HIV/HCV coinfection are the same as those for HCV-monoinfected patients.

Alternative regimen(s) for treatment-naive or treatment-experienced (prior PEG/RBV relapse) HIV/HCV- coinfected patients with genotype 1 who are eligible to receive IFN

Simeprevir (150 mg once daily) for 12 weeks and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) plus weekly PEG for 24 weeks (for treatment-naive and treatment-experienced with prior relapse to PEG/RBV) is an acceptable regimen for IFN-eligible HIV/HCV-coinfected persons with either (1) HCV genotype 1b or (2) HCV genotype 1a infection in whom the Q80K polymorphism is not detected prior to treatment. Simeprevir can only be used with the following antiretroviral drugs: raltegravir, rilpivirine, maraviroc, enfuvirtide tenofovir, emtricitabine, lamivudine, and abacavir.

Rating: Class IIa, Level B

Alternative regimen(s) for treatment-experienced (PEG/RBV nonresponders) HIV/HCV-coinfected patients with genotype 1 who are eligible for IFN

Sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) plus weekly PEG for 12 weeks is an acceptable regimen for IFN-eligible persons with HCV genotype 1 infection, regardless of subtype.

Rating: Class IIb, Level C

Alternative regimen(s) for treatment-naive and PEG/RBV relapser HIV/HCV-coinfected patients with genotype 1 who are ineligible or unwilling to receive IFN.

None

Alternative regimen(s) for treatment-experienced (PEG/RBV nonresponder) HIV/HCV-coinfected patients with genotype 1 who are ineligible to receive IFN.

Sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) for 24 weeks is an acceptable regimen for treatment-experienced (nonresponder) HIV/HCV-coinfected patients with HCV genotype 1 infection.

Rating: Class IIb, Level C

Alternative regimen(s) for treatment-naive and PEG/RBV relapser, HIV/HCV-coinfected patients with genotype 2 or 3 infection.

None

Alternative regimen(s) for treatment-experienced (PEG/RBV nonresponder) HIV/HCV-coinfected patients with genotype 2 or 3 infection who are eligible to receive IFN.

Sofosbuvir (400 mg once daily) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg] daily) plus weekly PEG for 12 weeks is an acceptable regimen for treatment-experienced IFN-eligible persons with HCV genotype 2 or 3 infection.

Rating: Class IIa, Level C

Alternative regimen(s) for treatment-naive and treatment-experienced HIV/HCV-coinfected patients with HCV genotype 4, 5, or 6 infection.

None

The TMC435-C212 is a Phase III, open-label, single-arm study investigating simeprevir plus PEG/RBV (fixed-dose ribavirin) in treatment-naive and treatment-experienced patients coinfected with HCV genotype-1 and HIV. (Dieterich, 2013) The study used an RGT design for treatment-naive and prior PEG/RBV relapsers; prior partial and null responders and all patients with cirrhosis (regardless of treatment history) received 48 weeks of therapy (SMV x 12 weeks plus PEG/RBV x 48 weeks). The primary analysis reported an overall SVR12 of 74% (treatment naive: 79%; prior relapsers, 87%: prior partial responders: 70%; prior null responders: 57%). Most (89%) eligible patients met criteria for RGT and were able to shorten therapy to 24 weeks, after which time 78% achieved SVR12. Lower SVR12 was reported in several clinically relevant subgroups: genotype 1a (71% vs 89% in genotype 1b); genotype 1a with the Q80K mutation at baseline (67%); advanced fibrosis or cirrhosis (64%); IL28B unfavorable genetic polymorphisms (68% and 61% for the CT and TT variants vs 96% for the favorable CC variant); high baseline HCV RNA (70% for >800,000 IU/mL or 93% for <800,000 IU/mL); and patients not receiving antiretroviral therapy (62% vs 75% in subjects on antiretroviral drugs). As with patients with HCV monoinfection, baseline resistance testing for the Q80K polymorphism should be performed in all patients harboring the genotype 1a subtype and a different regimen considered if the polymorphism is present. Virologic failures occurred; most failures (79%) were associated with the emergence of resistant-associated mutations.

The adverse event profile was similar to that of patients with HCV monoinfection, with a higher frequency of pruritus, rash, photosensitivity, and increased bilirubin than is observed in patients receiving PEG/RBV alone. Due to the complexity of antiretroviral drug-associated drug interactions with simeprevir, the longer course of PEG/RBV, the adverse effect profile, and the risk of resistance emergence with treatment failure,

simeprevir plus PEG/RBV is considered an alternative regimen for treatment-naive and prior PEG/RBV relapse patients with HIV coinfection with genotype HCV who cannot tolerate the recommended regimens. This regimen is not recommended in prior nonresponders or patients with cirrhosis because of observed lower response rates seen and the poor tolerability of 48 weeks of PEG/RBV. Due to diminished activity in vitro (for genotype 2 and 3) and insufficient data (for genotype 4) this regimen cannot be recommended for these genotypes.

Sofosbuvir plus PEG/RBV has not been studied in patients with HIV/HCV genotype 1 coinfection in whom previous IFN-based HCV therapy has failed. However, in a study of a limited number of patients (n=19), the efficacy of this regimen in treatment-naive subjects with HIV/HCV genotype 1 coinfection was equivalent to that in patients with HCV monoinfection. (Rodriguez-Torres, 2013) An exploratory FDA analysis estimated the SVR rate of this regimen to be 78% among a treatment-experienced population with HCV monoinfection, including 71% in those with multiple poor pretreatment response predictors. (US FDA, 2013b) These data, along with the absence of antiretroviral drug limitations, support inclusion of this regimen as a recommended option for treatment-experienced patients with HIV/HCV coinfection.

Sofosbuvir plus RBV has not been studied in prior HCV treatment-experienced patients with HIV/HCV genotype 1 coinfection. This regimen yielded an SVR12 rate of 76% among treatment-naive HIV/HCV genotype 1-coinfected patients. (Sulkowski, 2013b) However, responses to this regimen are expected to be lower in treatment-experienced coinfected subjects based on limited data in treatment-experienced HCV-monoinfected patients treated for 12 weeks with sofosbuvir (400 mg once daily) plus weight-based RBV (1000 mg to 1200 mg daily in divided doses). (Gane, 2013a) Further, response rates are expected to be lower than those associated with the recommended and alternative regimens. This regimen should be reserved for coinfected patients who cannot tolerate IFN and do not have antiretroviral regimen options compatible with simeprevir. These patients require expert consultation with careful consideration of fibrosis stage; in some cases, deferral of therapy may be a more appropriate action.

Sofosbuvir plus PEG/RBV has not been studied in patients with HIV/HCV genotype 2 or 3 coinfection in whom previous IFN-based HCV therapy has failed. However, recognizing the potential limitations of sofosbuvir plus RBV in more difficult to treat genotype 2 and 3 patients, particularly those with prior nonresponse and cirrhosis, the addition of IFN to the regimen can be considered for those patients who are eligible. The LONESTAR-2 (open-label, single-site, single-arm phase 2 trial) evaluated PEG (180 & #956;g weekly), sofosbuvir (400 mg once daily), and weight-based RBV (1000 mg to 1200 mg daily in divided doses) for 12 weeks in HCV-monoinfected treatment-experienced patients with genotype 2 or 3 infection. Cirrhosis was present at baseline in 55% of patients. Overall, SVR12 was achieved in 96% (22 of 23) of those with genotype 2 infection. SVR occurred in 93% (13/14) and 100% (9 of 9) of patients with and without cirrhosis, respectively. Because sofosbuvir is safe and effective when used to treat HIV/HCV-coinfected patients, the combination of sofosbuvir plus PEG/RBV for 12 weeks can be considered for appropriate genotype 2 and 3 HIV/HCV-coinfected patients.

The following regimens are NOT recommended for treatment-naive or treatment-experienced HIV/HCV-coinfected patients

PEG/RBV with or without telaprevir or boceprevir for 24 to 48 weeks

Rating: Class IIb, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Due to its prolonged treatment course, adverse effects, and poor response rates, PEG/RBV is no longer recommended for the treatment of patients with HCV genotypes 1, 2, 3, or 4 who are coinfected with HIV. Neither telaprevir nor boceprevir is approved for use in patients with HIV/HCV coinfection. However, when combined with PEG/RBV and used for 48 weeks, these drugs have reported efficacy and safety in patients with HIV/HCV genotype 1 coinfection similar to that in patients with HCV genotype 1 monoinfection. (Sulkowski, 2013d); (Sulkowski, 2013a) Ongoing Phase III trials will investigate the use of RGT for select patient groups. Telaprevir and boceprevir are each substrates and inhibitors of CYP3A4 and thus have substantial drug interactions with antiretroviral drugs. (van Heeswijk, 2011a); (van Heeswijk, 2011b); (Kakuda, 2012); (Johnson, 2013); (Kasserra, 2011); (Hulskotte, 2013); (Garraffo, 2013); (de Kanter, 2012); (Hammond, 2013); (Vourvahis, 2013) Due to the adverse effect profile, prolonged required course of PEG/RBV, and substantial drug interactions, these agents are no longer recommended for HIV/HCV-coinfected patients.

Because of their limited activity in vitro and in vivo against HCV genotypes 2 and 3, boceprevir, telaprevir, and simeprevir should not be used as therapy for HIV/HCV-coinfected patients with HCV genotype 2 or 3 infection. Boceprevir and telaprevir also have limited activity against HCV genotype 4 and should not be used as therapy for HIV/HCV coinfected patients with HCV genotype 4 infection. There are currently not enough data to support a recommendation for the use of simeprevir for genotype 4 infection in HIV/HCV-coinfected patients.

2. Patients with Cirrhosis

The summary of recommendations for patients with cirrhosis is in the BOX.

Compensated Cirrhosis

Treatment-naive patients with compensated cirrhosis, including those with hepatocellular carcinoma, should receive the same treatment as recommended for patients without cirrhosis.

Rating: Class I, Level A

This statement is supported by a number of studies (described above) that included patients with compensated cirrhosis who were evaluated in sub-group analyses.

Decompensated Cirrhosis

Patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C) ***should be referred to a medical practitioner with expertise in that condition (ideally***

in a liver transplant center).

Rating: Class I, Level C

If the decision to treat has been made, the recommended regimen for patients with any HCV genotype who have decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C***) who may or may not be candidates for liver transplantation, including those with hepatocellular carcinoma. This regimen should be used only by highly experienced HCV providers***

Daily sofosbuvir (400 mg) plus weight-based RBV (with consideration of the patient's creatinine clearance and hemoglobin level) for up to 48 weeks

Rating: Class IIb, Level B

In one study, 61 patients with HCV infection and hepatocellular carcinoma meeting MILAN criteria for liver transplant were treated with sofosbuvir plus RBV for up to 48 weeks. (Curry MP, 2013) At the time of treatment initiation, the median MELD score was 8 (range: 6-14), and 17 patients had CTP scores of 7 or 8 (CTP Class B). To date, 44 patients have undergone liver transplantation, of whom 41 (93%) had HCV RNA below the lower limit of quantification. At 12 weeks post-transplant, 23 of 37 (62%) had no detected HCV RNA consistent with prevention of recurrent HCV infection. In the post-transplant period, 10 patients experienced recurrent HCV infection. Among the 10 patients who experienced recurrent graft infection, 9 had HCV RNA not detected for less than 30 days pretransplant. The most common adverse effects were fatigue, anemia, and headache; adverse effects led to treatment discontinuation for 2 patients (3%).

In a sofosbuvir compassionate-use program for patients with severe recurrent HCV infection following liver transplantation who were predicted to have a less than 6-month survival, (Forns, 2013b) 44 patients were treated with sofosbuvir plus RBV 32 patients were also given PEG. At treatment initiation, the median MELD score was 16 (range: 6-43), and fibrosing cholestatic hepatitis was documented in 20 patients. After week 12 of treatment, 91% of patients treated with sofosbuvir plus RBV and 75% of those treated with the addition of PEG achieved HCV RNA less than the lower limit of quantification. Of 27 patients evaluated at 12 weeks post-treatment, 15 patients (56%) achieved SVR. Overall, 75% had improved or stable clinical liver disease including improvement in hyperbilirubinemia and coagulopathy as well as decrease in MELD score. In this very sick population, 8 patients died, most from liver disease progression.

The following regimens are NOT recommended for patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C***)***:

Any IFN-based therapy

Rating: Class III, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir-, boceprevir-, or simeprevir-based regimens

Rating: Class III, Level A

IFN should not be given to patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C) because of the potential for worsening hepatic decompensation. Neither telaprevir nor boceprevir should be used for this population because they must be coadministered with PEG/RBV. Very minimal data exist for the use of simeprevir in patients with decompensated cirrhosis. Until additional data become available, simeprevir should not be used in patients with decompensated cirrhosis.

3. Patients Who Develop Recurrent HCV Infection Post-Liver Transplantation

The summary of recommendations for patients who develop recurrent HCV infection post-liver transplantation is in the BOX.

Recommended regimen for treatment-naive patients with HCV genotype 1 in the allograft liver, including those with compensated cirrhosis

Daily sofosbuvir (400 mg) plus simeprevir (150 mg), with or without RBV (initial dose 600 mg/day, increased monthly by 200 mg/day as tolerated to weight-based dose of 1000 mg [<75 kg] to 1200 mg [≥75 kg] 1200 mg), for 12 weeks to 24 weeks is recommended for patients with compensated allograft HCV genotype 1 infection.

Rating: Class IIb, Level C

Recommended regimen for treatment-naive patients with HCV genotype 2 or 3 in the allograft liver, including those with compensated cirrhosis

Daily sofosbuvir (400 mg) and RBV (initial dose 600 mg/day, increased monthly by 200 mg/day as tolerated to weight-based dose of 1000 mg [<75 kg] to 1200 mg [≥75 kg] 1200 mg) with consideration of the patient's CrCl value and hemoglobin level for 24 weeks is recommended for patients with compensated allograft HCV genotype 2 or 3 infection.

Rating: Class IIb, Level C

Alternate regimen for treatment-naive patients with genotype 1 HCV in the allograft liver, including those with compensated cirrhosis.

Daily sofosbuvir (400 mg) and RBV (initial dose 600 mg/day, increased monthly by 200 mg/day as tolerated to weight-based dose of 1000 mg [<75 kg] to 1200 mg [≥75 kg] 1200 mg) with consideration of the patient's CrCl value and hemoglobin level, with or without PEG (in the absence of contraindication to its use), for 24 weeks is recommended for patients with compensated allograft HCV genotype 1 infection.

Rating: Class IIb, Level C

Simeprevir has not been studied with sofosbuvir in the posttransplant setting. The coadministration of single-dose cyclosporine with simeprevir resulted in a 19% increase in cyclosporine concentrations and no change in simeprevir concentrations (see simeprevir package insert). However, in an interim analysis of an ongoing study (TMC435HPC3016), concomitant use of simeprevir (plus daclastavir and RBV) with cyclosporine at steady state resulted in an approximately 6-fold increase in plasma concentrations of simeprevir compared with historical data of simeprevir in the absence of cyclosporine (personal communication). This interaction may be due to inhibition of OATP1B1, P-gp and CYP3A by cyclosporine. Although the current FDA labeling does not recommend against the coadministration of simeprevir and cyclosporine, given these new findings, simeprevir should not be coadministered with cyclosporine.

The coadministration of single-dose tacrolimus with simeprevir did not result in a significant change of tacrolimus concentrations (see simeprevir package insert). In an ongoing study, concomitant use of simeprevir with tacrolimus also resulted in a 2-fold increase in plasma concentrations of simeprevir compared with historical data (personal communication). Based on phase I studies, a 2-fold increase in simeprevir concentrations is unlikely to be clinically significant.

Clinicians may consider the use of sofosbuvir plus simeprevir in patients receiving tacrolimus with therapeutic drug monitoring, particularly in those expected to have difficulty tolerating RBV (eg, patients with impaired renal function and anemia). Consideration should be given to pretreatment resistance testing for the Q80K polymorphism in genotype 1a-infected patients.

In addition to the sofosbuvir compassionate use program (Forns, 2013a), 40 patients with recurrent HCV [illegible] were reported. Discontinued therapy due to adverse events. No deaths, graft loss, or episodes of rejection.

The addition of PEG to sofosbuvir plus RBV may also be considered in the absence of contraindications.

The following regimens are NOT recommended for treatment-naive patients with compensated allograft hepatitis C virus infection.

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir- or boceprevir- based regimens should not be used for patients

with compensated allograft hepatitis C virus infection.

Rating: Class III, Level A

Telaprevir or boceprevir should not be used in the post-liver transplant population because of surrounding toxicity and drug interactions with calcineurin inhibitors**.**

Decompensated Cirrhosis

Treatment-naive patients with decompensated allograft HCV infection should receive the same treatment as recommended for patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C).

Rating: Class I, Level C

4. Patients with Renal Impairment, Including Severe Renal Impairment (CrCl <30 mL/min) or ESRD Requiring Hemodialysis or Peritoneal Dialysis

Summary of Recommendations for Patients with Renal Impairment Including, Severe Renal Impairment (CrCl <30 mL/min) or ESRD Requiring Hemodialysis or Peritoneal Dialysis is found in the BOX.

When using sofosbuvir to treat or retreat HCV infection in patients with appropriate genotypes, no dosage adjustment is required for patients with mild to moderate renal impairment (CrCl ≥30 mL/min). Sofosbuvir is not recommended in patients with severe renal impairment/ESRD (CrCl <30 mL/min) or those who require hemodialysis, because no dosing data are currently available for this patient population.

Rating: Class IIa, level B

Sofosbuvir enters the hepatocyte, where it is metabolized to its active form, GS-461203. The downstream inactive nucleoside metabolite GS-331007 is almost exclusively eliminated from the body renally, mediated through a combination of glomerular filtration and active tubular secretion. Results of phase 2 and 3 sofosbuvir clinical trials have excluded patients with serum Cr level above 2.5 and/or CrCl level below <60 mL/min. The pharmacokinetics of a single dose of sofosbuvir 400 mg was assessed in persons not infected

with HCV (study P7977-0915) with mild (estimated glomerular filtration rate [eGFR] ≥50 and <80 mL/min/1.73m^2), moderate (eGFR ≥30 and <50 mL/min/1.73m^2), severe renal impairment (eGFR <30 mL/min/1.73m^2) and persons with ESRD requiring hemodialysis. Relative to persons with normal renal function (eGFR>80 mL/min/1.73m^2), the sofosbuvir AUC(0-inf) was 61%, 107%, and 171% higher in subjects with mild, moderate, and severe renal impairment, respectively. The GS-331007 AUC(0-inf) was 55%, 88%, and 451% higher, respectively. No safety signals have been seen under similar conditions. In subjects with ESRD (relative to subjects with normal renal function), sofosbuvir and GS-331007 AUC (0-inf) was 28% and 1280% higher, respectively, when sofosbuvir was dosed 1 hour before hemodialysis, compared with 60% and 2070% higher, respectively, when sofosbuvir was dosed 1 hour after hemodialysis. No dose adjustment is required for patients with mild or moderate renal impairment. The safety of sofosbuvir has not been established in patients with severe renal impairment or ESRD. Therefore, a dose recommendation cannot be provided for these populations at this time, although a dedicated study to evaluate optimal dosing of sofosbuvir in HCV-infected patients with severe renal impairment or ESRD on hemodialysis is currently underway.

When using simeprevir in treatment/retreatment of HCV-infected patients, no dosage adjustment is required for patients with mild to moderate to severe renal impairment. Simeprevir has not been studied in patients with ESRD, including those requiring hemodialysis.

Rating: Class IIa, level B

Simeprevir is primarily metabolized by liver CYP3A4, and renal clearance plays an insignificant role (<1%) in the elimination of simeprevir and its metabolites.

Simeprevir 150 mg daily for 7 days has been studied in non-HCV infected patients with severe renal impairment (eGFR<30 mL/min/1.73m^2) and healthy volunteers (eGFR> mL/min/1.73 m^2). For persons with severe renal impairment, simeprevir C_{min}, C_{max}, and AUC(24 hour) were 71%, 34%, and 62% higher, respectively, compared with matched healthy controls. Simeprevir exposure was higher in patients with severe renal impairment (steady-state by day 7), but no significant difference was observed in simeprevir plasma protein binding. Simeprevir was generally safe and well tolerated in subjects with severe renal impairment. Therefore, no dose adjustment of simeprevir is required in these patients. No clinically significant differences in pharmacokinetics were observed in HCV-uninfected participants with mild, moderate, or severe renal impairment. CrCl level was not identified as a significant covariate of simeprevir population pharmacokinetics in HCV-infected patients.Simeprevir has not been evaluated in patients receiving hemodialysis.

In patients with renal impairment/ESRD/HD, dosing of PEG and RBV should follow updated FDA recommendations or package insert recommendations based on calculated GFR. Caution should be used in administering RBV to these patients, and close monitoring of hemoglobin is required.

Rating: Class IIa, level B

HCV infection is a major health problem in patients with ESRD. The incidence of acute HCV infection during maintenance dialysis is much higher than that in the general population because of the risk for nosocomial transmission. The kidney is important for the catabolism and filtration of both IFN and RBV, and therefore, reduced doses of both PEG and RBV are warranted in patients with ESRD.

Impaired excretion of RBV occurs in patients with chronic kidney disease, as RBV is mostly eliminated by the kidney. Very little RBV is removed via dialysis. Thus, the drug can accumulate, exacerbating hemolysis in the dialysis population already at substantial risk for anemia. If a decision is made to use RBV in patients on maintenance hemodialysis, it should be used only after the implementation of several safety precautions, including (1) administering very low doses of RBV (200 mg daily), (2) monitoring hemoglobin levels on a weekly basis, (3) titrating epoetin alfa to treat anemia, and (4) providing intravenous iron supplementation to boost erythropoietin activity.

Dose adjustments needed for patients with renal impairment are summarized in the Renal Impairment Table.

Unique Patient Populations: HIV/HCV Coinfection Box. Summary of Recommendations for HIV/HCV Coinfected Patients Who are Being Treated for HCV, by Genotype

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Unique Patient Populations: HIV/HCV Coinfection Box. Summary of Recommendations for HIV/HCV Coinfected Patients Who are Being Treated for HCV, by Genotype

Genotype	Recommended	Alternative	NOT Recommended	Allowable Antiretroviral Therapy
1	**Treatment-naive and prior PEG/RBV relapsers** **IFN eligible:** SOF + PEG/RBV x 12 weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or	**Treatment naive and prior PEG/RBV relapsers** **IFN eligible:** SMV x 12 weeks + PEG/RBV x 24 weeks* IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below	TVR + PEG/RBV x 24 or 48 weeks (RGT) BOC + PEG/RBV x 28 or 48 weeks (RGT) PEG/RBV x 48 weeks SMV x 12 weeks + PEG/RBV x 48 wks	**For SOF use:** ALL except didanosine, zidovudine, or tipranavir **For SMV use:** LIMITED to raltegravir, rilpivirine, maraviroc, enfuvirtide, tenofovir, emtricitabine, lamivudine, abacavir

baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: SOF + RBV x 24 weeks SOF + SMV ± RBV x 12 weeks **Treatment experienced (prior PEG/RBV nonresponders) regardless of IFN eligibility:** SOF + SMV ± RBV x 12 weeks	90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: None **Treatment experienced (prior PEG/RBV nonresponders)** **IFN eligible:** SOF + PEG/RBV x 12 Weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: SOF + RBV x 24 Weeks

2	SOF + RBV x 12 weeks regardless of treatment history	**Treatment naive and prior PEG/RBV relapsers**: None **Treatment experienced (prior PEG/RBV nonresponders)** **IFN eligible:** SOF + PEG/RBV X 12 Weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: None	PEG/RBV x 24-48 weeks Any regimen with TVR, BOC, or SMV	ALL except didanosine, zidovudine, or tipranavir
3	SOF + RBV x 24 weeks regardless of treatment history	**Treatment naive and PEG/RBV relapsers:** None **Treatment**	PEG/RBV x 24 - 48 weeks Any regimen with TVR, BOC, or	ALL except didanosine, zidovudine, or tipranavir

		experienced (prior PEG/RBV nonresponders) **IFN eligible:** SOF + PEG/RBV X 12 Weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: None	SMV	
4	**Regardless of treatment history:** **IFN eligible:** SOF + PEG/RBV x 12 weeks IFN ineligible IFN ineligible is defined as one or more of the below: • Intolerance to	None	PEG/RBV x 48 weeks Any regimen with TVR or BOC	ALL except didanosine, zidovudine, or tipranavir

	IFN • Autoimmune hepatitis and other autoimmune disorders • Hypersensitivity to PEG or any of its components • Decompensated hepatic disease • Major uncontrolled depressive illness • A baseline neutrophil count below 1500/?L, a baseline platelet count below 90,000/?L or baseline hemoglobin below 10 g/dL • A history of preexisting cardiac disease: SOF + RBV x 24 weeks			
5 or 6	**Regardless of treatment history:** SOF + PEG/RBV x 12 weeks	None	PEG/RBV x 48 weeks Any regimen with TVR, BOC, or SMV	ALL except didanosine, zidovudine, or tipranavir

*For genotype 1a, baseline resistance testing for Q80K should be performed and alternative treatments should be considered if this mutation is present.

Unique Patient Populations: Cirrhosis Box. Summary of Recommendations for Patients with Cirrhosis

Unique Patient Populations: Cirrhosis Box. Summary of Recommendations for Patients with Cirrhosis

Treatment-naive patients with compensated cirrhosis, including those with hepatocellular carcinoma, should receive the same treatment as recommended for patients without cirrhosis.

Rating: Class I, Level A

Patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C) should be referred to a medical practitioner with expertise in that condition (ideally in a liver transplant center).

Rating: Class I, Level C

The recommended regimen for patients with any HCV genotype who have decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C) who may or may not be candidates for liver transplantation, including those with hepatocellular carcinoma. This regimen should be used only by highly experienced HCV providers

Daily sofosbuvir (400 mg) plus weight-based RBV (with consideration of the patient's creatinine clearance and hemoglobin level) for up to 48 weeks

Rating: Class IIb, Level B

The following regimens are NOT recommended for patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C):

Any IFN-based therapy

Rating: Class III, Level A

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir-, boceprevir-, or simeprevir-based regimens

Rating: Class III, Level A

Unique Patient Populations: Post-Liver Transplantation Box. Summary of Recommendations for Patients Who Develop Recurrent HCV Infection Post-Liver Transplantation

Unique Patient Population: Post-Liver Transplantation Box. Summary of Recommendations for Patients Who Develop Recurrent HCV Infection Post-Liver Transplantation

Recommended regimen for treatment-naive patients with HCV genotype 1 in the allograft liver, including those with compensated cirrhosis

Daily sofosbuvir (400 mg) plus simeprevir (150 mg), with or without RBV (initial dose 600 mg/day, increased monthly by 200 mg/day as tolerated to weight-based dose of 1000 mg [<75 kg] to 1200 mg [≥75 kg] 1200 mg), for 12 weeks to 24 weeks is recommended for patients with compensated allograft HCV genotype 1 infection.

Rating: Class IIb, Level C

Recommended regimen for treatment-naive patients with HCV genotype 2 or 3 in the allograft liver, including those with compensated cirrhosis

Daily sofosbuvir (400 mg) and RBV (initial dose 600 mg/day, increased monthly by 200 mg/day as tolerated to weight-based dose of 1000 mg [<75 kg] to 1200 mg [≥75 kg] 1200 mg) with consideration of the patient's CrCl value and hemoglobin level for 24 weeks is recommended for patients with compensated allograft HCV genotype 2 or 3 infection.

Rating: Class IIb, Level C

Alternate regimen for treatment-naive patients with genotype 1 HCV in the allograft liver, including those with compensated cirrhosis.

Daily sofosbuvir (400 mg) and RBV (initial dose 600 mg/day, increased monthly by 200 mg/day as tolerated to weight-based dose of 1000 mg [<75 kg] to 1200 mg [≥75 kg] 1200 mg) with consideration of the patient's CrCl value and hemoglobin level,

with or without PEG (in the absence of contraindication to its use), for 24 weeks is recommended for patients with compensated allograft HCV genotype 1 infection.

Rating: Class IIb, Level C

The following regimens are NOT recommended for treatment-naive patients with compensated allograft hepatitis C infection

Monotherapy with PEG, RBV, or a DAA

Rating: Class III, Level A

Telaprevir- or boceprevir- based regimens should not be used for patients with compensated allograft hepatitis C infection.

Rating: Class III, Level A

Treatment-naive patients with decompensated allograft HCV infection should receive the same treatment as recommended for patients with decompensated cirrhosis (moderate or severe hepatic impairment; CTP class B or C).

Rating: Class I, Level C

Unique Patient Populations: Renal Impairment Table. Dose Adjustments Needed for Patients with Renal Impairment

Unique Patient Populations: Renal Impairment Table. Dose Adjustments Needed for Patients with Renal Impairment

Renal Impairment	eGFR/CrCl level (mL/min/ 1.73 m^2)	Interferon	Ribavirin	Sofosbuvir	Simeprevir
Mild	50-80	180 µg PEG (2a); PEG (2b) 1.5 µg/kg	Standard	Standard	Standard
Moderate	30-50	180 µg PEG (2a); PEG alfa-2b1 µg/kg or 25% reduction	Alternating doses 200 and 400 mg every other day	Standard	Standard
Severe	<30	135 µg PEG (2a); PEG (2b)1 µg/kg or 50% reduction	200 mg/d	Data not available	Standard
ESRD/HD		PEG (2a) 135 µg/wk or PEG (2b) 1 µg/kg/wk or standard IFN 3 mU 3x/wk	200 mg/d	Data not available	Data not available

Unique Patient Populations: Renal Impairment Box. Summary of Recommendations for Patients with Renal Impairment Including Severe Renal Impairment (CrCl <30 ML/min) or ESRD Requiring Hemodialysis or Peritoneal Dialysis

Renal Impairment Box. Summary of Recommendations for Patients with Renal Impairment, Including Severe Renal Impairment (CrCl <30 ML/min) or ESRD Requiring Hemodialysis or Peritoneal Dialysis

When using sofosbuvir to treat or retreat HCV infection in patients with appropriate genotypes, no dosage adjustment is required for patients with mild to moderate renal impairment (CrCl ?30 mL/min). Sofosbuvir is not recommended in patients with severe renal impairment/ESRD (CrCl <30 mL/min) or those who require hemodialysis, because no dosing data are currently available for this patient population.

Rating: Class IIa, level B

When using simeprevir in treatment/retreatment of HCV-infected patients, no dosage adjustment is required for patients with mild to moderate to severe renal impairment. Simeprevir has not been studied in patients with ESRD, including those requiring hemodialysis.

Rating: Class IIa, level B

In patients with renal impairment/ESRD/HD, dosing of PEG and RBV should follow updated FDA recommendations or package insert recommendations based on calculated GFR. Caution should be used in administering RBV to these patients, and close monitoring of hemoglobin is required.

Rating: Class IIa, level B

COMING SOON: Management of Acute HCV Infection

COMING SOON: Management of Acute HCV Infection

REFERENCES

Aberg JA, Gallant JE, Ghanem KG, Emmanuel P, Zingman BS, Horberg MA. Primary Care Guidelines for the Management of Persons Infected With HIV: 2013 Update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis.* 2013.

Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med.* 1992;327(21):1490-1495.

Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep.* 2003;52(RR-3):1-13, 15.

Alvarez D, Dieterich DT, Brau N, Moores L, Ball L, Sulkowski MS. Zidovudine use but not weight-based ribavirin dosing impacts anaemia during HCV treatment in HIV-infected persons. *J Viral Hepat.* 2006;13:683-689.

American Heart Association. http://my.americanheart.org/idc/groups/ahamah-public/@wcm/@sop/documents/downloadable/ucm_319826.pdf. Accessed on January 27, 2014.

Amon JJ, Garfein RS, Ahdieh-Grant L, et al. Prevalence of hepatitis C virus infection among injection drug users in the United States, 1994-2004. *Clin Infect Dis.* 2008;46(12):1852-1858.

Arase Y, Suzuki F, Suzuki Y, et al. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology.* 2009;49(3):739-744.

Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med.* 2006;144(10):705-714.

Arora S, Thornton K, Murata G, et al. Outcomes of treatment for hepatitis C virus infection by primary care providers. *N Engl J Med.* 2011;364(23):2199-2207.

Aspinall EJ, Corson S, Doyle JS, et al. Treatment of hepatitis C virus infection among people who are actively injecting drugs: a systematic review and meta-analysis. *Clin Infect Dis.* 2013;57(Suppl 2):S80-S89.

Backus LI, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A sustained virologic response reduces risk of all-cause mortality in patients with hepatitis C. *Clin Gastroenterol Hepatol.* 2011;9(6):509-516.

Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med.* 2011;364(13):1207-1217.

Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C.

Hepatology. 2003;38(6):1449-1457.

Bellentani S, Pozzato G, Saccoccio G, et al. Clinical course and risk factors of hepatitis C virus related liver disease in the general population: report from the Dionysos study. *Gut.* 1999;44(6):874-880.

Benhamou Y, Bochet M, Di Martino V, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. *Hepatology.* 1999;30(4):1054-1058.

Benhamou Y, Di Martino V, Bochet M, et al. Factors affecting liver fibrosis in human immunodeficiency virus- and hepatitis C virus-coinfected patients: impact of protease inhibitor therapy. *Hepatology.* 2001;34(2):283-287.

Berenguer J, Álvarez-Pellicer J, Martin PM, et al. Sustained virological response to interferon plus ribavirin reduces liver-related complications and mortality in patients coinfected with human immunodeficiency virus and hepatitis C virus. *Hepatology.* 2009;50(2):407-413.

Berenguer M, Palau A, Aguilera V, Rayon JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. *Am J Transplant.* 2008;8(3):679-687.

Berenguer M, Schuppan D. Progression of liver fibrosis in post-transplant hepatitis C: mechanisms, assessment and treatment. *J Hepatol.* 2013;58(5):1028-1041.

Bochud PY, Cai T, Overbeck K, et al. Genotype 3 is associated with accelerated fibrosis progression in chronic hepatitis C. *J Hepatol.* 2009;51(4):655-666.

Bonkovsky HL, Snow KK, Malet PF, et al. Health-related quality of life in patients with chronic hepatitis C and advanced fibrosis. *J Hepatol.* 2007;46(3):420-431.

Boursier J, de L, V, Zarski JP, et al. Comparison of eight diagnostic algorithms for liver fibrosis in hepatitis C: new algorithms are more precise and entirely noninvasive. *Hepatology.* 2012;55(1):58-67.

Bräu N, Salvatore M, RÍos-Bedoya CF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. *J Hepatol.* 2006;44:47-55.

Bravo MJ, Vallejo F, Barrio G, et al. HCV seroconversion among never-injecting heroin users at baseline: no predictors identified other than starting injection. *Int J Drug Policy.* 2012;23(5):415-419.

Bruggmann P, Litwin AH. Models of care for the management of hepatitis C virus among people who inject drugs: one size does not fit all. *Clin Infect Dis.* 2013;57 Suppl 2:S56-S61.

Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology.* 2011;53(3):1020-1022.

Butt AA, McGinnis K, Skanderson M, Justice AC. A comparison of treatment eligibility for hepatitis

C virus in HCV-monoinfected versus HCV/HIV-coinfected persons in electronically retrieved cohort of HCV-infected veterans. *AIDS Res Hum Retroviruses.* 2011;27(9):973-979.

Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology.* 2012;142(6):1293-1302.

Castera L, Sebastiani G, Le BB, de L, V, Couzigou P, Alberti A. Prospective comparison of two algorithms combining non-invasive methods for staging liver fibrosis in chronic hepatitis C. *J Hepatol.* 2010;52(2):191-198.

Centers for Disease Control and Prevention. Recommendations for preventing transmission of infections among chronic hemodialysis patients. *MMWR Recomm Rep.* 2001;50(RR-5):1-43.

Centers for Disease Control and Prevention. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR Morb Mortal Wkly Rep.* 1998;47(RR-19):1-39.

Centers for Disease Control and Prevention. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR.* 2008;57(RR-8)

Centers for Disease Control and Prevention (CDC). Testing for HCV infection: an update of guidance for clinicians and laboratorians. *MMWR Morb Mortal Wkly Rep.* 2013;62(18):362-365.

Charlton M, Seaberg E, Wiesner R, et al. Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology.* 1998;28(3):823-830.

Charlton MR, Gane E, Manns M, et al. Sofosbuvir and Ribavirin for the Treatment of Established Recurrent Hepatitis C Infection After Liver Transplantation: Preliminary Results of a Prospective, Multicenter Study. *Hepatology: Special Issue: The 64th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2013.* 2013;Vol 58(4):1378A.

Charlton MR. E-mail communication. August 14, 2014.

Chew KW, Allen SA, Taylor LE, Rich JD, Feller E. Treatment outcomes with pegylated interferon and ribavirin for male prisoners with chronic hepatitis C. *J Clin Gastroenterol.* 2009;43(7):686-691.

Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Ann Intern Med.* 2013b;159(5):372.

Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: a systematic review. *Ann Intern Med.* 2013;158(11):807-820.

Chu CJ, Lee SD. Hepatitis B virus/hepatitis C virus coinfection: epidemiology, clinical features, viral interactions and treatment. *J Gastroenterol Hepatol.* 2008;23(4):512-520.

Clark BT, Garcia-Tsao G, Fraenkel L. Patterns and predictors of treatment initiation and completion in patients with chronic hepatitis C virus infection. *Patient Prefer Adherence.* 2012;6:285-295.

Coilly A, Roche B, Dumortier J, et al. Safety and efficacy of protease inhibitors to treat hepatitis C

after liver transplantation: A multicenter experience. *J.Hepatol.* 2014;60(1):78-86.

Coppola N, De PS, Pisaturo M, et al. Sustained virological response to antiviral treatment in chronic hepatitis C patients may be predictable by HCV-RNA clearance in peripheral blood mononuclear cells. *J Clin Virol.* 2013;58(4):748-750.

Corrao G, Arico S. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. *Hepatology.* 1998;27(4):914-919.

Crippin JS, McCashland T, Terrault N, Sheiner P, Charlton MR. A pilot study of the tolerability and efficacy of antiviral therapy in hepatitis C virus-infected patients awaiting liver transplantation. *Liver Transpl.* 2002;8(4):350-355.

Curry MP, et al. Pretransplant sofosbuvir and ribavirin to prevent recurrence of HCV infection after liver transplantation. 64th Annual Meeting of the American Association for the Study of Liver Disease. Nov 1-5, 2013, 2013; Washington, DC.

Curry M, Forns X, Chung RT, et al. Pretransplant sofosbuvir and ribavirin to prevent recurrence of HCV infection after liver transplantation. *Hepatology.* 2013b;58(S1):313A-317A.

de Kanter C, Blonk M, Colbers A et al. The influence of the HCV protease inhibitor boceprevir on the pharmacokinetics of the HIV integrase inhibitor raltegravir [Abstract 772LB]. 19th Conference on Retroviruses and Opportunistic Infections (CROI). March 5-8, 2012; Seattle, WA.

de Ledinghen V, Barreiro P, Foucher J, et al. Liver fibrosis on account of chronic hepatitis C is more severe in HIV-positive than HIV-negative patients despite antiretroviral therapy. *J Viral Hepat.* 2008;15(6):427-433.

Di Bisceglie A, Kuo A, Rustgi V, Sulkowski M. Virological Outcomes and Adherence to Treatment Algorithms in a Longitudinal Study of Patients with Chronic Hepatitis C Treated with Boceprevir or Telaprevir in the U.S. (HCV-TARGET). AASLD The Liver Meeting. Nov 1-5, 2013, 2013; Washington, DC; 2013.

Di Bisceglie AM, Shiffman ML, Everson GT, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med.* 2008;359(23):2429-2441.

Dienstag JL, Ghany MG, Morgan TR, et al. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. *Hepatology.* 2011;54(2):396-405.

Dieperink E, Ho SB, Heit S, Durfee JM, Thuras P, Willenbring ML. Significant reductions in drinking following brief alcohol treatment provided in a hepatitis C clinic. *Psychosomatics.* 2010;51(2):149-156.

Dieterich D, Rockstroh J, Orkin C, et al. Simeprevir (TMC435) plus peginterferon/ribavirin in patients co-infected with HCV genotype-1 andHIV-1: primary analysis of the C212 study. 14th European AIDs Conference (EACS 2013). Oct 16-19, 2013, 2013; Brussels.

Durier N, Nguyen C, White LJ. Treatment of hepatitis C as prevention: a modeling case study in Vietnam. *PLoS One.* 2012;7(4):e34548.

Everhart JE, Lok AS, Kim HY, et al. Weight-related effects on disease progression in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Gastroenterology.* 2009;137(2):549-557.

Everhart JE, Wright EC, Goodman ZD, et al. Prognostic value of Ishak fibrosis stage: findings from the hepatitis C antiviral long-term treatment against cirrhosis trial. *Hepatology.* 2010;51(2):585-594.

Everson GT. Treatment of patients with hepatitis C virus on the waiting list. *Liver Transpl.* 2003;9(11):S90-S94.

Everson GT, Hoefs JC, Seeff LB, et al. Impact of disease severity on outcome of antiviral therapy for chronic hepatitis C: Lessons from the HALT-C trial. *Hepatology.* 2006;44(6):1675-1684.

Everson GT, Trotter J, Forman L, et al. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. *Hepatology.* 2005;42(2):255-262.

Fabrizi F, Dixit V, Messa P. Antiviral therapy of symptomatic HCV-associated mixed cryoglobulinemia: meta-analysis of clinical studies. *J Med Virol.* 2013;85(6):1019-1027.

Fabrizi F, Martin P, Dixit V, Messa P. Meta-analysis of observational studies: hepatitis C and survival after renal transplant. *J Viral Hepat.* 2014;21(5):314-324.

Fabrizi F, Messa P, Martin P. Health-related quality of life in dialysis patients with HCV infection. *Int J Artif Organs.* 2009;32(8):473-481.

Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. *Hepatology.* 2002;36(1):3-10.

Fabrizi F, Takkouche B, Lunghi G, Dixit V, Messa P, Martin P. The impact of hepatitis C virus infection on survival in dialysis patients: meta-analysis of observational studies. *J Viral Hepat.* 2007;14(10):697-703.

Feld JJ, Liang TJ. Hepatitis C -- identifying patients with progressive liver injury. *Hepatology.* 2006;43(2 Suppl 1):S194-S206.

Fierer DS, Dieterich DT, Fiel MI, et al. Rapid progression to decompensated cirrhosis, liver transplant, and death in HIV-infected men after primary hepatitis C virus infection. *Clin Infect Dis.* 2013;56(7):1038-1043.

Fierer DS, Uriel AJ, Carriero DC, et al. Liver fibrosis during an outbreak of acute hepatitis C virus infection in HIV-infected men: a prospective cohort study. *J Infect Dis.* 2008;198(5):683-686.

Finelli L, Miller JT, Tokars JI, Alter MJ, Arduino MJ. National surveillance of dialysis-associated diseases in the United States, 2002. *Semin Dial.* 2005;18(1):52-61.

Fissell RB, Bragg-Gresham JL, Woods JD, et al. Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int.* 2004;65(6):2335-2342.

Fleischer R, Boxwell D, Sherman KE. Nucleoside analogues and mitochondrial toxicity. *Clin Infect*

Dis. 2004;38:e79-e80.

Fontana RJ, Sanyal AJ, Ghany MG, et al. Factors that determine the development and progression of gastroesophageal varices in patients with chronic hepatitis C. *Gastroenterology.* 2010;138(7):2321-31, 2331.

Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology.* 2002;122:889-896.

Forns X, Fontana RJ, Moonka D, et al. Initial Evaluation of the Sofosbuvir Compassionate Use Program for Patients with Severe Recurrent HCV Following Liver Transplantation. Special Issue: The 64th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2013. Epub Oct 1, 2013, 10-1-2013a;732A.

Forns X, Lawitz E, Zeuzem S, et al. Simeprevir (TMC435) with peg-interferon a-2a/ribavirin for treatment of chronic HCV genotype 1 infection in patients who relapsed after previous interferon-based therapy: efficacy and safety in patient sub-populations in the PROMISE phase III trial. 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2013). Nov 1-5, 2013, 2013b; Washington, DC.

Forns X, Fontana RJ, Moonka D, McHutchison JG, Symonds WT. Initial evaluation of the sofosbuvir compassionate use program for patients with severe recurrent HCV following liver transplantation. *Hepatology.* 2013c;58(S1):730A-760A.

Forns X, Navasa M, Rodes J. Treatment of HCV infection in patients with advanced cirrhosis. *Hepatology.* 2004;40(2):498.

Foster GR, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. *Hepatology.* 1998;27(1):209-212.

Foster GR, Hezode C, Bronowicki JP, et al. Telaprevir alone or with peginterferon and ribavirin reduces HCV RNA in patients with chronic genotype 2 but not genotype 3 infections. *Gastroenterology.* 2011;141(3):881-889.

Gane EJ, Stedman CA, Hyland RH, et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med.* 2013a;368(1):34-44.

Gane EJ, Stedman CA, Hyland RH, et al. Once daily sofosbuvir plus ribavirin for 12 and 24 weeks in treatment-naive patients with HCV infection: the QUANTUM study. Program and abstracts of the 48th Annual Meeting of the European Association for the Study of the Liver. April 24-28, 2013, 2013b; Amsterdam, the Netherlands.

Garcia-Bengoechea M, Basaras M, Barrio J, et al. Late disappearance of hepatitis C virus RNA from peripheral blood mononuclear cells in patients with chronic hepatitis C in sustained response after alpha-interferon therapy. *Am J Gastroenterol.* 1999;94(7):1902-1905.

Garcia-Tsao G, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology.* 2007;46(3):922-938.

Garraffo R, Poizot-Martin I, Piroth L et al. Pharmacokinetic (PK) interactions between Boceprevir (BOC) and Atazanavir/r (ATV/r) or Raltegravir (RAL) in HIV/HCV coinfected patients (pts). 14th International Workshop on Clinical Pharmacology on HIV Therapy. April 22-24, 2013; Amsterdam, the Netherlands.

Genentech Inc. COPEGUS (ribavirin) package insert. 2014a. http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=d370635f-5530-4d42-a019-d76b61639787. Accessed on August 28, 2014.

Genentech Inc. PEGASYS (peginterferon alfa-2a) package Insert. 2014b. http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=de61685e-2b8c-4e22-84bb-869e13600440. Accessed on August 28, 2014.

George SL, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology.* 2009;49(3):729-738.

Ghany MG, Kleiner DE, Alter H, et al. Progression of fibrosis in chronic hepatitis C. *Gastroenterology.* 2003;124(1):97-104.

Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology.* 2011;54(4):1433-1444.

Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology.* 2009;49(4):1335-1374.

Gilead Sciences I. Sofosbuvir [package insert]. 2013.Foster City, CA, Gilead Sciences, Inc.

Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in porphyria cutanea tarda: systematic review and meta-analysis. *J Hepatol.* 2003;39(4):620-627.

Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Systematic review: regression of lymphoproliferative disorders after treatment for hepatitis C infection. *Aliment Pharmacol Ther.* 2005;21(6):653-662.

Govindasamy D, Ford N, Kranzer K. Risk factors, barriers and facilitators for linkage to antiretroviral therapy care: a systematic review. *AIDS.* 2012;26(16):2059-2067.

Grady BP, Schinkel J, Thomas XV, Dalgard O. Hepatitis C virus reinfection following treatment among people who use drugs. *Clin Infect Dis.* 2013;57(Suppl 2):S105-S110.

Gumber SC, Chopra S. Hepatitis C: a multifaceted disease. Review of extrahepatic manifestations. *Ann Intern Med.* 1995;123(8):615-620.

Hammond KP, Wolfe P, Burton JR, Jr., et al. Pharmacokinetic interaction between boceprevir and etravirine in HIV/HCV seronegative volunteers. *J Acquir Immune Defic Syndr.* 2013;62(1):67-73.

Harris DR, Gonin R, Alter HJ, et al. The relationship of acute transfusion-associated hepatitis to the

development of cirrhosis in the presence of alcohol abuse. *Ann Intern Med.* 2001;134(2):120-124.

Hellard ME, Jenkinson R, Higgs P, et al. Modelling antiviral treatment to prevent hepatitis C infection among people who inject drugs in Victoria, Australia. *Med J Aust.* 2012;196(10):638-641.

Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med.* 2002;347(2):89-94.

Holmberg SD, Spradling PR, Moorman AC, Denniston MM. Hepatitis C in the United States. *N Engl J Med.* 2013;368(20):1859-1861.

Holmes HM, Hayley DC, Alexander GC, Sachs GA. Reconsidering medication appropriateness for patients late in life. *Arch Intern Med.* 2006;166(6):605-609.

Hosein SR, Wilson DP. HIV, HCV, and drug use in men who have sex with men. *Lancet.* 2013;382(9898):1095-1096.

Hourigan LF, Macdonald GA, Purdie D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology.* 1999;29(4):1215-1219.

Hsu YC, Lin JT, Ho HJ, et al. Antiviral treatment for hepatitis C virus infection is associated with improved renal and cardiovascular outcomes in diabetic patients. *Hepatology.* 2014;59(4):1293-1302.

Hulskotte EG, Feng HP, Xuan F, et al. Pharmacokinetic interactions between the hepatitis C virus protease inhibitor boceprevir and ritonavir-boosted HIV-1 protease inhibitors atazanavir, darunavir, and lopinavir. *Clin Infect Dis.* 2013;56(5):718-726.

Hung CH, Wang JH, Hu TH, et al. Insulin resistance is associated with hepatocellular carcinoma in chronic hepatitis C infection. *World J Gastroenterol.* 2010;16(18):2265-2271.

Islam MM, Topp L, Conigrave KM, et al. Linkage into specialist hepatitis C treatment services of injecting drug users attending a needle syringe program-based primary healthcare centre. *J Subst Abuse Treat.* 2012;43(4):440-445.

Jacobson IM, Dore GJ, Foster G, et al. Simeprevir (TMC435) with Peginterferon/ Ribavirin for Chronic HCV Genotype-1 Infection in Treatment-Naive Patients: Results From QUEST-1, a Phase III Trial. Digestive Disease Week. May 18-21, 2013; Orlando, FL.

Jacobson IM, Ghalib RH, Rodriguez-Torres M, et. SVR results of a once-daily regimen of simeprevir (TMC435) plus sofosbuvir (GS-7977) with or without ribavirin in cirrhotic and non-cirrhotic HCV genotype 1 treatment-naïve and prior null responder patients: The COSMOS study. *Hepatology: Special Issue: The 64th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2013.* 2013;58(4):1379A.

Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med.* 2013;368(20):1867-1877.

Jadoul M, Cornu C, van Ypersele de SC. Universal precautions prevent hepatitis C virus

transmission: a 54 month follow-up of the Belgian Multicenter Study. The Universitaires Cliniques St-Luc (UCL) Collaborative Group. *Kidney Int.* 1998;53(4):1022-1025.

Janssen Therapeutics. Simeprevir [package insert]. 2013.Titusville, NJ, Janssen Therapeutics.

Johnson M, Borland J, Chen S-J et al. Dolutegravir, Boceprevir, Telaprevir PK: The effect of Boceprevir and Telaprevir on Dolutegravir Pharmacokinetics, in Healthy Adult Subjects. 14th International Workshop on Clinical Phamacology of HIV Therapy. Apr 22-24, 2013, 2013; Amsterdam.

Johnson RJ, Gretch DR, Couser WG, et al. Hepatitis C virus-associated glomerulonephritis. Effect of alpha-interferon therapy. *Kidney Int.* 1994;46(6):1700-1704.

Johnson RJ, Gretch DR, Yamabe H, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med.* 1993;328(7):465-470.

Kakuda T, Leopold L, Nijs S. Pharmacokinetic interaction between etravirine or rilpivirine and telaprevir in healthy volunteers: a randomised, two-way crossover trial [Abstract O-18]. 13th International Workshop on Clinical Pharmacology of HIV Therapy. April 16-18, 2012; Barcelona, Spain.

Kanwal F, Kramer JR, Ilyas J, Duan Z, El-Serag HB. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. *Hepatology.* 2014;60(1):98-105.

Kasserra C, Hughes E, Treitel M, Gupta S, O'Mara E. Clinical Pharmacology of BOC: Metabolism, Excretion, and Drug-Drug Interactions. In: Proceedings from the 18th Conference on Retroviruses and Opportunistic Infections (CROI): February 27-March 2. 2011; Boston, MA. Abstract 118.

KDIGO. KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. *Kidney Int Suppl.* 2008;(109):S1-99.

Khokhar OS, Lewis JH. Reasons why patients infected with chronic hepatitis C virus choose to defer treatment: do they alter their decision with time? *Dig Dis Sci.* 2007;52(5):1168-1176.

Kirby B, Mathias A, Rossi S, et al. No clinically significant pharmacokinetic drug interactions between sofosbuvir (GS-7977) and HIV antiretrovirals atripla, rilpivirine, darunavir/ritonavir, or raltegravir in healthy volunteers. 63rd Annual Meeting of the American Association of the Study of Liver Diseases (AASLD) 2012. Nov 9-11, 2012, 2013; Boston, MA.

Kleiner DE. The liver biopsy in chronic hepatitis C: a view from the other side of the microscope. *Semin Liver Dis.* 2005;25(1):52-64.

Konerman MA, Mehta SH, Sutcliffe CG, et al. Fibrosis progression in human immunodeficiency virus/hepatitis C virus coinfected adults: prospective analysis of 435 liver biopsy pairs. *Hepatology.* 2014;59(3):767-775.

Lai JC, Kahn JG, Tavakol M, Peters MG, Roberts JP. Reducing infection transmission in solid organ transplantation through donor nucleic Acid testing: a cost-effectiveness analysis. *Am J*

Transplant. 2013;13(10):2611-2618.

Lalezari L, Nelson DR, Hyland RH, et al. Once daily sofosbuvir plus ribavirin for 12 and 24 weeks in treatment-naive patients with HCV infection: the QUANTUM study. Program and abstracts of the 48th Annual Meeting of the European Association for the Study of the Liver. April 24-28, 2013, 2013; Amsterdam, The Netherlands.

Lambers FA, Prins M, Thomas X, et al. Alarming incidence of hepatitis C virus re-infection after treatment of sexually acquired acute hepatitis C virus infection in HIV-infected MSM. *AIDS.* 2011;25(17):F21-F27.

Landau DA, Scerra S, Sene D, Resche-Rigon M, Saadoun D, Cacoub P. Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. *J Rheumatol.* 2010;37(3):615-621.

Larney S, Kopinski H, Beckwith CG, et al. Incidence and prevalence of hepatitis C in prisons and other closed settings: Results of a systematic review and meta-analysis. *Hepatology.* 2013;58(4):1215-1224.

Lawitz E, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med.* 2013a;369(7):678-679.

Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med.* 2013b;368(20):1878-1887.

Lawitz E, Poordad FF, Pang PS, et al. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet.* 2014;383(9916):515-523.

Lee SR, Kardos KW, Schiff E, et al. Evaluation of a new, rapid test for detecting HCV infection, suitable for use with blood or oral fluid. *J Virol Methods.* 2011;172(1-2):27-31.

Lewis JH, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: Results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. *Hepatology.* 2007;46(5):1453-1463.

Limketkai BN, Mehta SH, Sutcliffe CG, et al. Relationship of liver disease stage and antiviral therapy with liver-related events and death in adults coinfected with HIV/HCV. *JAMA.* 2012;308(4):370-378.

Linas BP, Wong AY, Schackman BR, Kim AY, Freedberg KA. Cost-effective screening for acute hepatitis C virus infection in HIV-infected men who have sex with men. *Clin Infect Dis.* 2012;55(2):279-290.

Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. AASLD practice guideline update. *Hepatology.* 2009;50(3):661-662.

Louie KS, St LS, Forssen UM, Mundy LM, Pimenta JM. The high comorbidity burden of the

hepatitis C virus infected population in the United States. *BMC Infect Dis.* 2012;12:86.

Macias J, Berenguer J, Japon MA, et al. Fast fibrosis progression between repeated liver biopsies in patients coinfected with human immunodeficiency virus/hepatitis C virus. *Hepatology.* 2009;50(4):1056-1063.

Maddison AR, Fisher J, Johnston G. Preventive medication use among persons with limited life expectancy. *Prog Palliat Care.* 2011;19(1):15-21.

Mahajan R, Liu SJ, Klevens RM, Holmberg SD. Indications for testing among reported cases of HCV infection from enhanced hepatitis surveillance sites in the United States, 2004-2010. *Am J Public Health.* 2013;103(8):1445-1449.

Mahale P, Kontoyiannis DP, Chemaly RF, et al. Acute exacerbation and reactivation of chronic hepatitis C virus infection in cancer patients. *J Hepatol.* 2012;57(6):1177-1185.

Manns MP, Pockros PJ, Norkrans G, et al. Long-term clearance of hepatitis C virus following interferon alpha-2b or peginterferon alpha-2b, alone or in combination with ribavirin. *J Viral Hepat.* 2013;20(8):524-529.

Marcellin P, Boyer N, Gervais A, et al. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med.* 1997;127(10):875-881.

Martin NK, Hickman M, Hutchinson SJ, Goldberg DJ, Vickerman P. Combination interventions to prevent HCV transmission among people who inject drugs: modeling the impact of antiviral treatment, needle and syringe programs, and opiate substitution therapy. *Clin Infect Dis.* 2013a;57(Suppl 2):S39-S45.

Martin NK, Vickerman P, Grebely J, et al. Hepatitis C virus treatment for prevention among people who inject drugs: Modeling treatment scale-up in the age of direct-acting antivirals. *Hepatology.* 2013b;58(5):1598-1609.

Mazzaro C, Little D, Pozzato G. Regression of splenic lymphoma after treatment of hepatitis C virus infection. *N Engl J Med.* 2002;347(26):2168-2170.

McGowan CE, Monis A, Bacon BR, et al. A global view of hepatitis C: physician knowledge, opinions, and perceived barriers to care. *Hepatology.* 2013;57(4):1325-1332.

Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med.* 2000;133(8):592-599.

Mehta SH, Lucas GM, Mirel LB, et al. Limited effectiveness of antiviral treatment for hepatitis C in an urban HIV clinic. *AIDS.* 2006;20(18):2361-2369.

Merchante N, Giron-Gonzalez JA, Gonzalez-Serrano M, et al. Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. *AIDS.* 2006;20(1):49-57.

Miller L, Fluker SA, Osborn M, Liu X, Strawder A. Improving access to hepatitis C care for urban, underserved patients using a primary care-based hepatitis C clinic. *J Natl Med Assoc*. 2012;104(5-6):244-250.

Mira JA, Rivero-Juárez A, López-Cortes LF, et al. Benefits from sustained virologic response to pegylated interferon plus ribavirin in HIV/hepatitis C virus-coinfected patients with compensated cirrhosis. *Clin Infect Dis.* 2013;56(11):1646-1653.

Moreno C, Herzode C, Marcellin P, et al. Simeprevir with peginterferon/ribavirin in treatment-naïve or experienced patients with chronic HCV genotype 4 infection: Interim results of a Phase III trial. 14th European AIDS conference Brussels Belgium Oct 2013. Oct 16-19, 2013, 2013; Brussels, Belgium.

Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med.* 2013;158(5 Pt 1):329-337.

Morgan TR, Ghany MG, Kim HY, et al. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. *Hepatology.* 2010;52(3):833-844.

Morisco F, Granata R, Stroffolini T, et al. Sustained virological response: a milestone in the treatment of chronic hepatitis C. *World J Gastroenterol.* 2013;19(18):2793-2798.

Morrill JA, Shrestha M, Grant RW. Barriers to the treatment of hepatitis C. Patient, provider, and system factors. *J Gen Intern Med.* 2005;20(8):754-758.

Moyer VA. Screening for HIV: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2013;159(1):51-60.

Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology.* 2010;52(1):79-104.

Neary MP, Cort S, Bayliss MS, Ware JE, Jr. Sustained virologic response is associated with improved health-related quality of life in relapsed chronic hepatitis C patients. *Semin Liver Dis.* 1999;19(Suppl 1):77-85.

Nelson PK, Mathers BM, Cowie B, et al. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet.* 2011;378(9791):571-583.

Neumann UP, Berg T, Bahra M, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J Hepatol.* 2004;41(5):830-836.

Noda K, Yoshihara H, Suzuki K, et al. Progression of type C chronic hepatitis to liver cirrhosis and hepatocellular carcinoma--its relationship to alcohol drinking and the age of transfusion. *Alcohol Clin Exp Res.* 1996;20(1 Suppl):95A-100A.

Nutt AK, Hassan HA, Lindsey J, Lamps LW, Raufman JP. Liver biopsy in the evaluation of patients with chronic hepatitis C who have repeatedly normal or near-normal serum alanine aminotransferase levels. *Am J Med.* 2000;109(1):62-64.

Ortiz V, Berenguer M, Rayon JM, Carrasco D, Berenguer J. Contribution of obesity to hepatitis C-related fibrosis progression. *Am J Gastroenterol.* 2002;97(9):2408-2414.

Osinusi A, Meissner EG, Lee YJ, et al. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. *JAMA.* 2013;310(8):804-811.

Ouwerkerk-Mahadevan S, Sekar V, Peeters M, Beumont-Mauviel M. The pharmokinetic interactions of HCV protease inhibitor TMC435 with RPV, TDF, EFV, or RAL in health volunteers [Abstract 49]. 19th Conference on Retroviruses and Opportunistic Infections (CROI). March 5-8, 2012; Seattle, Washington.

Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. *Hepatology.* 2002;36(5 Suppl 1):S65-S73.

Petta S, Camma C, Di M, V, et al. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. *Am J Gastroenterol.* 2008;103(5):1136-1144.

Picciotto FP, Tritto G, Lanza AG, et al. Sustained virological response to antiviral therapy reduces mortality in HCV reinfection after liver transplantation. *J Hepatol.* 2007;46(3):459-465.

Pineda JA, Romero-Gómez M, Díaz-García F, et al. HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. *Hepatology.* 2005;41:779-789.

Poordad F, Manns MP, Marcellin P, et al. Simeprevir (TMC435) with Peginterferon/Ribavirin for Treatment of Chronic HCV Genotype-1 Infection in Treatment-Naive Patients: Results From QUEST-2, a Phase III Trial. Digestive Disease Week. May 18-21, 2013, 2013; Orlando, FL.

Post JJ, Arain A, Lloyd AR. Enhancing assessment and treatment of hepatitis C in the custodial setting. *Clin Infect Dis.* 2013;57(Suppl 2):S70-S74.

Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet.* 1997;349(9055):825-832.

Poynard T, Cacoub P, Ratziu V, et al. Fatigue in patients with chronic hepatitis C. *J Viral Hepat.* 2002a;9(4):295-303.

Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology.* 2002b;122(5):1303-1313.

Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. *J Hepatol.* 2001;34(5):730-739.

Pradat P, Alberti A, Poynard T, et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. *Hepatology.* 2002;36(4 Pt 1):973-977.

Proeschold-Bell RJ, Patkar AA, Naggie S, et al. An integrated alcohol abuse and medical treatment model for patients with hepatitis C. *Dig Dis Sci.* 2012;57(4):1083-1091.

Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol.* 2002;97(10):2614-2618.

Reilley B, Leston J, Redd JT, Geiger R. Lack of Access to Treatment as a Barrier to HCV Screening: A Facility-Based Assessment in the Indian Health Service. *J Public Health Manag Pract.* 2013;

Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology.* 2006;43(2 Suppl 1):S113-S120.

Rodriguez-Torres M, Rodriguez-Orengo JF, Gaggar A, et al. Sofosbuvir and Peginterferon Alfa-2a/Ribavirin for Treatment-Naïve Genotype 1?4 HCV-Infected Patients Who Are Coinfected With HIV. 53rd ICAAC 2013. Sept 10-13, 2013, 2013; Denver, CO.

Rossaro L, Torruellas C, Dhaliwal S, et al. Clinical Outcomes of Hepatitis C Treated with Pegylated Interferon and Ribavirin via Telemedicine Consultation in Northern California. *Dig Dis Sci.* 2013;58(12):3620-3625.

Ruane P, Ain D, Meshrekey R, Stryker R. Sofosbuvir Plus Ribavirin in the Treatment of Chronic HCV Genotype 4 Infection in Patients of Egyptian . Ancestry AASLD The Liver Meeting 2013. Nov 2, 2013; Washington DC.

Ruane PJ, Ain D, Meshrekey R, Riad J, Soliman M, Mikhail S, Wolfe PR, Kersey K, Doehle B, Jiang D, Symonds WT. Sofosbuvir plus ribavirin, an interferon-free regimen, in the treatment of treatment-naive and treatment-experienced patients wtih chronic genotype 4 HCV infection [Abstract P1243]. 49th Annual Meeting of the European Association for the Study of the Liver. April 9-13, 2014; London, United Kingdom.

Saadoun D, Resche RM, Thibault V, et al. Peg-IFNa/ribavirin/protease inhibitor combination in hepatitis C virus associated mixed cryoglobulinemia vasculitis: results at week 24. *Ann Rheum Dis.* 2014;73(5):831-837.

Safdar K, Schiff ER. Alcohol and hepatitis C. *Semin Liver Dis.* 2004;24(3):305-315.

Sangiovanni A, Prati GM, Fasani P, et al. The natural history of compensated cirrhosis due to hepatitis C virus: a 17-year cohort study of 214 patients. *Hepatology.* 2006;43(6):1303-1310.

Sarkar S, Jiang Z, Evon DM, Wahed AS, Hoofnagle JH. Fatigue before, during and after antiviral therapy of chronic hepatitis C: results from the Virahep-C study. *J Hepatol.* 2012;57(5):946-952.

Schmidt AJ, Falcato L, Zahno B, et al. Prevalence of hepatitis C in a Swiss sample of men who have sex with men: whom to screen for HCV infection? BMC Public Health. 2014;14(1):3.

Schneider MD, Sarrazin C. Antiviral therapy of hepatitis C in 2014: do we need resistance testing? *Antiviral Res.* 2014;105:64-71.

Sebastiani G, Halfon P, Castera L, et al. SAFE biopsy: a validated method for large-scale staging of liver fibrosis in chronic hepatitis C. *Hepatology.* 2009;49(6):1821-1827.

Seem DL, Lee I, Umscheid CA, Kuehnert MJ. Excerpt from PHS guideline for reducing HIV, HBV and HCV transmission through organ transplantation. *Am J Transplant.* 2013;13(8):1953-1962.

Selph, S. and Chou, R. Impact of contacting study authors on systematic review conclusions: diagnostic tests for hepatic fibrosis. http://www.ncbi.nlm.nih.gov/books/NBK198806/. Accessed on July 11, 2014.

Shaw K, Gennat H, O'Rourke P, Del MC. Exercise for overweight or obesity. *Cochrane Database Syst Rev.* 2006;(4):CD003817.

Shiffman RN, Shekelle P, Overhage JM, Slutsky J, Grimshaw J, Deshpande AM. Standardized reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. Ann Intern Med. 2003;139(6):493-498.

Singal AG, Volk ML, Jensen D, Di Bisceglie AM, Schoenfeld PS. A sustained viral response is associated with reduced liver-related morbidity and mortality in patients with hepatitis C virus. *Clin Gastroenterol Hepatol.* 2010;8(3):280-8, 288.

Smith BD, Morgan RL, Beckett GA, et al. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. *MMWR Recomm Rep.* 2012;61(RR-4):1-32.

Spaulding AC, Weinbaum CM, Lau DT, et al. A framework for management of hepatitis C in prisons. *Ann Intern Med.* 2006;144(10):762-769.

Stein MR, Soloway IJ, Jefferson KS, Roose RJ, Arnsten JH, Litwin AH. Concurrent group treatment for hepatitis C: implementation and outcomes in a methadone maintenance treatment program. *J Subst Abuse Treat.* 2012;43(4):424-432.

Stone NJ, Robinson J, Lichtenstein AH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation.* 2013;

Sulkowski M, Pol S, Mallolas J, et al. Boceprevir versus placebo with pegylated interferon alfa-2b and ribavirin for treatment of hepatitis C virus genotype 1 in patients with HIV: a randomised, double-blind, controlled phase 2 trial. *Lancet Infect Dis.* 2013a;13(7):597-605.

Sulkowski M, Rodriguez-Torres M, Lalezari J, et al. All-Oral Therapy With Sofosbuvir Plus Ribavirin For the Treatment of HCV Genotype 1, 2, and 3 Infection in Patients Co-infected With HIV (PHOTON-1) . *Hepatology.* 2013b;58(Number 4 Suppl 1):313A.

Sulkowski M, Rodriguez-Torres M, Lalezari JP, et al. All-Oral Therapy with Sofosbuvir Plus Ribavirin for the treatment of HCV genotype 1,2 and 3 infection in patients coinfected with HIV (PHOTON-1). AASLD Annual Meeting 2013. Nov 1-5, 2013, 2013c; Washington, DC.

Sulkowski MS, Sherman KE, Dieterich DT, et al. Combination therapy with telaprevir for chronic hepatitis C virus genotype 1 infection in patients with HIV: a randomized trial. *Ann Intern Med.* 2013d;159(2):86-96.

Svoboda J, Andreadis C, Downs LH, Miller Jr WT, Tsai DE, Schuster SJ. Regression of advanced non-splenic marginal zone lymphoma after treatment of hepatitis C virus infection. *Leuk Lymphoma.* 2005;46(9):1365-1368.

Swain MG, Lai MY, Shiffman ML, et al. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. *Gastroenterology.* 2010;139(5):1593-1601.

Takahashi K, Nishida N, Kawabata H, Haga H, Chiba T. Regression of Hodgkin lymphoma in response to antiviral therapy for hepatitis C virus infection. *Intern Med.* 2012;51(19):2745-2747.

Takikawa H, Yamazaki R, Shoji S, Miyake K, Yamanaka M. Normalization of urinary porphyrin level and disappearance of skin lesions after successful interferon therapy in a case of chronic hepatitis C complicated with porphyria cutanea tarda. *J Hepatol.* 1995;22(2):249-250.

Terrault NA, Roland ME, Schiano T, et al. Outcomes of liver transplant recipients with hepatitis C and human immunodeficiency virus coinfection. *Liver Transpl.* 2012;18(6):716-726.

Thein HH, Yi Q, Dore GJ, Krahn MD. Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. *AIDS.* 2008;22(15):1979-1991.

Thomas DL. The challenge of hepatitis C in the HIV-infected person. *Annu Rev Med.* 2008;59:473-485.

Tyson GL, Kramer JR, Duan Z, Davila JA, Richardson PA, El-Serag HB. Prevalence and predictors of hepatitis B virus coinfection in a United States cohort of hepatitis C virus-infected patients. *Hepatology.* 2013;58(2):538-545.

Urbanus AT, van de Laar TJ, Stolte IG, et al. Hepatitis C virus infections among HIV-infected men who have sex with men: an expanding epidemic. *AIDS.* 2009;23(12):F1-F7.

US FDA. FDA Antiviral Drugs Advisory Committee Meeting October 25, 2013: Background Package for NDA 204671 Sofosbuvir (GS-7977). http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AntiviralDrugsAdvisoryCommittee/ucm371875.htm. Accessed on November 15, 2013a.

US FDA. FDA Introductory Remarks: Sofosbuvir NDA 204671. Presented on October 25, 2013. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AntiviralDrugsAdvisoryCommittee/UCM375285.pdf. Accessed on December 6, 2013b.

US Preventive Services Task Force. Screening for hepatitis C virus infection in adults: US Preventive Services Task Force recommendation statement. http://www.uspreventiveservicestaskforce.org/uspstf/uspshepc.htm. Accessed on October 28, 2013.

van de Laar T, Pybus O, Bruisten S, et al. Evidence of a large, international network of HCV transmission in HIV-positive men who have sex with men. *Gastroenterology.* 2009;136(5):1609-1617.

van de Laar TJ, Matthews GV, Prins M, Danta M. Acute hepatitis C in HIV-infected men who have sex with men: an emerging sexually transmitted infection. *AIDS.* 2010;24(12):1799-1812.

van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA.* 2012;308(24):2584-2593.

van Heeswijk R, Garg V, Vandevoorde A, Witek J, Dannemann B. The pharmacokinetic interaction between telaprevir and raltegravir in healthy volunteers. 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). September 17-20, 2011a; Chicago, IL.

van Heeswijk R, Vandevoorde A, Boogaerts G et al. Pharmacokinetic interactions between ARV agents and the investigational HCV protease inhibitor TVR in healthy volunteers [Abstract 119]. 18th Conference on Retroviruses and Opportunistic Infections (CROI). February 27-March 2, 2011b; Boston, MA.

Veldt BJ, Heathcote EJ, Wedemeyer H, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med.* 2007;147(10):677-684.

Vermeersch P, Van RM, Lagrou K. Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory. *J Clin Virol.* 2008;42(4):394-398.

Vourvahis M, Plotka A, Kantaridis C, Fang A, Heera J. The effect of boceprevir and telaprevir on the pharmacokinetics of maraviroc: an open-label, fixed-sequence study in healthy volunteers [Abstract O-17]. 14th International Workshop on Clinical Pharmacology of HIV Therapy. April 22-24, 2013; Amsterdam, Netherlands.

Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology.* 2003;38(2):518-526.

Wandeler G, Gsponer T, Bregenzer A, et al. Hepatitis C virus infections in the Swiss HIV Cohort Study: a rapidly evolving epidemic. *Clin Infect Dis.* 2012;55(10):1408-1416.

Ward JW. Testing for HCV: the first step in preventing disease transmission and improving health outcomes for HCV-infected individuals. *Antivir Ther.* 2012;17(7 Pt B):1397-1401.

Wedemeyer H, Duberg AS, Buti M, et al. Strategies to manage hepatitis C virus (HCV) disease burden. *J Viral Hepat.* 2014;21(Suppl 1):60-89.

Westin J, Lagging LM, Spak F, et al. Moderate alcohol intake increases fibrosis progression in untreated patients with hepatitis C virus infection. *J Viral Hepat.* 2002;9(3):235-241.

White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol.* 2008;49(5):831-844.

Whitlock EP, Polen MR, Green CA, Orleans T, Klein J. Behavioral counseling interventions in primary care to reduce risky/harmful alcohol use by adults: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med.* 2004;140(7):557-568.

Wiley TE, McCarthy M, Breidi L, McCarthy M, Layden TJ. Impact of alcohol on the histological and clinical progression of hepatitis C infection. *Hepatology.* 1998;28(3):805-809.

Williams IT, Bell BP, Kuhnert W, Alter MJ. Incidence and transmission patterns of acute hepatitis C in the United States, 1982-2006. *Arch Intern Med.* 2011;171(3):242-248.

Witt MD, Seaberg EC, Darilay A, et al. Incident hepatitis C virus infection in men who have sex with men: a prospective cohort analysis, 1984-2011. *Clin Infect Dis.* 2013;57(1):77-84.

Yoneda M, Saito S, Ikeda T, et al. Hepatitis C virus directly associates with insulin resistance independent of the visceral fat area in nonobese and nondiabetic patients. *J Viral Hepat.* 2007;14(9):600-607.

Younossi ZM, Stepanova M, Gerber L, Nader F, Frost S, Hunt SL. P717 Improvement of central fatigue is associated with sustained virologic response (SVR) following sofosbuvir (SOF) containing regimens. *J Hepatol.* 2014;60(1):S308.

Younossi ZM, Stepanova M, Henry L, et al. Effects of sofosbuvir-based treatment, with and without interferon, on outcome and productivity of patients with chronic hepatitis C. *Clin Gastroenterol Hepatol.* 2013;[Epub ahead of print]

Zarski JP, Bohn B, Bastie A, et al. Characteristics of patients with dual infection by hepatitis B and C viruses. *J Hepatol.* 1998;28(1):27-33.

Zeuzem S, Andreone P, Pol S, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med.* 2011;364(25):2417-2428.

Zeuzem S, Berg T, Gane E, et al. Simeprevir Increases Rate of Sustained Virologic Response Among Treatment-Experienced Patients with HCV Genotype-1 Infection: a Phase IIb Trial. *Gastroenterology.* 2013a;

Zeuzem S, Dusheiko GM, Salupere R. Sofosbuvir + ribavirin for 12 or 24 weeks for patients with HCV genotype 2 or 3: the VALENCE trial. Hepatology: Special Issue: The 64th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2013. Nov 1-5, 2013, 10-15-2013b;733A; Washington, DC.

Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology.* 2005;41(1):48-54.

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Transient elastography (FibroScan) for evaluating liver fibrosis

April 2008

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Transient elastography (FibroScan) for evaluating liver fibrosis

Target group

- All patients with chronic liver disease in particular those with suspected liver fibrosis associated with hepatitis B and C, non-alcoholic steatohepatitis (NASH), alcoholic steatohepatitis (ASH) and non-alcoholic fatty liver disease (NAFLD).

Technology description

FibroScan is an active medical diagnostic aid, which makes non-invasive, painless and immediate measurements of the stiffness (or elasticity) of the hepatic parenchyma. A FibroScan test is conducted by placing a probe in an intercostal space over the patient's liver triggering a shear wave, which is tracked through the liver using the ultrasound element of the probe (FibroScan is currently of limited use in obese patients). FibroScan can be performed by trained medical or paramedical staff. Each test typically takes less than 15 minutes in total. The acquisition speed is very high and the acquisitions are therefore not biased by cardiac or respiratory movements as they are with ultrasound imaging. FibroScan can be used as an initial screening tool to reduce the number of patients requiring liver biopsy and also to measure ongoing response to treatment. In some patients the initial screening results may be sufficiently well-defined to provide a confident assessment of the patient's stage of liver fibrosis.

Innovation and/or advantages

Unlike with a needle biopsy of the liver no anaesthetic is needed with FibroScan and the patient can be discharged immediately. No special infrastructure or specialist consumables are required for FibroScan. Whilst many patients will still require a biopsy, the company believe that a reduction in biopsies of between 40 to 60% may be observed when FibroScan is employed.

The most significant advantage of FibroScan may be that many more patients could be assessed at much more frequent intervals compared with liver biopsies[a]. The patient cohorts who would most benefit may be those with recurrent disease (whatever the aetiology) post-transplant and those with viral hepatitis[a].

Developer

EchoSens (Artemis Medical Ltd. are the UK distributor).

Availability, launch or marketing dates, and licensing plans:

CE marked. Currently installed in 12 NHS hospitals. Most of the systems in use within the NHS have been funded by either pharmaceutical companies and/or charity donations. The company predict that the initial uptake is likely to be in the major hepatology centres (approximately 35 systems). The second phase requirement would be into DGHs with gastroenterology departments. Expert opinion suggests that national guidelines regarding the use of FibroScan are needed.

NHS or Government priority area:

No NHS or government priority area was identified.

[a] Expert opinion

Relevant guidance

- The Centre for Evidence-based Purchasing (CEP) is currently conducting an evidence review of FibroScan (publication date not known).

NICE Technology Appraisals

- Hepatitis B (chronic) – tenofovir disoproxil fumarate. Proposed 17th wave.
- Hepatitis B (chronic) – adefovir dipivoxil and pegylated interferon alpha-2a. 2006[1] (review date February 2009).
- Hepatitis C – peginterferon alfa and ribavirin. 2006[2] (review date being considered). This is an extension of Hepatitis C – pegylated interferons, ribavirin and alfa interferon. 2004.

NICE Interventional Procedures

- Extracorporeal albumin dialysis for acute-on-chronic liver failure
- Laparoscopic liver resection
- Living-donor liver transplantation
- Radiofrequency-assisted liver resection

- Haute Autorité de Santé[b]. Guidelines for the diagnosis of uncomplicated cirrhosis. 2007[3].
- SIGN. Management of hepatitis C. A national clinical guideline. 2006[4].
- British Society for Gastroenterology. Guidelines on the use of liver biopsy in clinical practice. 2004[5].
- The Canadian Agency for Drugs and Technologies in Health (CADTH). Transient elastography (FibroScan) for non-invasive assessment of liver fibrosis. 2006[6].

  Stated that the diagnostic performance of FibroScan is good for identifying severe fibrosis or cirrhosis, but is less accurate for milder presentations. FibroScan is a promising technology, but large multi-centre trials comparing a range of emerging non-invasive fibrosis staging technologies are required.
- The Australia and New Zealand Horizon Scanning Network (ANZHSN). MR and transient elastography for the non-invasive assessment of liver fibrosis. 2006[7].

  Stated that elastography (magnetic resonance and transient) appears to be a reliable technique for diagnosing severe liver fibrosis. For conditions such as viral hepatitis however, antiviral therapy should ideally begin in the early stages of fibrosis. Given the low quality of evidence outlining the ability of elastography to detect mild or moderate fibrosis, it is recommended that the technology be archived[c].

Clinical need and burden of disease

Hepatitis C

Liver fibrosis is associated with significant morbidity and mortality. The major cause is hepatitis C. Hepatitis C virus (HCV) causes chronic hepatitis in about 80% of those infected. Recent estimates suggest that approximately 200,000 to 500,000 people are infected with HCV in England and Wales[2]. In 2005 the Department of Health estimated that only 47,000 people with HCV infection had been diagnosed and only 7,000 had been treated.

People infected with HCV are often asymptomatic, but about 20% will develop overt hepatitis. The rate of progression of the disease is slow but variable, usually taking about

[b] French National Authority for Health.
[c] The company have received enquiries from Australia regarding FibroScan.

20–50 years from the time of infection. About 30% of those who are infected develop cirrhosis within 20–30 years, and a small percentage of these people are at a high risk of developing hepatocellular carcinoma. A third may never progress to cirrhosis or will not progress for at least 50 years.

Hepatitis B

The Department of Health estimates that about 180,000 people in the UK have chronic hepatitis B[1]. There are about 7,700 new cases of chronic hepatitis B each year. Of these, around 300 people were infected within the UK; the remainder (mainly immigrants to the UK) were infected abroad, generally in areas of high prevalence where the virus is frequently transmitted from mother to child.

People with active chronic hepatitis B are at increased risk of liver cirrhosis and primary liver cancer.

Alcoholic Liver Disease (ALD)

In 2005, 4,160 people died in England and Wales from alcoholic liver disease, an increase of 37% since 1999[8]. The process is initially silent, but when liver disease has developed it presents as an acute illness with a 25-50% immediate mortality rate. In England, around 39,180 people are admitted to hospital with alcoholic liver disease each year.

There are three main stages of ALD: minimal change, or fatty liver; alcoholic hepatitis; and cirrhosis. Severe alcoholic steatohepatitis (ASH) is the major complication of advanced ALD and has a high mortality even when treated with corticosteroids.

Non-alcoholic fatty liver disease (NAFLD)

The prevalence of NAFLD is approximately 20-30% in western countries[9], which would approximate to between 10.7 – 16.1M people in England and Wales. It covers a number of conditions, including non-alcoholic steatohepatitis (NASH). Severe obesity, type 2 diabetes, hypertension and/or dyslipidaemia are major risk factors. NASH is usually benign and very slowly progressive but can result in the development of fibrosis in up to 40% of patients, or cirrhosis in 5-10% of patients.

Existing comparators and treatments

The gold standard for the assessment of fibrosis in chronic liver conditions is currently a liver biopsy. Liver biopsies, which are associated with a degree of sampling error, are generally performed under local anaesthesia and require a short hospital stay. Liver biopsy is an invasive technique with a risk of serious adverse events due to bleeding and other complications and therefore should only be performed if the benefits outweigh the risks (in terms of altering treatment or disease outcome).

An alternative for liver biopsy is the non-invasive prediction of the severity of liver disease using combinations of clinical and biochemical parameters. Recent SIGN guidelines[4] recommend that:

- biochemical markers should not be used as an alternative to liver biopsy for the staging of intermediate grades of fibrosis
- biochemical tests may be used as an alternative to liver biopsy to diagnose cirrhosis or to direct screening for complications of fibrosis.

Guidelines from France recommend that once a patient has been referred to a specialist, and a firm diagnosis cannot be made from the clinical and biological observations, a liver

biopsy or a non-invasive test has to be carried out[3]. The recommended first-line test for the chronic untreated hepatitis C patient with no comorbidities is a non-invasive procedure (either FibroTest[d] or FibroScan)[3].

Hepatitis C

The decision to treat a person with hepatitis C does not depend on a liver biopsy, however the clinician may recommend a biopsy for other reasons or if a strategy of watchful waiting is chosen.

Hepatitis B

The hepatitis B surface antibody (anti-HBs) is the most common test for hepatitis B. Other tests detect the presence of viral antigens. Liver biopsy is an accepted part of the diagnosis and management of patients with chronic hepatitis B and can be used for grading fibrosis and inflammation.

Alcoholic Liver Disease (ALD)

If ALD is suspected, liver function tests, blood counts, and hepatitis serology are performed. No specific test exists for alcoholic liver disease. The role and timing of liver biopsy in patients with suspected ALD remains uncertain. In patients with evidence of liver damage and a history of alcohol excess, a liver biopsy is helpful in determining the degree of liver damage.

Non-alcoholic fatty liver disease (NAFLD)

NAFLD is often asymptomatic, and may be identified through abnormal liver function tests (LFTs) or by abdominal ultrasound. The role of liver biopsy for this indication is not clearly established. Histology remains the gold standard for making the important distinction between simple steatosis (which is generally non-progressive and readily reversible) and NASH.

Efficacy and safety

There are numerous published trials, conference presentations, ongoing trials and several non-systematic reviews pertaining to FibroScan. Two systematic reviews were identified, which included 4 studies[10] and 9 studies[11] respectively (see table for details of the latter review) – these reviews had 3 studies in common.

Title	Ultrasound-based transient elastography for the detection of hepatic fibrosis
Status	Published 2007[11]
Design	Systematic review and meta-analysis
Description of study	Studies reporting accuracy of ultrasound-based transient elastography compared with liver biopsy were identified through (i) search of PubMed (MEDLINE), EMBASE, Cochrane Library, Database of Abstracts of Reviews of Effects, Web of Science, SCOPUS, American College of Physicians Journal Club, Google Scholar databases from database inception to January 2007, and (ii) review of abstracts and proceedings from the American Association for the Study of Liver Disease, European Association for the Study of the Liver, and Digestive Disease Week annual meetings between 2003-2006. N=9 studies (2,083 patients) identified.
Primary outcome	Diagnostic test performance of ultrasound-based transient elastography for the detection of cirrhosis (stage IV fibrosis) vs no cirrhosis (stages 0-III fibrosis) compared

[d] FibroTest combines the variables of age and gender with 5 biomarkers. Reported to ACTS in January 2004 for Hepatitis C and updated for chronic liver conditions in July 2005 (ACTS decided the topic did not meet the selection criteria).

	with the reference standard of liver biopsy.
Secondary outcomes	Diagnostic test performance of ultrasound-based transient elastography for detecting stages II-IV fibrosis vs stage 0-I fibrosis.
Key results	For patients with stage IV fibrosis (9 studies), the pooled estimates for sensitivity were 87% (95% CI, 84%-90%), specificity 91% (95% CI, 89%-92%), positive likelihood ratio 11.7 (95% CI, 7.9-17.1), and negative likelihood ratio 0.14 (95% CI, 0.10-0.20). For patients with stages II-IV fibrosis (7 studies), the pooled estimates for sensitivity were 70% (95% CI, 67%-73%), specificity 84% (95% CI, 80%-88%), positive likelihood ratio 4.2 (95% CI, 2.4-7.2), and negative likelihood ratio 0.31 (95% CI, 0.23-0.43). Diagnostic threshold (or cut-off value) bias was identified as an important cause of heterogeneity for pooled results in both patient groups.

Estimated cost and cost impact

FibroScan has an initial capital cost of £49,950 (+VAT) plus an annual maintenance cost of £3,000 (+VAT) from year 2 onwards. The cost per test will vary depending on the usage of the machine. The more the machine is used the lower the cost per patient.

In patients younger than 70 without complications the cost for an inpatient biopsy (HRG code G05) is £710 (elective spell) and £2,213 (non-elective spell)[12].

Potential or intended impact – speculative

Patients

☐ Reduced morbidity	☐ Reduced mortality or increased survival	☑ Improved quality of life for patients and/or carers
☑ Quicker, earlier or more accurate diagnosis or identification of disease	☐ Other:	☐ None identified

Services

☑ Increased use	☐ Service reorganisation required	☑ Staff or training required – FibroScan assessments generally undertaken by existing staff. Training is typically 3-4 hrs per operator.
☐ Decreased use	☐ Other:	☐ None identified

Costs

☐ Increased unit cost compared to alternative	☐ Increased costs: more patients coming for treatment	☑ Increased costs: capital investment needed
☐ New costs:	☑ Savings: FibroScan permits assessments to be performed in many more patients than alternative means would realistically allow. Once purchased, the cost per patient is primarily only that of the operator	☐ Other:

References

[1]National Institute for Health and Clinical Excellence. Adefovir dipivoxil and pegylated interferon alpha-2a for the treatment of chronic hepatitis B. Technology appraisal TA96. February 2006.

[2] National Institute for Health and Clinical Excellence. Peginterferon alfa and ribavirin for the treatment of mild chronic hepatitis C. Technology appraisal TA106. August 2006.

[3] Fontaine H, Petitprez K, Roudot-Thoraval F & Trinchet J-C. Guidelines for the diagnosis of uncomplicated cirrhosis. Gastroenterol Clin Biol 2007;31:504-509.
[4] Scottish Intercollegiate Guidelines Network (SIGN). Management of hepatitis C. No. 92. 2006. http://www.sign.ac.uk . Accessed 10/04/08.
[5] British Society of Gastroenterology. Guidelines on the use of Liver Biopsy in Clinical Practice. http://www.bsg.org.uk/bsgdisp1.php?id=ad4bb7b46fc7b3518cb4&h=1&sh=1&i=1&b=1&m=00023 Accessed on 02/04/08.
[6] Canadian Agency for Drugs and Technologies in Health (CADTH). Transient elastography (FibroScan) for non-invasive assessment of liver fibrosis. Issues in Emerging Health Technologies. Issue 90, September 2006.
[7] The Australia and New Zealand Horizon Scanning Network (ANZHSN). MR and transient elastography for the non-invasive assessment of liver fibrosis. Prioritising Summary. Volume 14, No. 5, September 2006.
[8] British Liver Trust. Fighting Liver Disease. http://www.britishlivertrust.org.uk/home Accessed on 04/04/08.
[9] Harrison SA & Day CP. Benefits of lifestyle modifications in NAFLD. Gut 2007;56:1760-1769.
[10] Shaheen AA, Wan AF & Myers RP. FibroTest and FibroScan for the prediction of hepatitis c-related fibrosis: a systematic review of diagnostic test accuracy. American Journal of Gastroenterology 2007;102:2589-2600.
[11] Talwalker JA, Kurtz DM, Schoenleber SJ et al. Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis. Clinical Gastroenterology and Hepatology 2007;5:1214-1220.
[12] NHS Tariff. 2007-2008.

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Issues in Emerging Health Technologies

Transient Elastography (FibroScan) for Non-invasive Assessment of Liver Fibrosis

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Summary

- ✓ **Liver fibrosis is associated with significant morbidity and mortality. The major cause is hepatitis C, which affects 240,000 Canadians.**
- ✓ **Assessing the degree of liver fibrosis is critical to its management. Liver biopsy, an invasive procedure, is considered to be the diagnostic gold standard.**
- ✓ **FibroScan is a rapid, non-invasive technology that uses low frequency vibration and ultrasound to assess the stiffness of liver tissue.**
- ✓ **The diagnostic performance of FibroScan is good for identifying severe fibrosis or cirrhosis, but it is less accurate for milder presentations.**
- ✓ **FibroScan is a promising technology, but large multi-centre trials comparing a range of emerging non-invasive fibrosis staging technologies are required.**

The Technology

Fibrosis of the liver, which is associated with significant morbidity and mortality, occurs as a result of an exaggerated healing response triggered by chronic insults to the liver, such as injury, infection, and inflammation. Fibrosis of the liver develops when scar tissue replaces normal liver tissue. It can be localized or diffuse, and it can progress to cirrhosis, which increases the risk of developing liver cancer. Liver function is impaired. Assessing the degree of fibrosis is important when making decisions about starting treatment, assessing treatment response, and screening for liver cancer.[1-3]

FibroScan (EchoSens, Paris, France) is a non-invasive test that draws on the physics of transient elastography to assess liver fibrosis. A specialized ultrasound transducer placed over the liver transmits a mild amplitude, low frequency vibration. The vibration creates an elastic shear wave that moves through the underlying liver tissue. Its velocity is measured using pulse-echo ultrasound. Shear waves propagate more quickly in stiff tissue, and fibrotic tissue is stiff. FibroScan takes <5 minutes to perform, and produces immediate, operator-independent results.[4]

Regulatory Status

FibroScan is not currently licensed for use in Canada (Kathleen Savage, Health Canada, Ottawa: personal communication, 2006 May 3).

Patient Group

Liver fibrosis can be caused by many diseases, but most of the studies examining the effectiveness of FibroScan have focused on patients infected with hepatitis C (HCV). Up to 3% of the world's population are infected with HCV, and hepatitis C becomes chronic in 85% of infected adults.[5] In the US, HCV accounts for more cases of chronic liver disease than any other single cause.[6] In Canada, about 240,000 people are infected with HCV, with a prevalence estimated at 0.8% of the population.[7]

Current Practice

Liver biopsy is the gold standard for assessing the degree of fibrosis. Biopsy is recommended, but not mandated, in practice guidelines addressing the management of HCV.[8-10] No information on the proportion of HCV patients who receive a liver biopsy is available, but it is likely to be a subset of the HCV population. The clinical progression of HCV is typically slow, and infected persons may be asymptomatic for ≥20 years.[5,6] Furthermore, a biopsy may be unnecessary for HCV genotypes 2 and 3, which respond well to treatment.[8]

Liver biopsy is not easily accepted by patients. Pain is reported by one-third, complications occur in three per 1,000 biopsies, and death occurs in three per 10,000 biopsies. In addition, liver biopsy is subject to sampling error, because the typical biopsy specimen represents only 1/50,000 of the total liver volume, and discrepancies of ≥1 fibrosis stage have been identified in 33% of the samples taken from the right and left lobes of the same patient. There can also be significant intra- and inter-pathologist variation in the interpretation of biopsy samples.[11]

The Evidence

Seven observational studies address the relative performance of FibroScan, blood tests, or combinations thereof versus liver biopsy. Most of these were conducted in France.[12-18] There is overlap in the authorship of five of the seven studies.[12,14,15,17,18] Four studies[12,13,16,18] include ≤75 patients in the statistical analysis, while the others include 183 to 711 participants.[14,15,17] Most[12-16] include only HCV patients, one[17] includes patients with chronic liver disease of any origin, and one[18] includes only patients who are HIV and HCV co-infected.

The studies suggest that FibroScan results are reproducible across operators and time.[12,13] All the studies report that FibroScan's diagnostic performance is good, with Colletta *et al.*[16] indicating that it agrees perfectly with liver biopsy. This finding has sparked debate.[3,19] Five studies[12,14,15,17,18] present AUROC (area under the receiver operating characteristic curve) values, a commonly used index of diagnostic accuracy where values close to 1.0 represent high diagnostic accuracy. In terms of FibroScan's ability to discriminate degrees of fibrosis, as staged on the Metavir scale (F0 to F4 where F0=no fibrosis, and F4=cirrhosis), the AUROC ranges across the studies were F≥2, 0.72 to 0.88; F≥3, 0.90 to 0.91; and, F=4, 0.95 to 0.99. These results suggest that FibroScan performs well in identifying severe fibrosis or cirrhosis, but is less accurate in identifying lesser degrees of fibrosis. This is important because F2 is a threshold for initiating treatment.[9,10]

Two studies consider the performance of FibroScan relative to or with blood tests.[15,16] Castera *et al.*[15] reported that the diagnostic accuracy of FibroScan, FibroTest®, (BioPredictive, Paris, France) and APRI (aspartate transaminase to platelets ratio index) are of the same order, and that diagnostic accuracy is maximized when FibroScan and FibroTest are used in tandem. Colletta *et al.*[16] found FibroScan to be superior to FibroTest among HCV patients with normal aminotransferases.

These are early results, so more research is needed. Most of the studies conducted to date are small, focus on a subset of patients with chronic liver disease, fail to consider the full range of non-invasive tests, and arrive at differing thresholds for discriminating among the degrees of fibrosis. It is also unclear whether the studies were independent of industry involvement.

Adverse Effects

FibroScan is a non-invasive test, and no adverse effects have been reported.

Administration and Cost

FibroScan can be performed by trained medical or paramedical staff. No studies have addressed the optimal frequency of testing. The only available cost figures for FibroScan were published in 2004. Estimates include equipment costs of €71,760 (C$100,464), annual maintenance contract costs of €5,400 (C$7,560), and negligible costs for consumables. Although details of the calculations are not provided, it is estimated that at 20 and 150 examinations annually, the cost per examination would be €1,000 (C$1,400) and €100 (C$140) respectively.[20] These estimates focus on equipment-related expenditures, and seem to exclude staffing or indirect costs.

Liver biopsy costs are reported to range from €703 to €1,566 (C$984 to C$2,192) in European centres, and US$1,032 to US$2,745 (C$1,146 to C$3,047) in US centres, depending on whether complications occur.[11,21,22] Canadian costs may be lower; two large British Columbia health care organizations report direct costs of C$334 and C$337, and total costs of C$432 and C$472 respectively for liver biopsy (Larry Okotinsky, Fraser Health, Surrey, BC: personal communication, 2006 May 15; Chris Haack, Providence Health Care, Vancouver: personal communication, 2006 May 15).

Concurrent Development

Laboratory tests for liver fibrosis, based on direct and indirect serum markers, continue to be evaluated. Opinions about the reliability of these tests are mixed, but their development is seen as promising.[11,23,24] Another development is magnetic resonance elastography, which may offer an opportunity to assess a larger volume of the liver, and provide three-dimensional information.[25]

Rate of Technology Diffusion

FibroScan is not widely diffused in any jurisdiction, but if its effectiveness is confirmed, the technology will probably diffuse rapidly, largely on the basis of its low capital cost, widespread providers' familiarity with ultrasound technologies, and consumer demand.

The pattern of diffusion may be irregular, because the geographic prevalence of HCV varies significantly. The economics of FibroScan will be most favourable in centres that perform a significant volume of liver biopsies.

Morbid obesity or narrow intercostal spaces (the area between the ribs) preclude the use of FibroScan in 5% to 8% of patients.[14,15] This has led to speculation that the rate of uncompleted tests will be higher in jurisdictions such as the US, where the average body mass index is high.[3,14,23] This might affect diffusion, but the manufacturer is developing transducers for use in patients who are obese, or who have narrow intercostal spaces.[4,14]

Implementation Issues

The potential for non-invasive fibrosis staging is promising, but which technology or combination of technologies will be most useful is unclear. Once licensed, FibroScan could enter the Canadian health care system, as none of its features pose a significant barrier to uptake.

Although it might be argued that FibroScan does not need to be used more often than liver biopsy, it could be compelling to use FibroScan more frequently based on its rapid and non-invasive nature. This would be true for baseline fibrosis evaluations, but might also extend to ongoing monitoring, despite the lack of proof for FibroScan's utility for this purpose. If other non-invasive tools for assessing fibrosis gain acceptance, the market for FibroScan may be fragmented. Such scenarios might negatively affect the economics of FibroScan, or where non-invasive technologies are used in tandem, increase the total volume of tests conducted. This situation would be compounded if the indications for FibroScan expand beyond liver fibrosis due to HCV. This trend is evident in the literature.[17]

The degree to which FibroScan can replace liver biopsy is still unclear. Ziol *et al.*[14] and Castera *et al.*[15] indicate that the use of FibroScan alone or in tandem with another test would have permitted 42% and 77% of their respective study populations to avoid biopsy. Given the lack of information about who receives biopsy and for what reason, it is impossible to extrapolate their findings to clinical practice. Any shift to non-invasive testing will affect the system's capacity to perform liver biopsies, and perhaps require a concentration of such specialized services.

References

1. Bissell, JM, Maher, JJ. In: Zahim D, Boyer TD, editors. *Hepatology: a textbook of liver disease*. 4th ed. Philadelphia;Toronto: Saunders; 2003. p.395-416.
2. Di Sario, A, et al. *European Review for Medical & Pharmacological Sciences* 2004;8(1):11-8.
3. Ghany MG, et al. *Hepatology* 2005;42(4):759-61.
4. Castera L, et al. *Medscape General Medicine* 2005;7(4):4p.
5. Hepatitis C: medical information update. Canadian Liver Foundation. National Hepatitis C Education Program. *Can J Public Health* 2000;91 Suppl 1:S4-S9.
6. Davis GL. In: Schiff ER, Sorrell MF, Maddrey WC, editors. *Schiff's diseases of the liver*. Philadelphia: Lippincott, Williams & Wilkins; 2003.
7. Zou S, et al. *Can J Public Health* 2003;94(2):127-9.
8. Sherman M, et al. *Management of viral hepatitis: a Canadian consensus conference*. Toronto: Canadian Association for the Study of the Liver; 2004. Available: http://www.phac-aspc.gc.ca/hepc/hepatitis_c/pdf/ccc_04/pdf/consensus_04.pdf .
9. Strader DB, et al. *Hepatology* 2004;39(4):1147-71.
10. Dienstag JL, et al. *Gastroenterology* 2006;130(1):225-30.
11. Blanc JF, et al. *Hepatol Res* 2005;32(1):1-8.
12. Sandrin L, et al. *Ultrasound Med Biol* 2003;29(12):1705-13.
13. Saito H, et al. *Hepatol Res* 2004;29(2):97-103.
14. Ziol M, et al. *Hepatology* 2005;41(1):48-54.
15. Castéra L, et al. *Gastroenterology* 2005;128(2):343-50.
16. Colletta C, et al. *Hepatology* 2005;42(4):838-45.
17. Foucher J, et al. *Gut* 2006;55(3):403-8.
18. de Ledinghen V, et al. *J Acquir Immune Defic Syndr* 2006;41(2):175-9.
19. Castera L, et al. *Hepatology* 2006;43(2):373-4.
20. *Quantification of liver fibrosis using ultrasound transient elastography: rapid assessment procedure*. Paris: CEDIT; 2004. Available: http://cedit.aphp.fr/servlet/siteCeditGB?Destination=reco&numArticle=04.10/Av1/04 .
21. Comit, d'Evaluation et de Diffusion des Innovations Technologiques (CEDIT). *Biological markers of liver fibrosis in hepatitis c:CEDIT recommendations*. Paris: CEDIT; 2004. Available: http://cedit.aphp.fr/servlet/siteCeditGB?Destination=reco&numArticle=02.08/Re1/04 .
22. Dienstag JL. *Hepatology* 2002;36(5 Suppl 1):S152-S160.
23. Kelleher TB, et al. *Clin Liver Dis* 2005;9(4):667-83, vii.
24. Thuluvath PJ, et al. *Am J Gastroenterol* 2005;100(9):1981-3.
25. Huwart L, et al. *NMR Biomed* 2006;19(2):173-9.

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Research Article

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Elastography for the diagnosis of severity of fibrosis in chronic liver disease: A meta-analysis of diagnostic accuracy

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Background & Aims: Transient elastography is a non-invasive method, for the assessment of hepatic fibrosis, developed as an alternative to liver biopsy. We studied the performance of elastography for diagnosis of fibrosis using meta-analysis.

Methods: MEDLINE, EMBASE, SCI, Cochrane Library, conference abstracts books, and article references were searched. We included studies using biopsy as a reference standard, with the data necessary to calculate the true and false positive, true and false negative diagnostic results of elastography for a fibrosis stage, and with a 3-month maximum interval between tests. The quality of the studies was rated with the QUADAS tool.

Results: We identified 40 eligible studies. Summary sensitivity and specificity was 0.79 (95% CI 0.74–0.82) and 0.78 (95% CI 0.72–0.83) for F2 stage and 0.83 (95% CI 0.79–0.86) and 0.89 (95% CI 0.87–0.91) for cirrhosis. After an elastography result at/over the threshold value for F2 or cirrhosis ("positive" result), the corresponding post-test probability for their presence (if pre-test probability was 50%) was 78%, and 88% respectively, while, if values were below these thresholds ("negative" result), the post-test probability was 21% and 16%, respectively. No optimal stiffness cut-offs for individual fibrosis stages were validated in independent cohorts and cut-offs had a wide range and overlap within and between stages.

Conclusions: Elastography theoretically has good sensitivity and specificity for cirrhosis (and less for lesser degrees of fibrosis); however, it should be cautiously applied to everyday clinical practice because there is no validation of the stiffness cut-offs for the various stages. Such validation is required before elastography is considered sufficiently accurate for non-invasive staging of fibrosis.

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Introduction

Liver fibrosis represents the final common outcome of chronic liver injury and is often progressive, eventually evolving into cirrhosis [1]. Cirrhosis is the severest form of fibrosis with the worst clinical outcomes.

Currently, histological examination of a liver biopsy specimen is the reference standard for the diagnosis, staging, and monitoring of liver fibrosis [2]. However, it is invasive and can lead to fatal bleeding [2].

Transient elastography is a non-invasive method of quantifying fibrosis developed as an alternative to liver biopsy. Ultrasound elastography analyses ultrasound frequency waves which are related to the elasticity (deforming capacity) of the liver. It is simple, highly reproducible, and can be completed in 10 min in an outpatient setting with no significant expertise [3]. Magnetic resonance elastography involves measuring the elasticity of liver tissues using complex algorithms [4]. An increasing number of studies have evaluated the accuracy of elastography in the staging of fibrosis and compared it to liver biopsy.

In the present study, we used meta-analysis to assess the performance of elastography in the diagnosis of severity of liver fibrosis using liver biopsy as the reference standard.

Methods

Criteria for the selection of studies

We included full papers and abstracts, without language restrictions that (1) evaluated elastography in the diagnosis of severity of liver fibrosis or monitoring thereof, using liver biopsy as the reference standard, and (2) reported on data necessary to calculate the true positive false positive, true negative and false negative diagnostic results of elastography for the diagnosis of a fibrosis stage based on a defined cut-off point for liver stiffness. If such data were unavailable, the corresponding author was contacted via e-mail to provide them; if he/she failed to reply, the study was excluded. We also excluded studies reporting on <10 patients, or in which the maximum time interval between performing elastography and liver biopsy was >3 months.

Literature search

MEDLINE (Pubmed), Embase, Science Citation Index expanded, and The Cochrane Hepato-Biliary Group Controlled Trials Register, the Cochrane Central Register of Controlled Trials in The Cochrane Library were searched until May 2009. Conference abstracts from the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) were manually searched from 2007 to 2009. Reference lists of identified studies and reviews were also hand-searched. Search terms for each database are shown in the Appendix.

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A Meta-analysis of Transient Elastography for the Detection of Hepatic Fibrosis

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Objectives: The use of transient elastography to assess liver stiffness measurement (LSM) has now become widely available for the diagnosis of liver fibrosis as a rapid, noninvasive test (it is still not approved for use in the United States). It has previously been showed as an accurate method of representing the state of liver fibrosis with concomitant evaluation of liver biopsy and the histologic scoring system METAVIR. We performed a meta-analysis to further assess its use in comparisou with liver biopsy.

Methods: Studies from the literature were analyzed with a median liver stiffness value in kilopascal given for fibrosis stages according to histopathologic findings on biopsy and best discriminant cutoff levels in kilopascals for significant fibrosis ($\geq$ F2) and cirrhosis (F4).

Results: A total of 22 studies were selected comprising 4430 patients; chronic hepatitis C infection was the most common etiology of fibrosis. The pooled estimates for significant fibrosis ($\geq$ F2) measured 7.71 kPa (LSM cutoff value) with a sensitivity of 71.9% [95% confidence interval (CI): 71.4%-72.4%] and specificity of 82.4% (95% CI: 81.9-82.9%), whereas for cirrhosis (F4) the results showed a cutoff of 15.08 kPa with a sensitivity of 84.45% (95% CI: 84.2-84.7%) and specificity of 94.69% (95% CI: 94.3%-95%).

Conclusions: Further evaluation of transient elastography to assess LSM is required in prospective studies to potentially increase the sensitivity and establish its clinical utility.

Key Words: transient elastography, liver stiffness measurement, liver fibrosis, meta-analysis

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Liver fibrosis is characterized by the accumulation of an extracellular matrix that distorts the liver architecture. The major etiologies of liver fibrosis are chronic liver disease such as viral hepatitis, alcohol abuse, nonalcobolic steatobepatitis, and autoimmune disease. The progression of liver fibrosis increases the stiffness of the liver and the resistance of liver blood flow.[1] Insufficiency of liver blood flow results in liver failure with eventual cirrhosis—at which stage liver transplantation may be the only therapy to avoid a fatal outcome.[2] For this reason, an accurate assessment of liver fibrosis is vital in predicting the prognosis and the timing of commencing appropriate treatment to prevent disease progression.

Liver biopsy remains the gold standard method for assessing liver fibrosis. However, it is often difficult to undertake on a repeated basis because of the invasive nature and associated complications such as pain and bleeding. In addition, tissue samples are often too small and diagnostic opinions may differ among pathologists. Histologic quantification of liver fibrosis is also difficult because of diagnostic variability.[3] Consequently, new methods for the assessment of liver fibrosis are required.

Transient elastography (TE, FibroScan) for assessing liver stiffness measurement (LSM) has become available for the evaluation of liver fibrosis as a rapid, noninvasive method. It has been previously shown to accurately represent the state of liver fibrosis as concomitantly evaluated by the histologic scoring system METAVIR on liver biopsy.[4] FibroScan findings are generally more accurate than serum markers at predicting liver fibrosis,[5–14] the only exception being showed in one study[5] in which the combined use of TE and FibroTest offered the best diagnostic performance, both for significant fibrosis and cirrhosis.

To investigate the use of TE further, we performed a meta-analysis/systematic review to assess the validity of previously published studies in the detection of hepatic fibrosis.

METHODS

We searched medical databases (MEDLINE, Current Contents, PubMed, Cochrane library, Google Scholar), textbooks, and medical reports (UN, WHO) for relevant articles (English language). We also scanned the bibliographies of studies, reviewed abstract books/CDs for conferences and contacted individuals for personal opinions and up-to-date information. Search terms included "liver fibrosis and transient elastography," "hepatic fibrosis and transient elastography," and the terms were also interchanged—"transient elastography" to "elastography" or "fibroscan" or "elastometry" and "liver/hepatic fibrosis" to only "fibrosis."

All studies that reported data required for meta-analyses were identified and included—information providing

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Liver Stiffness Is Associated With Risk of Decompensation, Liver Cancer, and Death in Patients With Chronic Liver Diseases: A Systematic Review and Meta-analysis

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This article has an accompanying continuing medical education activity on page e89. Learning Objective—At the end of this activity, the successful learner will be able to summarize the prognostic utility of liver stiffness in predicting risk of clinically relevant, liver-related events in patients with chronic liver disease.

BACKGROUND & AIMS: **Liver stiffness measurement (LSM), using elastography, can independently predict outcomes of patients with chronic liver diseases (CLDs). However, there is much variation in reporting and consistency of findings. We performed a systematic review and meta-analysis to evaluate the association between LSM and outcomes of patients with CLDs.**

METHODS: **We performed a systematic review of the literature, through February 2013, for studies that followed up patients with CLDs prospectively for at least 6 months and reported the association between baseline LSM and subsequent development of decompensated cirrhosis or hepatocellular carcinoma (HCC), as well as mortality. Summary relative risk (RR) estimates per unit of LSM and 95% confidence intervals (CIs) were estimated using the random effects model.**

RESULTS: **Our final analysis included 17 studies, reporting on 7058 patients with CLDs. Baseline LSM was associated significantly with risk of hepatic decompensation (6 studies; RR, 1.07; 95% CI, 1.03–1.11), HCC (9 studies; RR, 1.11; 95% CI, 1.05–1.18), death (5 studies; RR, 1.22; 95% CI, 1.05–1.43), or a composite of these outcomes (7 studies; RR, 1.32; 95% CI, 1.16–1.51). We observed considerable heterogeneity among studies—primarily in the magnitude of effect, rather than the direction of effect. This heterogeneity could not be explained by variations in study locations, etiologies and stages of CLD, techniques to measure liver stiffness, adjustment for covariates, or method of imputing relationship in the meta-analysis.**

CONCLUSIONS: **Based on a meta-analysis of cohort studies, the degree of liver stiffness is associated with risk of decompensated cirrhosis, HCC, and death in patients with CLDs. LSM therefore might be used in risk stratification.**

Keywords: Elastography; Prognosis; Outcomes; Cirrhosis; Cancer.

Cirrhosis is the eighth leading cause of mortality worldwide and is associated with significant morbidity.[1,2] Two distinct stages of cirrhosis with different prognostic implications have been defined: compensated cirrhosis (stage 4 fibrosis with or without esophageal varices) and decompensated cirrhosis (variceal bleeding, hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, and/or hepatorenal syndrome).[3] Most of the morbidity and mortality in patients with cirrhosis is attributable to transitioning to a decompensated state. In addition, the presence of cirrhosis increases the risk of hepatocellular carcinoma (HCC). Liver transplantation (LT) is the only effective treatment modality once these outcomes develop for highly selected candidates. Hence, early recognition of patients with chronic liver disease (CLD) at high risk for developing these outcomes in a noninvasive manner is warranted to allow implementation of optimal preventative management strategies that may modify the natural course of disease.[4]

Unfortunately, objective markers of hepatic synthetic function that accurately predict an individual's risk of transitioning to a decompensated state are lacking.[5] Hepatic venous pressure gradient (HVPG) is a potential prognostic

Abbreviations used in this paper: CI, confidence interval; CLD, chronic liver disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; HVPG, hepatic venous pressure gradient; LSM, liver stiffness measurement; LT, liver transplantation; MELD, model for end-stage liver disease; MRE, magnetic resonance elastography; RR, relative risk; TE, transient elastography.

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Review History
Definitions
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Policy

Aetna considers transient elastography (e.g., FibroScan) medically necessary for distinguishing hepatic cirrhosis from noncirrhosis in persons with hepatitis C or other chronic liver diseases. Performance of transient elastography more than twice per year is considered not medically necessary. Performance of transient elastography within six months following a liver biopsy is considered not medically necessary. Transient elastography is considered experimental and investigational for all other indications.

Aetna considers serum marker tests (e.g., FibroMAX, FibroSpect, FibroTest-ActiTest, HCV-FibroSure, HepaScore, and NASH FibroSure) experimental and investigational for detecting or monitoring hepatic fibrosis in persons with hepatitis C or other chronic liver diseases (e.g., non-alcoholic fatty liver disease) because their effectiveness for these indications has not been established.

Background

Serum Markers of Hepatic Fibrosis:

Biochemical marker combinations are being developed as alternatives to liver biopsy in patients with chronic hepatitis C and other chronic liver diseases, including chronic hepatitis B, alcoholic liver disease, or non-alcoholic steatosis. Non-invasive tests are being developed to replace liver biopsy, and thus avoid the risk of biopsy-related adverse events. Non-invasive tests also have the potential to avoid limitations of liver biopsy, including the risk of sampling errors and inter- and intra-pathologist variability.

The FibroSpect II (Prometheus Laboratories, San Diego, CA) is a non-invasive diagnostic panel to assist in the detection of liver fibrosis. The FibroSpect II uses a combination of components in the fibrogenic cascade, such as hyaluronic acid, TIMP-1 (tissue inhibitor of metalloproteinase), and alpha-2-macroglobulin. The test is intended to differentiate mild fibrosis from more severe disease. The manufacturer's website provides "performance data" on the FibroSpect II Test, but this information is unpublished.

The FibroTest (Biopredictive, Houilles, France) and the ActiTest (Biopredictive, Houilles, France), marketed in the United States as the HCV-FibroSure Test (LabCorp, Burlington, NC), are the most thoroughly studied serum biochemical test for the assessment of fibrosis and necroinflammatory activity, respectively. The HCV-FibroSure Test includes the following five markers, as well as age and gender: alpha2-macroglobulin, haptoglobin, gamma-glutamyl transpeptidase (GGT), total

bilirubin, apolipoprotein A1, plus alanine aminotransferase (ALT). Rossi et al (2003) reported on the results of FibroTest scores of 125 patients with hepatitis C. Of these, 57 had FibroTest scores either less than 0.1 (indicating no fibrosis) or greater than 0.6 (indicating significant fibrosis). Although 33 of the 125 patients had FibroTest scores of less than 0.1 and were therefore deemed unlikely to have fibrosis, 6 (18 %) had significant fibrosis. Conversely, of the 24 patients with scores of greater than 0.6 who were likely to have significant fibrosis, 5 (21 %) had mild fibrosis. The investigators concluded that, "[o]f the 125 patients in the cohort, 57 (46 %) could have avoided liver biopsy"; but discrepant results were recorded in 11 of those 57 (19 %). In other words, discrepancies with the biopsy gold standard were found in one-fifth of patients. There are no prospective clinical outcome studies of the HCV-FibroSure in the management of patients with hepatitis C or other chronic liver diseases.

An National Institutes of Health Consensus Statement on Management of Hepatitis C (NIH, 2002) concluded that liver biopsy is useful in defining baseline abnormalities of liver disease and in enabling patients and healthcare providers to reach a decision regarding antiviral therapy. The NIH Consensus Statement concludes that noninvasive tests are not adequate substitutes for liver biopsy.

Various noninvasive tests of hepatic fibrosis have been examined for monitoring patients with chronic hepatitis C virus (HCV) infection. These include routinely available laboratory tests, such as liver- associated chemistries, platelet count, and prothrombin time, as well as specific serum markers of fibrosis and inflammation not currently widely available or well validated. No single test or panel of serologic markers can provide an accurate assessment of intermediate stages of hepatic fibrosis. Similarly, quantitative tests of liver function and radiologic imaging of the liver are sensitive for diagnosing advanced cirrhosis but are not useful in assessing hepatic fibrosis and early cirrhosis.

In a review on newer markers for hepatocellular carcinoma, Marrero and Lok (2004) stated that there is a scarcity of longitudinal studies evaluating the ability of biomarkers to detect pre-clinical disease. There is an urgent need for novel biomarkers for the detection of early hepatocellular carcinoma.

Suzuki et al (2005) stated that “use of an accurate serum marker for severe hepatic fibrosis may also improve accuracy of non-invasive diagnostic models. Hyaluronic acid, a serum marker for severe hepatic fibrosis, has been reported to have a high diagnostic performance in assessing the severity of hepatic fibrosis in patients with alcoholic liver disease. In this issue, a non-invasive diagnostic model including hyaluronic acid was shown to have excellent performance in excluding the presence of medium to large esophageal varices in severe alcohol abusers. Based on current evidence, the strategy of using a non-invasive diagnostic model together with a serum marker for severe hepatic fibrosis may improve cost-benefit in the prevention of variceal hemorrhage among patients with alcoholic liver disease. The independent verification of such diagnostic models is needed”.

Evidence based guidelines on the management of hepatitis C from the American Association for the Study of Liver Diseases (Strader et al, 2004) stated: "Although liver fibrosis markers are commercially available, they are currently insufficiently accurate to support their routine use. Until sensitive serum markers can be developed that will define all stages of fibrosis and mirror the information derived from liver biopsy, the procedure remains the only means of defining the severity of damage from HCV infection in many patients".

Serum gamma glutamyltransferase (GGT) is elevated in individuals with acute and chronic alcohol toxicity. Serum GGT assay may be useful in evaluating patients when heavy drinking is suspected but the patient denies it (NIAAA, 2005).

Wilson et al (2006) stated that although most HCV infections are acquired by injection drug use, prospective data on the progression of liver fibrosis are sparse. In this study, baseline liver biopsies were obtained on a random sample of 210 out of 1,667 HCV-positive injection drug users (IDUs). Subjects were followed biannually, with a second biopsy offered to those eligible. Paired biopsies were scored 0 to 6 (modified Ishak score), significant fibrosis was defined as score 3 or greater, and

progression of fibrosis was defined as an increase 2 or more units or clinical evidence of end-stage liver disease. Predictive values of blood markers (FibroSure, aspartate aminotransferase-to-platelet-ratio index (APRI) and alanine aminotransferase (ALT)) were assessed for detection of contemporaneous and future liver fibrosis. Among 119 prospectively followed IDUs, 96 % were African American; 97 % HCV genotype 1a/b; 27 % HIV-infected, and median age was 42 years. Most (90.7 %) did not have significant liver fibrosis at first biopsy. Although predictive value for detecting insignificant fibrosis at first biopsy was greater than 95 % for FibroSure, APRI, and ALT, specificities were 88.9 %, 72.7 %, and 72.7 %, respectively. After 4.2 years median follow-up, 21 % had progression of fibrosis, which was significantly associated with serum level of HCV RNA and ALT. No serological test had predictive value greater than 40 % for contemporaneous or future significant fibrosis. Even initial biopsy result had only a 30.4 % value for predicting future significant fibrosis. The authors concluded that significant liver fibrosis and progression were detected in some, but not most, IDUs in this cohort. In this setting with low fibrosis prevalence, FibroSure, ALT, and APRI tests predict insignificant fibrosis; however, further work is needed to find non-invasive markers of significant liver fibrosis.

Nourani and Pockros (2007) noted that biochemical markers are a potentially useful alternative to liver biopsy in patients with chronic hepatitis C aged 65 years and older. Furthermore, Rossi et al (2007) stated that an obstacle to widespread adoption of serum marker models (e.g., FibroSpect) for assessing liver fibrosis has been the lack of uniform performance indicators, such as diagnostic odds ratios and likelihood ratios. At present, serum marker models are not considered sufficiently reliable to replace liver biopsy in patients with chronic liver disease.

Shaheen and Myers (2008) performed a systematic review and meta-analysis of the diagnostic accuracy of fibrosis marker panels in patients with HIV/hepatitis C coinfection. Random effects meta-analyses and areas under summary receiver operating characteristics curves (AUC) examined test accuracy for detecting significant fibrosis (F2 to F4) and cirrhosis. Heterogeneity was explored using meta-regression. Five studies (n = 574) including 4 fibrosis measures (APRI [n = 4 studies], Forns' [n = 2], FibroTest [n = 1], SHASTA [n = 1]) met the inclusion criteria. The prevalence of significant fibrosis and cirrhosis were 51 % and 16 %, respectively. For the prediction of significant fibrosis, the summary AUC was 0.82 (95 % confidence interval [CI]: 0.78 to 86) and diagnostic odds ratio was 7.8 (5.1 to 11.9). For cirrhosis, these figures were 0.83 (0.69 to 0.97) and 11.0 (4.6 to 26.2), respectively. Meta-regression including study factors (methodological quality and biopsy adequacy), patient characteristics (age, gender, CD4 count), and fibrosis measure failed to identify important predictors of accuracy. The authors concluded that available fibrosis marker panels have acceptable performance for identifying significant fibrosis and cirrhosis in HIV/HCV-coinfected patients but are not yet adequate to replace liver biopsy. They noted that additional studies are needed to identify the optimal measure.

Smith and Sterling (2009) reviewed non-invasive measures and their ability to replace biopsy for assessing hepatic fibrosis in patients with chronic HCV. A systematic review of PUBMED and EMBASE was carried out through 2008 using the following search terms: HCV, liver, elastography, hepatitis, Fibroscan, SPECT, non-invasive liver fibrosis, ultrasonography, Doppler, MRI, Fibrotest, Fibrosure, Actitest, APRI, Forns and breath tests, alone or in combination. These investigators identified 151 studies: 87 using biochemical, 57 imaging and 7 breath tests either alone or in combination. The authors concluded that great strides are being made in the development of accurate non-invasive methods for determination of fibrosis. Although no single non-invasive test or model developed to date can match that information obtained from actual histology (i.e., inflammation, fibrosis, steatosis), combinations of 2 modalities of non-invasive methods can reliably differentiate between minimal and significant fibrosis, and thereby avoid liver biopsy in a significant percentage of patients.

Carlson and colleagues (2009) evaluated the clinical and economic outcomes of non-invasive testing strategies in the diagnosis of significant liver fibrosis (Metavir score greater than or equal to 2) compared with liver biopsy. These researchers developed a decision analytic model of non-invasive testing strategies in a hypothetical patient population with genotype 1 hepatitis C virus infection, with no contraindications to liver biopsy. The testing strategies included a testing algorithm using the Fibrosure test, a non-invasive measure of fibrosis, followed by liver biopsy for

patients with indeterminate results, Fibrospect II, and Fibroscan. The primary outcomes were sensitivity, specificity, diagnostic accuracy (true positive + true negatives/total patients), and costs, evaluated from the health-care payer perspective. The testing algorithm using Fibrosure was the most accurate non-invasive strategy with a sensitivity, specificity, and overall accuracy of 84 %, 87 %, and 86 %, respectively. In comparison with liver biopsy alone, there was a cost savings of approximately $770/person with the Fibrosure testing algorithm, but a net decrease in accuracy of 14 %. Fibrospect II and Fibroscan had poorer accuracy (decreases of 12 % and 4 %, respectively) and lower costs (-$138 and -$357, respectively) compared with the Fibrosure algorithm. In uncertainty analyses in which biopsy sampling error was considered, the Fibrosure algorithm remained consistently less accurate (5 to 14 % decrease). The authors concluded that the results of this study suggested that compared with liver biopsy, non-invasive testing algorithms can result in short-term cost savings, but the consequences of misdiagnosis in terms of health outcomes and treatment costs might out-weigh the short-term gains in cost and convenience.

Adams (2011) stated that fibrosis prediction is an essential part of the management of patients with chronic liver disease. Serum biomarkers offer a number of advantages over the traditional standard of fibrosis assessment of liver biopsy, including safety, cost-savings and wide spread accessibility. Current biomarker algorithms include indirect surrogate measures of fibrosis, including aminotransaminases and platelet count, or direct measures of fibrinogenesis or fibrinolysis such as hyaluronic acid and tissue inhibitor of metalloproteinase-1. A number of algorithms have now been validated across a range of chronic liver disease including chronic viral hepatitis, alcoholic and non-alcoholic fatty liver disease. Furthermore, several models have been demonstrated to be dynamic to changes in fibrosis over time and are predictive of liver-related survival and overall survival to a greater degree than liver biopsy. Current limitations of biomarker models include a significant indeterminate range, and a predictive ability that is limited to only a few stages of fibrosis. Utilization of these biomarker models requires knowledge of patient co-morbidities which may produce false positive or negative results in a small proportion of individuals. Furthermore, knowledge of the underlying prevalence of fibrosis in the patient population is required for interpretation of the positive or negative predictive values of a test result. Novel proteins identified by proteomic technology and genetic polymorphisms from genome association studies offer the possibility for further refinement and individualization of biomarker fibrosis models in the future.

Sebastiani and associates (2011) examined the effect of etiology and stages of hepatic fibrosis on the performance of fibrosis biomarkers. A total of 2,411 patients with compensated chronic liver disease (CLD) (hepatitis C virus [HCV] = 75.1 %, hepatitis B virus [HBV] = 10.5 %, non-alcoholic steato-hepatitis [NASH] = 7.9 %, HIV/HCV = 6.5 %) were consecutively enrolled in 9 centers. APRI, Forns'index, Lok index, AST-to-ALT ratio, Fib-4, platelets and Fibrotest-Fibrosure were tested against liver biopsy, considered the gold standard. The effect of the stages of hepatic fibrosis to diagnose significant fibrosis and cirrhosis (greater than or equal to F2 and F4, respectively) was investigated through difference between advanced and non-advanced fibrosis stages (DANA). Performance was expressed as observed area under the ROC curve (ObAUROC) and AUROC adjusted for DANA (AdjAUROC). Performance of APRI and Fibrotest-Fibrosure was higher than other biomarkers. In all etiologies, AdjAUROC was higher than ObAUROC. APRI showed its best performance in HCV mono-infected cases, with an AdjAUROC of 0.77 and 0.83 for greater than or equal to F2 and F4, respectively. In HBV and NASH patients, its performance was poor (AdjAUROC < 0.70). Performance of Fibrotest-Fibrosure was good in all etiologies for both greater than or equal to F2 and F4 (AdjAUROC > 0.73), except for greater than or equal to F2 in NASH (AdjAUROC = 0.64). Performance of all biomarkers was reduced in HCV cases with normal ALT. The authors concluded that etiology is a major factor influencing the performance of liver fibrosis biomarkers. Even after correction for DANA, APRI and Fibrotest-Fibrosure exhibit the best performance. However, liver biopsy is not replaceable, especially to diagnose greater than or equal to F2 and in HCV carriers with normal ALT.

Adams et al (2005) stated that staging hepatic fibrosis by liver biopsy guides prognosis and treatment of hepatitis C, but is invasive and expensive. These researchers sought to create an algorithm of serum markers that accurately and reliably predict liver fibrosis stage among hepatitis C patients. A total of 10 biochemical markers were measured at time of liver biopsy in 117 untreated hepatitis C patients (training set). Multi-variate logistic regression and receiver-operating

characteristic (ROC) curve analyses were used to create a predictive model for significant fibrosis (METAVIR F2, F3, and F4), advanced fibrosis (F3 and F4), and cirrhosis (F4). The model was validated in 104 patients from other institutions. A model (HepaScore) of bilirubin, gamma-glutamyltransferase, hyaluronic acid (HA), alpha(2)-macroglobulin, age, and sex produced areas under the ROC curves (AUCs) of 0.85, 0.96, and 0.94 for significant fibrosis, advanced fibrosis, and cirrhosis, respectively. In the training set, a score greater than or equal to 0.5 (range of 0.0 to 1.0) was 92 % specific and 67 % sensitive for significant fibrosis, a score of less than 0.5 was 81 % specific and 95 % sensitive for advanced fibrosis, and a score of less than 0.84 was 84 % specific and 71 % sensitive for cirrhosis. Among the validation set, the AUC for significant fibrosis, advanced fibrosis, and cirrhosis were 0.82, 0.90, and 0.89, respectively. A score greater than or equal to 0.5 provided a specificity and sensitivity of 89 % and 63 % for significant fibrosis, whereas scores less than 0.5 had 74 % specificity and 88 % sensitivity for advanced fibrosis, respectively. The authors concluded that a model of 4 serum markers plus age and sex provides clinically useful information regarding different fibrosis stages among hepatitis C patients.

Stevenson et al (2012) evaluated the diagnostic accuracy, cost-effectiveness, and effect on patient outcomes of 4 non-invasive tests for liver fibrosis [the Enhanced Liver Fibrosis (ELF) test (Siemens Healthcare Diagnostic Inc., Tarrytown, NY), FibroTest (BioPredictive, Paris, France), FibroMAX (BioPredictive, Paris, France) and transient elastography (FibroScan; produced by EchoSens, Paris, France and distributed in the United Kingdom. by Artemis Medical Ltd, Kent, UK)] in patients suspected of having ALD. A systematic review was undertaken to identify studies reporting the diagnostic and prognostic accuracy of the ELF test, FibroTest, FibroMAX, and FibroScan for the identification of liver fibrosis and associated conditions in patients with suspected ALD. The following databases were searched in January 2010: MEDLINE (from 1950 to January 2010), MEDLINE In-Process & Other Non-Indexed Citations (from 1950 to January 2010), EMBASE (from 1980 to January 2010), Cochrane Database of Systematic Reviews (from 1996 to January 2010), Cochrane Central Register of Controlled Trials (from 1898 to January 2010), Cochrane Methodology Register (from 1904 to January 2010), Database of Abstracts of Reviews of Effects (from 1995 to January 2010), HTA Database (from 1995 to January 2010), NHS Economic Evaluation Database (from 1995 to January 2010), Cumulative Index to Nursing and Allied Health Literature (from 1982 to January 2010), Web of Knowledge and Science Citation Index (from 1969 to January 2010). Study quality was assessed using the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) checklist. Owing to the heterogeneity of the studies, no formal meta-analysis was undertaken. A de novo mathematical model was constructed to estimate the incremental costs and incremental quality-adjusted life-years (QALYs) associated with alternative strategies compared with a biopsy-all strategy. The tests were assessed first as a replacement for liver biopsy, and secondly as an additional test prior to liver biopsy. A total of 36 scenarios were assessed for each non-invasive test strategy, which varied the sensitivity of biopsy, the anxiety associated with biopsy, sensitivity and specificity values and whether or not the biopsy was percutaneous or transjugular. For each scenario, threshold levels were reported where biopsying all patients was more cost-effective than the strategy for 2 parameters (the decreased level of abstinence associated with the strategy compared with biopsying all and the level of incidental QALY gain associated with biopsy). No studies were identified that specifically assessed the ELF test, although a study was identified that evaluated the diagnostic accuracy of the European Liver Fibrosis Test (essentially, the ELF test with the addition of age to the algorithm) compared with biopsy. Three studies of FibroTest, no relevant studies of FibroMAX, and 6 studies of FibroScan assessing accuracy compared with biopsy in patients with known or suspected alcohol-related liver disease were identified. In all studies, the number of patients with suspected ALD was small, meaning that the estimated sensitivities and specificities were not robust. No conclusive estimate of the cost per QALY of each non-invasive test could be provided. Scenarios exist in which each of the strategies analyzed is more cost-effective than biopsying all patients and, in contrast, scenarios exist in which each strategy is less cost-effective than biopsying all patients. The authors concluded that no conclusive result can be provided on the most cost-effective strategy until further data are available. A large number of parameters require data; however, the following were selected as being of most importance: (i) the sensitivity and specificity of each non-invasive liver test (NILT) against biopsy at validated and pre-selected cut-off thresholds; (ii) the influence of potential confounding variables such as current drinking behavior and the degree of hepatic

inflammation on the performance of NILTs; and (iii) the likelihood, and magnitude, of decreases in abstinence rates associated with a diagnosis of significant ALD by diagnostic modality and the incidental gains in QALYs that may be associated with biopsy.

Sebastiani and Alberti (2012) chronic hepatitis C represents a major cause of progressive liver disease that can eventually evolve into cirrhosis and its end-stage complications. Formation and accumulation of fibrosis in the liver is the common pathway that leads to evolutive liver disease. Precise staging of liver fibrosis is essential for patient management in clinical practice because the presence of bridging fibrosis represents a strong indication for anti-viral therapy, while cirrhosis requires a specific follow-up. Liver biopsy has always represented the standard of reference for assessment of hepatic fibrosis, but it has limitations: it is invasive, costly and prone to sampling errors. Recently, blood markers and instrumental methods have been proposed for the non-invasive assessment of liver fibrosis in hepatitis C. However, international guidelines do not recommend the widespread use of non-invasive methods for liver fibrosis in clinical practice. This is because of, in some cases, unsatisfactory accuracy and incomplete validation of others. Some studies suggested that the effectiveness of non-invasive methods for assessing liver fibrosis may increase when they are combined, and a number of sequential and synchronous algorithms have been proposed for this purpose, with the aim of reducing rather than substituting liver biopsies. This may represent a rational and reliable approach for implementing noninvasive assessment of liver fibrosis in clinical practice. It could allow more comprehensive first-line screening of liver fibrosis in hepatitis C than would be feasible with liver biopsy alone.

Usluer et al (2012) compared the results of 9 non-invasive serum biomarkers with liver biopsies to predict liver fibrosis stage. HCV-RNA-positive, HCV genotype 1, treatment-naive patients with chronic HCV infections were included from 14 centers (n = 77). The platelet count, AST/ALT ratio (AAR), cirrhosis discriminate score (CDS), FIB4, APRI, age-platelet (AP) index, Goteborg University cirrhosis index (GUCI), FibroTest, and ActiTest were calculated and compared to histologic findings. All serum biomarkers, except AAR, were weakly or moderately correlated with liver biopsy results (ISHAK fibrosis score). The mean scores of FibroTest, FIB4, APRI, and AP index were significantly different between F0-F2 and F3-F4 groups and the NPVs of the F3-F4 group were 95 %, 85 %, 85 %, and 8 3%, respectively, for these serum biomarkers. The authors concluded that these findings suggested that serum biomarkers may help to diagnose significant fibrosis but inadequate to detect fibrosis in early stages.

Bhogal and Sterling (2012) noted that several blood tests, algorithms, and imaging tests have been studied as non-invasive markers to stage fibrosis in hepatitis C. In patients without suspicion for cirrhosis, 2 non-invasive methods can be used to predict presence of absence of significant liver fibrosis; however, liver biopsy remains the gold standard.

Chladek et al (2012) compared the results of serial measurements of serum fibrosis markers during the remission-induction phase of treatment with methotrexate (MTX) to those of patients on biological therapy and long-term MTX therapy (greater than 2 years). Serum concentrations of HA, N-terminal propeptide of collagen type III (PIIINP) and the results of 2 multi-test algorithms FibroTest and HepaScore were evaluated in patients with chronic plaque psoriasis (n = 24, age: 28 to 79 years, baseline Psoriasis Area Severity Index [PASI] 13.5, range of 2.2 to 33) at baseline and weeks 16 and 26 after the start of pharmacokinetically-guided therapy with MTX (Group A). Patients on established therapy with biologics (n = 15, Group B) and long-term MTX users (n = 10, Group C) with the mean baseline PASI scores of 0.9 and 1.2 were studied in parallel cohorts. At baseline, HA, HepaScore and PIIINP were correlated with PASI of Group A patients. At weeks 16 and 26, HA decreased by 48 % and 40 % ($p < 0.001$) and HepaScore by 31 ($p < 0.01$) and 20 % ($p < 0.05$) respectively. PASI75 (greater than or equal to 75 % improvement from baseline PASI) was observed in 76 % of Group A patients by week 26 and the absolute decreases in PASI and both fibrosis markers were correlated (HA: $r = 0.49$, $p = 0.018$, HepaScore: $r = 0.47$, $p = 0.022$). In contrast, no significant within-group differences were found in HA and HepaScore results of patients in the groups B and C. PIIINP and FibroTest were stable in all groups. The authors concluded that the fibrosis markers HA and HepaScore (the multiple test algorithm that includes HA) are less liver specific and more prone to reflect psoriasis activity than PIIINP and FibroTest.

Chou and Wasson (2013) stated that many blood tests have been proposed as alternatives to liver biopsy for identifying fibrosis or cirrhosis. These investigators evaluated the diagnostic accuracy of blood tests to identify fibrosis or cirrhosis in patients with HCV infection. Data sources included MEDLINE (1947 to January 2013), the Cochrane Library, and reference lists. Studies that compared the diagnostic accuracy of blood tests with that of liver biopsy were selected. Investigators abstracted and checked study details and quality by using pre-defined criteria. A total of 172 studies evaluated diagnostic accuracy. For identifying clinically significant fibrosis, the platelet count, age-platelet index, APRI, FibroIndex, FibroTest, and Forns index had median positive likelihood ratios of 5 to 10 at commonly used cut-offs and areas under the ROC curve (AUROCs) of 0.70 or greater (range of 0.71 to 0.86). For identifying cirrhosis, the platelet count, age-platelet index, APRI, and HepaScore had median positive likelihood ratios of 5 to 10 and AUROCs of 0.80 or greater (range of 0.80 to 0.91). The GUCI and the Lok index had slightly lower positive likelihood ratios (4.8 and 4.4, respectively). In direct comparisons, the APRI was associated with a slightly lower AUROC than the FibroTest for identifying fibrosis and a substantially higher AUROC than the aspartate aminotransferase-alanine aminotransferase ratio for identifying fibrosis or cirrhosis. The authors concluded that many blood tests are moderately useful for identifying clinically significant fibrosis or cirrhosis in HCV-infected patients. Drawbacks of this study included only English-language articles were included, and most studies had methodological limitations, including failure to describe blinded interpretation of liver biopsy specimens and inadequate description of enrollment methods.

Rossi et al (2013) noted that serum HA and biochemical models that require HA analysis are commonly used as predictors of liver fibrosis in patients with chronic liver disease, however biological variation data for HA are deficient. Four serial serum samples were obtained at weekly intervals from healthy volunteers and patients with chronic hepatitis B, chronic hepatitis C and NAFLD (20 in each group). The within-individual week-to-week variation (CVI) and reference change values for HA, αâ,,-macroglobulin and HepaScore were obtained. HepaScore was calculated from HA, α2-macroglobulin, bilirubin and γ-glutamyltransferase activity. Hyaluronic acid displayed large within-individual variation, the CVI values were 62 % in healthy subjects, 38 % in hepatitis C, 37 % in hepatitis B, and 36 % in NAFLD patients. HepaScore CVIs were 43 % in healthy subjects, 24 % in hepatitis C, 28 % in hepatitis B, and 39 % in NAFLD patients. Moreover, αâ,,-Macroglobulin was much less variable with CVIs ranging from 4.4 % to 7.6 %. Bland-Altman plots of week-to-week variations showed rates of significant disagreement for samples collected in any 2 successive weeks varied from 5 % in NAFLD patients to 8.3 % in healthy subjects. The authors concluded that when using non-fasting serum samples, HA and to a lesser extent, the HepaScore model displayed large within-individual variations in both health and chronic liver disease. This information is critical for interpreting the significance of both single measurements and changes in serial measurements.

Grattagliano et al (2013) stated that the diagnostic utilities of ultrasonography (US), fatty liver index (FLI) and an algorithm of 9 serum markers (FibroMAX) were evaluated in family practice to non-invasively characterize patients with NAFLD. A multi-center study was conducted by enrolling 259 consecutively observed patients (age of 51 +/- 10 years) with clinical and ultrasonographic features of NAFLD. Patients had mild (16.2 %), moderate (69.9 %), or severe (13.9 %) liver steatosis and 60.2 % had hyper-transaminasemia. The percent of patients with overweight, obesity, diabetes, hypertension, and dyslipidemia were 42.7 %, 46.5 % (4.2 % severe obesity), 24.7 %, 40.9 %, and 56.4 %, respectively. Lean patients (10.8 %) had normal transaminases in 2/3 of the cases. A multi-variate logistic regression (including age greater than 50 years, BMI greater than 30 kg/m2, homeostasis model assessment [HOMA] greater than 3, and hyper-transaminasemia) identified 12.3 % of patients at risk for steatohepatitis. With a sensitivity of 50 % and specificity of 94.7 %, FibroMAX identified 34 patients (13.1 %) with likely advanced fibrosis and found that over 28 % of patients with moderate (ultrasonographic) steatosis were likely to be carrying severe steatosis. Steatotest score was significantly associated with BMI, waist circumference, ALT, triglycerides, and FLI. FibroTest correlated only with ALT. Fatty liver index identified 73.4 % of patients as likely to be carrying a fatty liver. The authors concluded that NAFLD should be systematically searched and characterized in all patients with metabolic disturbances and cardiovascular risk. Asymptomatic subjects at risk also should be screened for NAFLD. They stated that FibroMAX is

a promising non-invasive diagnostic tool in family medicine for identifying patients at risk for NAFLD who require targeted follow-up.

Furthermore, an UpToDate review on "Tests used for the noninvasive assessment of hepatic fibrosis" (Curry and Afdhal, 2013) states that "While tremendous progress has been made in improving the accuracy of serum markers of hepatic fibrosis, they cannot yet supplant direct analysis of the liver. The ideal fibrosis marker is one that is specific, biologically based, noninvasive, easily repeated in all patients, correlates well with disease severity and outcome, and is not confounded by co-morbidities or drugs. Although this ideal has nearly been reached, no serum test has emerged as the perfect marker of fibrosis; all the serum tests have limitations …. Overall, the serum assay approaches remain promising, in part because these tests may represent an integrated readout of liver activity, rather than a minute sampling of the type obtained by conventional liver biopsy".

Transient Elastography:

Transient elastography (FibroScan) is a rapid, non-invasive, bedside method to evaluate liver fibrosis by measuring liver stiffness. This device is based on 1-dimensional transient elastography, a technique that uses both ultrasound (5 MHz) and low-frequency (50 Hz) elastic waves, whose propagation velocity is directly related to elasticity. In cirrhotic patients, liver stiffness measurements range from 12.5 to 75.5 kPa (kPa). However, the clinical relevance of these values is unknown.

In a prospective study, de Ledinghen et al (2006) evaluated the accuracy of liver stiffness measurement for the detection of fibrosis and cirrhosis in HIV/hepatitis C virus (HCV)-coinfected patients and compared its accuracy with other non-invasive methods. These researchers studied 72 consecutive HIV patients with chronic hepatitis C who had a simultaneous liver biopsy and liver stiffness measurement by transient elastography (FibroScan; Echosens, Paris, France) for the assessment of liver fibrosis. Liver stiffness values ranged from 3.0 to 46.4 kPa. Liver stiffness was significantly correlated to fibrosis stage (Kendall tau-b = 0.48; $p < 0.0001$). The area under the receiver operating characteristic (AUROC) curve of liver stiffness measurement was 0.72 for F > or = 2 and 0.97 for F = 4. For the diagnosis of cirrhosis, AUROC curves of liver stiffness measurement were significantly higher than those for platelet count ($p = 0.02$), aspartate aminotransferase/ALT ratio ($p = 0.0001$), Aspartate aminotransferase-to-Platelet Ratio Index ($p = 0.01$), and FIB-4 ($p = 0.004$). The authors concluded that liver stiffness measurement is a promising noninvasive method for the assessment of fibrosis in HIV-infected patients with chronic HCV infection. They also noted that its use for the follow-up of these patients should be further evaluated.

Foucher and colleagues (2006) assessed the accuracy of FibroScan for the detection of cirrhosis in patients with chronic liver disease. A total of 711 patients with chronic liver disease were studied. Etiologies of chronic liver diseases were hepatitis C virus or hepatitis B virus infection, alcohol, non-alcoholic steatohepatitis, other, or a combination of the above etiologies. Liver fibrosis was evaluated according to the METAVIR score. Stiffness was significantly correlated with fibrosis stage ($r = 0.73$, $p < 0.0001$). Areas under the receiver operating characteristic curve (95 % CI) were 0.80 (0.75 to 0.84) for patients with significant fibrosis (F > 2), 0.90 (0.86 to 0.93) for patients with severe fibrosis (F3), and 0.96 (0.94 to 0.98) for patients with cirrhosis. Using a cut off value of 17.6 kPa, patients with cirrhosis were detected with a positive predictive value and a negative predictive value (NPV) of 90 %. Liver stiffness was significantly correlated with clinical, biological, and morphological parameters of liver disease. With an NPV greater than 90 %, the cut off values for the presence of esophageal varices stage 2/3, cirrhosis Child-Pugh B or C, past history of ascites, hepatocellular carcinoma, and esophageal bleeding were 27.5, 37.5, 49.1, 53.7, and 62.7 kPa, respectively. The authors concluded that FibroScan is a promising non-invasive method for detection of cirrhosis in patients with chronic liver disease. They noted that its use for the follow-up and management of these patients could be of great interest and should be evaluated further.

Corpechot and associates (2006) assessed the diagnostic performance of liver stiffness measurement

(LSM) for the determination of fibrosis stage in chronic cholestatic diseases. A total of 101 patients with primary biliary cirrhosis (PBC, n = 73) or primary sclerosing cholangitis (PSC, n = 28) were prospectively enrolled in a multi-center study. All patients underwent liver biopsy (LB) and LSM. Histological and fibrosis stages were assessed on LB by two pathologists. LSM was performed by FibroScan. Efficiency of LSM for the determination of histological and fibrosis stages were determined by a ROC curve analysis. Analysis failed in 6 patients (5.9 %) because of unsuitable LB (n = 4) or LSM (n = 2). Stiffness values ranged from 2.8 to 69.1 kPa (median of 7.8 kPa). LSM was correlated to both fibrosis (Spearman's rho = 0.84, p < .0001) and histological (0.79, p < .0001) stages. These correlations were still found when PBC and PSC patients were analyzed separately. Areas under ROC curves were 0.92 for fibrosis stage (F) > or = 2, 0.95 for F > or = 3 and 0.96 for F = 4. Optimal stiffness cutoff values of 7.3, 9.8, and 17.3 kPa showed F > or = 2, F > or = 3 and F = 4, respectively. LSM and serum hyaluronic acid level were independent parameters associated with extensive fibrosis on LB. The authors concluded that FibroScan is a simple and reliable non-invasive means for assessing biliary fibrosis. They stated that it should be a promising tool to assess anti-fibrotic therapies in PBC or PSC.

The Canadian Agency for Drugs and Technologies in Health (CADTH) performed an evaluation on FibroScan for non-invasive assessment of liver fibrosis (Murtagh and Foster, 2006). It stated that the diagnostic performance of FibroScan is good for identifying severe fibrosis or cirrhosis, but it is less accurate for milder presentations. It concluded that FibroScan is a promising technology, but large multi-center studies comparing a range of emerging non-invasive fibrosis staging technologies are needed. An earlier assessment by the French Committee for Evaluation and Diffusion of Innovative Technologies (CEDIT, 2004) reached similar conclusions, stating that the absence of conclusive evidence concerning the diagnostic value of FibroScan argues against its immediate dissemination. More recently, an assessment by the French National Authority for Health (HAS, 2007) concluded that additional studies are necessary to evaluate the comparative cost-effectiveness of different methods of assessing liver fibrosis (e.g., FibroTest, FibroScan, and biopsy). A technology assessment by the Malasian Ministry of Health (Darus, 2008) reached similar conclusions about the need for additional research for the Fibroscan.

de Franchis et al (2007) stated that transient elastography (Fibroscan) might be of value for the non-invasive diagnosis of cirrhosis; however, its reproducibility needs to be further validated. Furthermore, Berrutti et al (2007) noted that FibroScan is a new, non-invasive method to evaluate liver stiffness and, consequently, the degree of liver fibrosis. Since its use in the clinical setting is of great interest, further studies should define the exact role of this procedure.

de Lédinghen et al (2007) assessed the feasibility of liver stiffness measurement and compared FibroScan, FibroTest, and APRI with liver biopsy for the diagnosis of cirrhosis in children with chronic liver diseases. A total of 116 consecutive children with chronic liver diseases were prospectively included. All except 1 child (58 boys, mean age of 10.7 years) could have non-invasive tests for fibrosis: FibroScan, FibroTest, and APRI, and, when necessary, a liver biopsy (n = 33). FibroScan, FibroTest, and APRI were correlated with clinical or biological parameters of chronic liver diseases, but the FibroScan marker correlated most with all parameters. By histology, the METAVIR fibrosis category score was F1 in 7 cases, F2 in 8 cases, F3 in 6 cases, and F4 in 12 cases. FibroScan, FibroTest, and APRI were significantly correlated with the METAVIR fibrosis score. For the diagnosis of cirrhosis, AUC was 0.88, 0.73, and 0.73 for FibroScan, FibroTest, and APRI, respectively. The authors concluded that these findings indicated that liver stiffness measurement is feasible in children and is related to liver fibrosis. A specific probe dedicated to children and slender patients has thus been developed and is currently under evaluation. The FibroScan equipped with this specific probe could become a useful tool for the management of chronic liver diseases in children.

Shaheen et al (2007) stated that the accurate diagnosis of HCV-related fibrosis is crucial for prognostication and treatment decisions. Due to the limitations of biopsy, non-invasive alternatives including FibroTest and FibroScan have beenn developed. These investigators systematically reviewed studies describing the accuracy of these tests for predicting HCV-related fibrosis. Studies comparing FibroTest or FibroScan versus biopsy in HCV patients were identified via an electronic search. Random effects meta-analyses and AUC were examined to characterize test accuracy for

significant fibrosis (F2 to F4) and cirrhosis. Heterogeneity was explored using meta-regression. A total of 12 studies were identified, 9 for FibroTest (n = 1,679) and 4 for FibroScan (n = 546). In heterogeneous analyses for significant fibrosis, the AUCs for FibroTest and FibroScan were 0.81 (95 % confidence interval [CI] 0.78 to 84) and 0.83 (0.03 to 1.00), respectively. At a threshold of approximately 0.60, the sensitivity and specificity of the FibroTest were 47 % (35 % to 59 %) and 90 % (87 % to 92%). For FibroScan (threshold approximately 8 kPa), corresponding values were 64 % (50 % to 76 %) and 87 % (80 % to 91 %), respectively. Methodological quality, the length of liver biopsy specimens, and inclusion of special populations did not explain the observed heterogeneity. However, the diagnostic accuracy of both measures was associated with the prevalence of significant fibrosis and cirrhosis in the study populations. For cirrhosis, the summary AUCs for FibroTest and FibroScan were 0.90 (95 % CI not calculable) and 0.95 (0.87 to 0.99), respectively. The authors concluded that FibroTest and FibroScan have excellent utility for the identification of HCV-related cirrhosis, but lesser accuracy for earlier stages. They noted that refinements are necessary before these tests can replace liver biopsy.

Sagir et al (2008) noted that transient elastography (also known as FibroScan) is a rapid, non-invasive, and reproducible method for measuring liver stiffness, which correlates with the degree of liver fibrosis in patients with chronic hepatitis. However, whether FibroScan is useful in the detection of pre-existing liver fibrosis/cirrhosis in patients presenting with acute liver damage is unclear. In this study, patients with acute liver damage of different etiologies were analyzed. Liver stiffness was measured during the acute phase of the liver damage and followed-up to the end of the acute phase. A total of 20 patients were included in the study. In 15 of the 20 patients, initial liver stiffness values measured by FibroScan during the acute phase of the liver damage were suggestive of liver cirrhosis. However, none of these 15 patients showed any signs of liver cirrhosis in the physical examination, ultrasound examination, or liver histology [performed in 11 of 15 (73 %) patients]. A significant difference was observed in the initial bilirubin levels (5.8 +/- 6.5 mg/dL versus 15.7 +/- 11.8 mg/dL; p = 0.042) and age (32.4 +/- 17.5 years versus 49.7 +/- 15.8 years; p = 0.042) between patients with liver stiffness below or above 12.5 kPa. Six patients with initially high liver stiffness were followed-up to abatement of the acute hepatitic phase; in all of them, liver stiffness values decreased to values below the cut-off value for liver cirrhosis. The authors concluded that transient elastography frequently yields pathologically high values in patients with acute liver damage and is unsuitable for detecting cirrhosis/fibrosis in these patients.

Han and Yoon (2008) stated that although liver biopsy is still the gold standard for assessing hepatic fibrosis, it has some technical limitations and risks. Furthermore, the dynamic process of liver fibrosis resulting from progression and regression can not be quantified by liver biopsy. Thus, alternative, simple, reliable and non-invasive tests are needed to assess the stage of fibrosis. Several non-invasive direct and indirect serum markers able to predict the presence of significant fibrosis or cirrhosis in patients with chronic liver disease with considerable accuracy have been reported. However, since most of these markers require complicated calculations, clinical application is difficult. Transient elastography (FibroScan) is a new method for the evaluation of liver stiffness. It is based on changes in tissue elasticity induced by hepatic fibrosis. The authors noted that based on accumulating clinical data, clinical applications of elastography will increase in the near future.

Sporea and colleagues (2008) stated that evaluation of liver fibrosis can be performed by FibroTest, elastography (FibroScan), and by LB, which is considered to be the "gold standard". At the present, there are 3 techniques for performing LB: percutaneous, transjugular, and laparoscopic. The percutaneous LB can be performed blind, ultrasound (US)-guided or US-assisted. There 2 two main categories of specialists who perform LB: gastroenterologists (hepatologists) and radiologists, and the specialty of the individual who performs the LB determines if the LB is performed under ultrasound guidance or not. There are 2 types of biopsy needles used for LB: cutting needles (Tru-Cut, Vim-Silverman) and suction needles (Menghini, Klatzkin, Jamshidi). The rate of major complications after percutaneous LB ranges from 0.09 % to 2.3 %, but the echo-guided percutaneous liver biopsy is a safe method for the diagnosis of chronic diffuse hepatitis (cost-effective as compared to blind biopsy) and the rate of complications seems to be related to the experience of the physician and the type of the needle used (Menghini type needle seems to be safer). The authors stated that maybe in a few years non-invasive markers of fibrosis will be used,

but at this time, most authorities in the field consider LB to be useful and necessary for the evaluation of chronic hepatopathies, despite the fact that it is not a perfect test.

Castera and associates (2008) stated that transient elastography (TE, FibroScan) is a novel non-invasive method that has been proposed for the assessment of hepatic fibrosis in patients with chronic liver diseases, by measuring liver stiffness. It is a rapid and user-friendly technique that can be easily performed at the bedside or in the outpatient clinic with immediate results and good reproducibility. Limitations include failure in approximately 5 % of cases, mainly in obese patients. So far, TE has been mostly validated in chronic hepatitis C, with diagnostic performance equivalent to that of serum markers for the diagnosis of significant fibrosis. Combining TE with serum markers increases diagnostic accuracy and as a result, LB could be avoided for initial assessment in most patients with chronic hepatitis C. These investigators stated that this strategy warrants further evaluation in other etiological types of chronic liver diseases. Transient elastography appears to be an excellent tool for early detection of cirrhosis and may have prognostic value in this setting. As TE has excellent patient acceptance it could be useful for monitoring fibrosis progression and regression in the individual case, but more data are awaited for this application. Guidelines are needed for the use of TE in clinical practice.

In a meta-analysis, Friedrich-Rust et al (2008) examined the performance of TE for the staging of liver fibrosis. Literature data bases and international conference abstracts were searched. Inclusion criteria were as follows: evaluation of TE, LB as reference, and assessment of the area under the receiver operating characteristic curve (AUROC). The meta-analysis was performed using the random-effects model for the AUROC, summary receiver operating curve techniques, as well as meta-regression approaches. A total of 50 studies were included in the analysis. The mean AUROC for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis were 0.84 (95 % CI: 0.82 to 0.86), 0.89 (95 % CI: 0.88 to 0.91), and 0.94 (95 % CI: 0.93 to 0.95), respectively. For the diagnosis of significant fibrosis a significant reduction of heterogeneity of the AUROC was found when differentiating between the underlying liver diseases ($p < 0.001$). Other factors influencing the AUROC were the scoring system used and the country in which the study was performed. Age, body mass index, and biopsy quality did not have a significant effect on the AUROC. The authors concluded that TE can be performed with excellent diagnostic accuracy and independent of the underlying liver disease for the diagnosis of cirrhosis. However, for the diagnosis of significant fibrosis, a high variation of the AUROC was found that is dependent on the underlying liver disease. A critique of this meta-analysis by the Centre for Reviews and Dissemination (CRD, 2010) stated that, although sensitivity and specificity data appeared to have been available for many of the studies included in this meta-analysis, the meta-analysis focused on pooled estimates of AUROC. The CRD noted that use of this measure of overall accuracy results in a loss of clinically important information about test performance; it was unclear how many inaccurate test results were due to false positive and how many to false negatives. The CRD critique stated that generation of summary receiver operating characteristic (SROC) curves using a bivariate of hierarchical model may have been more appropriate to this data set; the CRD noted that such models allow generation of summary estimates of sensitivity and specificity as well as potential to assess the significance of sources of heterogeneity. The CRD concluded that these limitations in the analysis mean that the conclusions of this meta-analysis should be interpreted with caution.

Abenavoli et al (2008) noted that in clinical practice there are currently 3 methods for the evaluation of liver fibrosis. First, LB is still considered as the "gold standard" method. Second, serological markers and their mathematical combination were suggested in the last years as an alternative to LB. Third, TE was proposed recently. This technique (TE) is based on the progression speed of an elastic shear wave within the liver. The authors concluded that currently, there are just a few studies capable of evaluating the effectiveness of TE in evaluating chronic liver diseases, mainly in patients infected with HCV. Its application must also be studied in the monitoring of patients suffering from chronic HCV infection and subjected to treatments that can modify their degree of liver fibrosis.

Muñoz et al (2009) evaluated the correlation between values of Fibroscan, liver biopsy, and clinical data among HCV-positive renal transplant patients. A total of 24 HCV/RNA-positive patients with a previous liver biopsy were selected to undergo Fibroscan (transient elastography) and a clinical

evaluation of liver function. Fibroscan values were expressed in kilopascals (kPa). As 2 patients were eliminated due to obesity or ascites, these investigators analyzed 22 patients. Thirteen patients (59 %) with fibrosis F0-F1 (METAVIR score) by biopsy and normal liver function showed a mean Fibroscan score of 5.2 kPa (range of 2.3 to 6.8 kPa). Three patients (13.6 %) exhibited F2 by biopsy and normal liver function with a mean Fibroscan score of 8.2 kPa (range of 7.3 to 8.9 kPa). Three patients (13.6 %) with F3 by biopsy and abnormal liver function showed a high mean Fibroscan score of 10.9 kPa (range of 10.5 to 11.6 kPa). The last 3 patients (13.6 %) with F4 (cirrhosis) by biopsy and abnormal clinical data showed the highest mean Fibroscan value of 14.2 kPa (range of 8.9 to 18 kPa). The authors concluded that among renal transplant patients with HCV, the values of Fibroscan seem to correlate with the degree of fibrosis by biopsy and with clinical liver function. Thus, Fibroscan may be useful to follow patients with LD. However, these results should be analyzed with caution due to the small number of cases and retrospective nature of the study.

Andersen and colleagues (2009) stated that liver biopsy is considered the "golden standard" for assessment of hepatic fibrosis. However, the procedure has limitations because of inconvenience and rare but serious complications as bleeding. Furthermore, sampling errors are frequent, and inter-observer variability often poses problems. Recently, transient elastography has been developed to assess fibrosis. The device measures liver elasticity, which correlates well with the degree of fibrosis. Studies have shown that transient elastography is more accurate in diagnosing cirrhosis than minor-to-moderate fibrosis. Most of the studies have been conducted on patients with chronic hepatitis but a few studies have also covered fibrosis and cirrhosis due to other etiologies, and they also demonstrated the high sensitivity and specificity. The authors concluded that transient elastography for assessment of fibrosis may turn out to be a valuable diagnostic procedure and follow-up of patients with chronic liver diseases.

Breton et al (2009) examined the feasibility and reliability of liver stiffness measurement in children with liver diseases. Liver stiffness measurements were performed on 72 children, from 4 to 18 years of age, with potential hepatic fibrosis disease. The clinical, biological, ultrasonographic, and endoscopic parameters were noted to identify children with portal hypertension syndrome. The APRI (ASAT-to-platelet ratio index) test was calculated according to the standard formula. An APRI test score higher than 1.5 indicates significant hepatic fibrosis. METAVIR scoring from 14 liver biopsies was compared to the liver stiffness using the Kappa statistic. A total of 28 patients had viral hepatitis, 20 cystic fibrosis, 16 chronic liver cholestasis, 5 autoimmune hepatitis, and 3 patients had liver fibrosis with uncertain etiology. FibroScan measurements were available in all children. There was good agreement between FibroScan and pathological studies (weighted kappa = 0.814). Only 9 children had portal hypertension syndrome with an average measurement of liver stiffness significantly higher than children without portal hypertension (26.5kPa versus 6.4kPa; $p < 0.01$). The APRI test for 6 out of 9 patients scored higher than 1.5. The authors concluded that these findings indicate that liver stiffness measurement is feasible in children and seems to be related to liver fibrosis. They stated that larger prospective studies are needed to validate this FibroScan method.

In a meta-analysis of transient elastography for the detection of hepatic fibrosis, Stebbing et al (2010) evaluated its use in comparison with liver biopsy. Studies from the literature were analyzed with a median liver stiffness value in kilopascal given for fibrosis stages according to histopathologic findings on biopsy and best discriminant cut-off levels in kilopascals for significant fibrosis (greater than or equal to F2) and cirrhosis (F4). A total of 22 studies were selected comprising 4,430 patients; chronic hepatitis C infection was the most common etiology of fibrosis. The pooled estimates for significant fibrosis (greater than or equal to F2) measured 7.71 kPa (LSM cut-off value) with a sensitivity of 71.9 % [95 % CI: 71.4 % to 72.4 %] and specificity of 82.4 % (95 % CI: 81.9 % to 82.9 %), whereas for cirrhosis (F4) the results showed a cut-off of 15.08 kPa with a sensitivity of 84.45 % (95 % CI: 84.2 % to 84.7 %) and specificity of 94.69 % (95 % CI: 94.3% to 95 %). The authors concluded that further evaluation of transient elastography to assess LSM is needed in prospective studies to potentially increase the sensitivity and establish its clinical utility.

Myers (2009) noted that non-alcoholic fatty liver disease (NAFLD) is the most common cause of

chronic liver disease, affecting about 30 % of Western populations and a frequent indication for liver transplantation. The histological spectrum of NAFLD includes simple steatosis, which has a benign prognosis, and non-alcoholic steatohepatitis, a more aggressive form of liver injury that may progress to cirrhosis and its complications. At present, the only widely accepted means of differentiating these lesions, including the severity of hepatic fibrosis, is liver biopsy. However, due to the invasiveness of this procedure, the rising prevalence of NAFLD, and the expected availability of effective therapies for this condition, the identification of non-invasive tools for the diagnosis and staging of NAFLD has emerged as a major clinical and research priority. The author summarized important advances in this field during the past decade, including the development of biomarkers of hepatic fibrosis, apoptosis, and inflammation; novel imaging techniques such as transient elastography; and high-throughput technologies including proteomics and genomics. Future studies must focus on the development of accurate, inexpensive, and reliable tools that can differentiate the major histologic determinants of NAFLD; are responsive to changes in NAFLD severity due to therapeutic intervention and time; and have prognostic significance. Until such tools are developed, liver biopsy remains an important tool in the assessment of patients with NAFLD.

Myers and co-workers (2010) determined the feasibility and performance of TE in a North American cohort of patients with chronic liver disease. Liver stiffness measurements were obtained using TE in 260 patients with chronic hepatitis B or C, or NAFLD from 4 Canadian hepatology centers. The accuracy of TE compared with liver biopsy for the prediction of significant fibrosis (Metavir fibrosis score of F2 or greater), bridging fibrosis (Metavir fibrosis score of F3 or greater) and cirrhosis (Metavir fibrosis score of F4) was assessed using area under ROC curves (AUROCs), and compared with the aspartate aminotransferase-to-platelet ratio index. The influence of ALT levels and other factors on liver stiffness was determined using linear regression analyses. Failure of TE occurred in 2.7 % of patients, while liver biopsies were inadequate for staging in 0.8 %. Among the remaining 251 patients, the AUROCs of TE for Metavir fibrosis scores of F2 and F3 or greater, and F4 were 0.74 (95 % CI: 0.68 to 0.80), 0.89 (95 % CI: 0.84 to 0.94), and 0.94 (95 % CI: 0.90 to 0.97), respectively. Liver stiffness measurement was more accurate than the aminotransferase-to-platelet ratio index for bridging fibrosis (AUROC 0.78) and cirrhosis (AUROC 0.88), but not significant fibrosis (AUROC 0.76). At a cut-off of 11.1 kPa, the sensitivity, specificity, and positive and negative predictive values for cirrhosis (prevalence 11 %) were 96 %, 81 %, 39 % and 99 %, respectively. For significant fibrosis (prevalence 53 %), a cut-off of 7.7 kPa was 68 % sensitive and 69 % specific, and had a positive predictive value of 70 % and a negative predictive value of 65 %. Liver stiffness was independently associated with ALT, body mass index and steatosis. The optimal LSM cut-offs for cirrhosis were 11.1 kPa and 11.5 kPa in patients with ALT levels lower than 100 U/L and 100 U/L or greater, respectively. For fibrosis scores of F2 or greater, these figures were 7.0 kPa and 8.6 kPa, respectively. The authors concluded that the major role of TE is the exclusion of bridging fibrosis and cirrhosis. However, TE can not replace biopsy for the diagnosis of significant fibrosis. Because liver stiffness may be influenced by significant ALT elevation, body mass index and/or steatosis, tailored liver stiffness cut-offs may be necessary to account for these factors.

Cholongitas et al (2010) systematically reviewed the literature regarding non-invasive tests (NIT) following liver transplantation. These investigators identified 14 studies evaluating NIT based on serum markers and/or liver imaging techniques: 10 studies assessed NIT in recipients with recurrent HCV infection for fibrosis and 4 studies evaluated predictors of progression of fibrosis in recurrent HCV. Transient Elastography had good discrimination for significant fibrosis (median AUROC: 0.88). Among the serum NIT, APRI had good performance (median AUROC: 0.75). Trnasient elastography performed better than serum (direct and indirect) NIT for significant fibrosis with median AUROC 0.88 (versus 0.66, $p < 0.001$), median sensitivity 0.86 (versus 0.56, $p = 0.002$), median NPV 0.90 (versus 0.74, $p = 0.05$) and median PPV 0.80 (versus 0.63, $p = 0.02$). Transient elastography compared to indirect serum NIT, had better performance, but was not superior to APRI score. Finally, direct, compared to indirect NIT, were not significantly different except for specificity: median: 0.83 versus 0.69, respectively, $p = 0.04$. The authors concluded that NIT could become an important tool in clinical management of liver transplant recipients, but whether they can improve clinical practice needs further evidence. Their optimal combination with

liver biopsy and assessment of collagen content requires investigation.

Stebing et al (2010) performed a meta-analysis to further assess the use of TE in comparison with liver biopsy. Studies from the literature were analyzed with a median liver stiffness value in kilopascal given for fibrosis stages according to histopathologic findings on biopsy and best discriminant cutoff levels in kilopascals for significant fibrosis (greater than or equal to F2) and cirrhosis (F4). A total of 22 studies were selected comprising 4,430 patients; chronic hepatitis C infection was the most common etiology of fibrosis. The pooled estimates for significant fibrosis greater than or equal to F2) measured 7.71 kPa (LSM cut-off value) with a sensitivity of 71.9 % [95 % CI: 71.4 % to 72.4 %] and specificity of 82.4 % (95 % CI: 81.9 % to 82.9%), whereas for cirrhosis (F4) the results showed a cut-off of 15.08 kPa with a sensitivity of 84.45 % (95 % CI: 84.2 % to 84.7 %) and specificity of 94.69 % (95 % CI: 94.3 % to 95 %). The authors concluded that further evaluation of TE to assess LSM is needed in prospective studies to potentially increase the sensitivity and establish its clinical utility.

Thabut et al (2011) noted that severe portal hypertension is responsible for complications and death. Although measurement of the hepatic venous pressure gradient is the most accurate method for evaluating the presence and severity of portal hypertension, this technique is considered invasive and is not routinely performed in all centers. Several non-invasive techniques have been proposed to measure portal hypertension. Certain methods evaluate elements related to the pathogenesis of portal hypertension through the measurement of hyperkinetic syndrome, or they examine the development of hepatic fibrosis through the measurement of increased intra-hepatic vascular resistance. Other methods assess the clinical consequences of portal hypertension, such as the presence of esophageal varices or the development of porto-systemic shunts. Methods evaluating increased hepatic vascular resistance are fairly accurate and primarily involve the detection of hepatic fibrosis by serum markers and transient elastography. The radiological assessment of hyperkinetic syndrome probably has value but is still under investigation. The assessment of severe portal hypertension by the presence of varices may be performed with simple tools such as biological assays, computed tomography, and esophageal capsules. More sophisticated procedures seem promising but are still under development. Screening tools for large populations must be simple, whereas more complicated procedures could help in the follow-up of already diagnosed patients. Although most of these non-invasive methods effectively identify severe portal hypertension, methods for diagnosing moderate portal hypertension need to be developed; this shows that further investigation is needed in this field.

Tsochatzis et al (2011) studied the performance of elastography for diagnosis of fibrosis using meta-analysis. MEDLINE, EMBASE, SCI, Cochrane Library, conference abstracts books, and article references were searched. These investigators included studies using biopsy as a reference standard, with the data necessary to calculate the true- and false-positive, true- and false-negative diagnostic results of elastography for a fibrosis stage, and with a 3-month maximum interval between tests. The quality of the studies was rated with the QUADAS tool. These researchers identified 40 eligible studies. Summary sensitivity and specificity was 0.79 (95 % CI: 0.74 to 0.82) and 0.78 (95 % CI: 0.72 to 0.83) for F2 stage and 0.83 (95 % CI: 0.79 to 0.86) and 0.89 (95 % CI: 0.87 to 0.91) for cirrhosis. After an elastography result at/over the threshold value for F2 or cirrhosis ("positive" result), the corresponding post-test probability for their presence (if pre-test probability was 50 %) was 78 %, and 88 %, respectively, while, if values were below these thresholds ("negative" result), the post-test probability was 21 % and 16 %, respectively. No optimal stiffness cut-offs for individual fibrosis stages were validated in independent cohorts and cut-offs had a wide range and overlap within and between stages. The authors concluded that elastography theoretically has good sensitivity and specificity for cirrhosis (and less for lesser degrees of fibrosis); however, it should be cautiously applied to everyday clinical practice because there is no validation of the stiffness cut-offs for the various stages. They stated that such validation is required before elastography is considered sufficiently accurate for non-invasive staging of fibrosis.

An UpToDate review on "Epidemiology, clinical features, and diagnosis of nonalcoholic steatohepatitis" (Sheth and Chopra, 2012) states that "A potentially useful non-invasive method for excluding advanced fibrosis is measurement of liver stiffness with transient elastography.

However, the approach is not widely available and has not been extensively studied in NASH".

Poca et al (2011) stated that prognostic markers of compensated cirrhosis should mainly investigate factors involved with progression to decompensation because death in cirrhosis is related with decompensation. Portal hypertension plays a crucial role in the pathophysiology of most complications of cirrhosis. Accordingly, hepatic venous pressure gradient (HVPG) monitoring has strong prognostic value. An HVPG of greater than or equal to 10 mm Hg determines a significantly higher risk of developing decompensation. Esophageal varices also can develop when the HVPG is greate than or equal to 10 mm Hg, although an HVPG greater than or equal to 12 mm Hg is required for variceal bleeding to occur. Monitoring the changes induced by the treatment of portal hypertension on HVPG, provides strong prognostic information. In compensated cirrhosis hemodynamic response is appropriate when the HVPG decreased to less than 10 mm Hg or by less than 10 % from baseline, because the incidence of complications such as bleeding or ascites significantly decrease when these targets are achieved. Whether serum markers, such as the FibroTest, may be valuable to predict decompensation should be established. Transient elastography is a promising technique that has shown an excellent accuracy to detect severe portal hypertension. However, whether it can adequately determine clinically significant portal hypertension, and risk of developing varices and decompensation, should be established. Magnetic resonance elastography is also promising.

Pesce and co-workers (2012) noted that portal hypertension has been reported as a negative prognostic factor and a relative contraindication for liver resection. These researchers considered a possible role of fibrosis evaluation by transient elastography (FibroScan) and its correlation with portal hypertension in patients with cirrhosis, and discussed the use of this technique in planning therapeutic options in patients with hepato-cellular carcinoma (HCC). A total of 77 patients with cirrhosis, 42 (54.5 %) of whom had HCC, were enrolled in this study during 2009 to 2011. The group included 46 (59.7 %) men. The mean age of the sample was 65.2 years. The principle etiology of disease was HCV-related cirrhosis (66.2 %). Liver function was assessed according to Child-Pugh classification. In all patients liver stiffness (LS) was measured using FibroScan. The presence of portal hypertension was indirectly defined as: (i) esophageal varices detectable on endoscopy; (ii) splenomegaly (increased diameter of the spleen to greater than or equal to 12 cm), or (iii) a platelet count of less than 100,000 platelets/mm(3). Median LS in all patients was 27.9 kPa. Portal hypertension was recorded as present in 37 patients (48.1 %) and absent in 40 patients (51.9 %). Median LS values in HCC patients with and without portal hypertension were 29.1 kPa and 19.6 kPa, respectively ($r = 0.26$, $p < 0.04$). Liver stiffness was used to implement the Barcelona Clinic Liver Cancer algorithm in decisions about treatment. The authors concluded that the evaluation of liver fibrosis by transient elastography may be useful in the follow-up of patients with cirrhosis and a direct correlation with portal hypertension may aid in the evaluation of surgical risk in patients with HCC and in the choice of alternative therapies.

In a multi-center study, Barbero-Villares et al (2012) evaluated the presence of significant liver fibrosis by transient elastography (FibroScan) in inflammatory bowel disease (IBD) patients treated with methotrexate. Cross-sectional study including IBD patients treated with methotrexate from different hospitals. Clinical and analytical data, duration of treatment, and cumulative dose of methotrexate were obtained. Liver stiffness was assessed by FibroScan. The cut-off value for significant liver fibrosis (according to METAVIR) was F greater than or equal to 2: 7.1 kPa. In the study, 46 patients were included, 30 women (65 %), with a mean age of 43 +/- 10 years; 31 patients had Crohn's disease (67.4 %), 13 ulcerative colitis (28.3 %), and 2 indeterminate colitis (4.3 %). The mean cumulative dose of methotrexate was 1,242 +/- 1,349 mg, with a mean treatment duration of 21 +/- 24 months. The mean value of liver stiffness was 4.7 +/- 6.9 kPa. There were 35 patients (76.1 %) with F01, 8 patients (17.4 %) with F = 2, and 3 patients with F greater than or equal to 3 (6.5 %). There were no differences in liver stiffness depending on sex, age, type of IBD, or cumulative dose of methotrexate. The authors concluded that (i) development of advanced liver fibrosis in IBD patients treated with methotrexate is exceptional, (ii) there were no differences in liver stiffness depending on the type of IBD or the cumulative dose of methotrexate, and (iii) FibroScan may be potentially useful for evaluation and follow-up of liver fibrosis in methotrexate-treated patients.

Lee and colleagues (2013) evaluated and compared the ability of serum HA and human cartilage glycoprotein-39 (YKL-40) values, as well as TE findings, to predict advanced hepatic fibrosis in a cohort from a single pediatric center. Subjects who underwent liver biopsy analysis within 12 months before enrollment were eligible for this prospective study. Hyaluronic acid and YKL-40 measurements were obtained within 1 month of TE. A METAVIR score of F3 or F4 was considered to indicate advanced fibrosis. A total of 128 patients (51 % males) aged 1.4 months to 27.6 years (22 % aged less than 2 years) were enrolled. Thirty-one subjects had data on HA and YKL-40; and 97 subjects had data on both blood tests and TE. For the prediction of advanced fibrosis, the AUC values were 0.83 for TE, 0.72 for HA, and 0.52 for YKL-40. The AUC of 0.83 for TE was statistically significantly greater than the AUCs for HA ($p = 0.03$) and YKL-40 ($p < 0.0001$). Optimal cut-off points for predicting F3-F4 fibrosis were 8.6 kPa for TE ($p < 0.0001$), 43 ng/ml for HA ($p < 0.0001$), and 26.2 ng/ml for YKL-40 ($p = 0.85$). The combination of TE and HA was not better than TE alone for predicting advanced fibrosis ($p = 0.15$). The authors concluded that in this study, which evaluated TE, HA, and YKL-40 to predict liver fibrosis in children in the United States, YKL-40 had no predictive value and TE was superior to HA, but the addition of HA did not improve the performance of TE. They stated that these findings suggested that TE and HA may be useful non-invasive tools for assessing liver fibrosis in children.

Guidelines on the management of hepatiis C from the American Association for the Study of LIver Disease (2014) state that "[n]on-invasive methods frequently used to estimate liver disease severity include a liver-directed physical exam (normal in most patients), routine blood tests (eg, serum alanine transaminase, albumin, bilirubin, international normalized ratio levels, and complete cell blood counts with platelets), serum fibrosis marker panels, liver imaging (eg, ultrasound, computed tomography scan), and liver elastography. . . . Liver elastography can provide instant information regarding liver stiffness at the point-of-care but can only reliably distinguish cirrhosis from non-cirrhosis."

CPT Codes / HCPCS Codes / ICD-9 Codes

Transient elastography (e.g., FibroScan):

CPT codes covered if selection criteria are met:

0346T

Serum marker tests:

CPT codes not covered for indications listed in the CPB:

83520

83883

Other CPT codes related to the CPB:

47000

47001

47100

82977

ICD-9 codes covered if selection criteria are met:

070.44	Chronic hepatitis C with hepatic coma
070.54	Chronic hepatitis C without mention of hepatic coma
571.0 - 571.9	Chronic liver disease and cirrhosis
V42.7	Organ or tissue replaced by transplant, liver

ICD-9 codes not covered for indications listed in the CPB:

070.0 - 070.43, 070.49 - 070.53, 070.59 - 070.9	Viral hepatitis
155.0	Malignant neoplasm of liver, primary [hepatocellular carcinoma]

The above policy is based on the following references:

Serum Markers:

1. National Institutes of Health (NIH). Management of hepatitis C: 2002. Rockville, MD: NIH; August 26, 2002.
2. Gebo K, Jenckes M, Chander G, et al. Management of chronic hepatitis C. Evidence Report/Technology Assessment 60. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); 2002.
3. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: A prospective study. Lancet. 2001;357(9262):1069-1075.
4. Munteanu M, Messous D, Thabut D, et al. Intra-individual fasting versus postprandial variation of biochemical markers of liver fibrosis (FibroTest) and activity (ActiTest). Comp Hepatol. 2004;3:3.
5. Poynard T, Munteanu M, Imbert-Bismut F, et al. Prospective analysis of discordant results between biochemical markers and biopsy in patientswith chronic hepatitis C. Clin Chem. 2004;50(8):1344-1355.
6. Callewaert N, Van Vlierberghe H, Van Hecke A, et al. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. Nat Med. 2004;10(4):429-434.
7. Imbert-Bismut F, Messous D, Thibaut V, et al. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and reference ranges in healthy blood donors. Clin Chem Lab Med. 2004;42(3):323-333.
8. Le Calvez S, Thabut D, Messous D, et al. The predictive value of Fibrotest vs. APRI for the diagnosis of fibrosis in chronic hepatitis C. Hepatology. 2004;39(3):862-863.
9. Myers RP, Tainturier MH, Ratziu V, et al. Prediction of liver histological lesions with biochemical markers in patients with chronic hepatitis B. J Hepatol. 2003;39(2):222-230.
10. Rossi E, Adams L, Prins A, et al. Validation of the FibroTest biochemical markers score in assessing liver fibrosis in hepatitis C patients. Clin Chem. 2003;49(3):450-454.
11. Halfon P, Imbert-Bismut F, Messous D, et al. A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (FibroTest) and activity (ActiTest) in patients with chronic liver disease. Comp Hepatol. 2002;1(1):3.
12. National Horizon Scanning Centre (NHSC). FibroTest-ActiTest, a diagnostic test for liver fibrosis in patients with hepatitis C -- horizon scanning review. Birmingham, UK: NHSC; 2004.
13. Mundy L, Merlin T, Parrella A. FibroTest-ActiTest: A diagnostic test for liver fibrosis in patients with hepatitis C. Horizon Scanning Prioritising Summary - Volume 6. Adelaide, SA: Adelaide Health Technology Assessment (AHTA) on behalf of National Horizon Scanning Unit (HealthPACT and MSAC); 2004.
14. National Horizon Scanning Centre (NHSC). FibroTest-ActiTest for diagnosis and monitoring of fibrosis in chronic liver conditions -- horizon scanning review. Birmingham, UK: NHSC; 2005.
15. Marrero JA, Lok AS. Newer markers for hepatocellular carcinoma. Gastroenterology. 2004;127(5 Suppl 1):S113-S119.
16. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. AASLD Practice Guidelines. Hepatol. 2004;39(4):1147-1171.
17. Suzuki A, Mendes F, Lindor K. Diagnostic model of esophageal varices in alcoholic liver disease. Eur J Gastroenterol Hepatol. 2005;17(3):307-309.
18. Parrella A, Mundy L, Hiller J. FibroTest-ActiTest: A diagnostic test for liver fibrosis in patients with hepatitis C (12 month update). Horizon Scanning Prioritising Summary - Volume 11. Adelaide, SA: Adelaide Health Technology Assessment (AHTA) on behalf of

National Horizon Scanning Unit (HealthPACT and MSAC); 2005.
19. National Institutes of Health (NIH), National Institute on Alcohol Abuse and Alcoholism (NIAAA). Helping patients who drink too much, A Clinician's Guide, 2005 Ed. Bethesda, MD: NIH; 2005. Available at: http://pubs.niaaa.nih.gov/publications/Practitioner/CliniciansGuide2005/clinicians_guide.htm. Accessed October 3, 2006.
20. Nourani S, Pockros PJ. How should hepatitis C be managed in patients aged 65 years and older? Nature Clin Practice Gastroenterol Hepatol. 2007;4:22-23. Available at: http://www.nature.com/ncpgasthep/journal/v4/n1/full/ncpgasthep0682.html. Accessed eptember 28, 2007.
21. Rossi E, Adams LA, Bulsara M, Jeffrey GP. Assessing liver fibrosis with serum marker models. Clin Biochem Rev. 2007; 28(1): 3–10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1904421. Accessed September 28, 2007.
22. Shaheen AA, Myers RP. Systematic review and meta-analysis of the diagnostic accuracy of fibrosis marker panels in patients with HIV/hepatitis C coinfection. HIV Clin Trials. 2008;9(1):43-51.
23. Wilt TJ, Shamliyan T, Shaukat A, et al. Management of chronic hepatitis B. Prepared by the Minnesota Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ) under Contract No. 290-02-0009. AHRQ Publication No. 09-E002. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); October 2008.
24. Smith JO, Sterling RK. Systematic review: non-invasive methods of fibrosis analysis in chronic hepatitis C. Aliment Pharmacol Ther. 2009;30(6):557-576.
25. Carlson JJ, Kowdley KV, Sullivan SD, et al. An evaluation of the potential cost-effectiveness of non-invasive testing strategies in the diagnosis of significant liver fibrosis. J Gastroenterol Hepatol. 2009;24(5):786-791.
26. Adams LA. Biomarkers of liver fibrosis. J Gastroenterol Hepatol. 2011;26(5):802-809.
27. Sebastiani G, Castera L, Halfon P, et al. The impact of liver disease aetiology and the stages of hepatic fibrosis on the performance of non-invasive fibrosis biomarkers: An international study of 2411 cases. Aliment Pharmacol Ther. 2011;34(10):1202-1216.
28. Adams LA, Bulsara M. Rossi E, et al. Hepascore an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem. 2005;51(10):1867-1873.
29. Stevenson M, Lloyd-Jones M, Morgan MY, Wong R. Non-invasive diagnostic assessment tools for the detection of liver fibrosis in patients with suspected alcohol-related liver disease: A systematic review and economic evaluation. Health Technol Assess. 2012;16(4):1-174.
30. Sebastiani G, Alberti A. How far is noninvasive assessment of liver fibrosis from replacing liver biopsy in hepatitis C? J Viral Hepat. 2012;19 Suppl 1:18-32.
31. Usluer G, Erben N, Aykin N, et al; Study Group. Comparison of non-invasive fibrosis markers and classical liver biopsy in chronic hepatitis C. Eur J Clin Microbiol Infect Dis. 2012;31(8):1873-1878.
32. Bhogal H, Sterling RK. Staging of liver disease: Which option is right for my patient? Infect Dis Clin North Am. 2012;26(4):849-861.
33. Chladek J, Simkova M, Vanecckova J, et al. Assessment of methotrexate hepatotoxicity in psoriasis patients: A prospective evaluation of four serum fibrosis markers. J Eur Acad Dermatol Venereol. 2012 Aug 7. [Epub ahead of print]
34. Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: A systematic review. Ann Intern Med. 2013;158(11):807-820.
35. Rossi E, Adams LA, Ching HL, et al. High biological variation of serum hyaluronic acid and Hepascore, a biochemical marker model for the prediction of liver fibrosis. Clin Chem Lab Med. 2013;51(5):1107-1114.
36. Grattagliano I, Ubaldi E, Napoli L, et al. Utility of noninvasive methods for the characterization of nonalcoholic liver steatosis in the family practice. The "VARES" Italian multicenter study. Ann Hepatol. 2013;12(1):70-77.Curry MP, Afdhal NH. Tests used for the noninvasive assessment of hepatic fibrosis. Last reviewed June 2013. UpToDate Inc., Waltham, MA.

Transient Elastography (FibroScan):

1. Comite d'Evaluation et de Diffusion des Innovations Technologiques (CEDIT). Quantification of hepatic fibrosis by transient elastography (Fibroscan (R)) - preliminary report [summary]. Ref. 04.10/AV1/04. Paris, France: CEDIT; 2004. Available at: http://cedit.aphp.fr/english/index_present.html. Accessed October 5, 2006.
2. de Ledinghen V, Douvin C, Kettaneh A, et al. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. J Acquir Immune Defic Syndr. 2006;41(2):175-179.
3. Foucher J, Chanteloup E, Vergniol J, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): A prospective study. Gut. 2006;55(3):403-408.
4. Corpechot C, El Naggar A, Poujol-Robert A, et al. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. Hepatology. 2006;43(5):1118-1124.
5. Adelaide Health Technology Assessment on behalf of National Horizon Scanning Unit (HealthPACT and MSAC). MR and transient elastography for the non-invasive assessment of liver fibrosis; horizon scanning prioritising summary - volume 14. Adelaide, SA: Adelaide Health Technology Assessment (AHTA) on behalf of National Horizon Scanning Unit (HealthPACT and MSAC); 2006.
6. Murtagh J, Foster V. Transient elastography (FibroScan) for non-invasive assessment of liver fibrosis. Issues in Emerging Health Technologies: Issue 90. Ottawa, ON: Canadian Agency for Drugs and Technologies in Health (CADTH); September 2006. Available at: http://www.cadth.ca/media/pdf/442_fibroscan_cetap_e.pdf. Accessed October 3, 2006.
7. de Franchis R, Dell'Era A. Non-invasive diagnosis of cirrhosis and the natural history of its complications. Best Pract Res Clin Gastroenterol. 2007;21(1):3-18.
8. Berrutti M, Ciancio A, Smedile A, et al. Assessment of liver fibrosis in the clinical setting: Something is changing? Minerva Gastroenterol Dietol. 2007;53(1):111-114.
9. Haute Autorite de Sante (HAS)/French National Authority for Health. Assessment of non-invasive techniques to measure liver fibrosis in chronic viral hepatitis C and B. Saint-Denis La Plaine, France: HAS; 2007.
10. de Lédinghen V, Le Bail B, Rebouissoux L, et al. Liver stiffness measurement in children using FibroScan: Feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. J Pediatr Gastroenterol Nutr. 2007;45(4):443-450.
11. Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: A systematic review of diagnostic test accuracy. Am J Gastroenterol. 2007;102(11):2589-2600.
12. Sagir A, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatology. 2008;47(2):592-595.
13. Han KH, Yoon KT. New diagnostic method for liver fibrosis and cirrhosis. Intervirology. 2008;51 Suppl 1:11-16.
14. Sporea I, Popescu A, Sirli R. Why, who and how should perform liver biopsy in chronic liver diseases. World J Gastroenterol. 2008;14(21):3396-3402.
15. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol. 2008;48(5):835-847.
16. Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient elastography for the staging of liver fibrosis: A meta-analysis. Gastroenterology. 2008;134(4):960-974.
17. Centre for Reviews and Dissemination (CRD). Performance of transient elastography for the staging of liver fibrosis; A meta-analysis. Database of Abstracts of Systematic Reviews (DARE). York, UK: University of York; 2010.
18. Abenavoli L, Addolorato G, Riccardi L, et al. Elastography assessment in patients with chronic HCV infection. Int J Clin Pract. 2008;62(7):1108-1112.
19. Darus PN. FibroScan. Technology Review. Putrajaya, Malaysia: Health Technology Assessment Unit, Medical Development Division, Ministry of Health Malaysia (MaHTAS); June 2008.
20. National Horizon Scanning Centre. Transient elastography (FibroScan) for evaluating liver fibrosis. Horizon Scanning Technology Briefing. Birmingham, UK: National Horizon Scanning Centre (NHSC); 2008.
21. Myers RP. Noninvasive diagnosis of nonalcoholic fatty liver disease. Ann Hepatol. 2009;8 Suppl 1:S25-S33.
22. Vergniol J, Foucher J, Castéra L, et al. Changes of non-invasive markers and FibroScan

values during HCV treatment. J Viral Hepat. 2009;16(2):132-140.
23. Castera L. Transient elastography and other noninvasive tests to assess hepatic fibrosis in patients with viral hepatitis. J Viral Hepat. 2009;16(5):300-314.
24. Sánchez Antolin G, Garcia Pajares F, Vallecillo MA, et al. FibroScan evaluation of liver fibrosis in liver transplantation. Transplant Proc. 2009;41(3):1044-1046.
25. Muñoz R, Ramírez E, Fernandez I, et al. Correlation between fibroscan, liver biopsy, and clinical liver function in patients with hepatitis C virus infection after renal transplantation. Transplant Proc. 2009;41(6):2425-2426.
26. Andersen ES, Christensen PB, Weis N. Transient elastography for liver fibrosis diagnosis. Eur J Intern Med. 2009;20(4):339-342.
27. Breton E, Bridoux-Henno L, Guyader D, et al. Value of transient elastography in noninvasive assessment in children's hepatic fibrosis. Arch Pediatr. 2009;16(7):1005-1010.
28. Zhang YG, Wang BE, Wang TL, Ou XJ. Assessment of hepatic fibrosis by transient elastography in patients with chronic hepatitis B. Pathol Int. 2010;60(4):284-290.
29. Stebbing J, Farouk L, Panos G, et al. A meta-analysis of transient elastography for the detection of hepatic fibrosis. J Clin Gastroenterol. 2010;44(3):214-219.
30. Degos F, Perez P, Roche B, et al. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: A multicenter prospective study (the FIBROSTIC study). J Hepatol. 2010;53(6):1013-1021.
31. Myers RP, Elkashab M, Ma M, Transient elastography for the noninvasive assessment of liver fibrosis: A multicentre Canadian study. Can J Gastroenterol. 2010;24(11):661-670.
32. Cholongitas E, Tsochatzis E, Goulis J, Burroughs AK. Noninvasive tests for evaluation of fibrosis in HCV recurrence after liver transplantation: A systematic review. Transpl Int. 2010;23(9):861-870.
33. Thabut D, Moreau R, Lebrec D. Noninvasive assessment of portal hypertension in patients with cirrhosis. Hepatology. 2011;53(2):683-694.
34. Tsochatzis EA, Gurusamy KS, Ntaoula S, et al. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: A meta-analysis of diagnostic accuracy. J Hepatol. 2011;54(4):650-659.
35. Poca M, Puente A, Graupera I, Villanueva C. Prognostic markers in patients with cirrhosis and portal hypertension who have not bled. Dis Markers. 2011;31(3):147-154.
36. Sheth SG, Chopra S. Epidemiology, clinical features, and diagnosis of nonalcoholic steatohepatitis. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed June 2012.
37. Pesce A, Scilletta R, Branca A, et al. Does transient elastography (FibroScan®) have a role in decision making in hepatocellular carcinoma? HPB (Oxford). 2012;14(6):403-408.
38. Barbero-Villares A, Mendoza Jimenez-Ridruejo J, Taxonera C, et al; Madrid Group for the Study of Inflammatory Bowel Disease ENICMAD. Evaluation of liver fibrosis by transient elastography (Fibroscan®) in patients with inflammatory bowel disease treated with methotrexate: A multicentric trial. Scand J Gastroenterol. 2012;47(5):575-579.
39. Lee CK, Perez-Atayde AR, Mitchell PD, et al. Serum biomarkers and transient elastography as predictors of advanced liver fibrosis in a United States cohort: The Boston Children's Hospital experience. J Pediatr. 2013 Jun 4. [Epub ahead of print]
40. Canadian Agency for Drugs and Technologies in Health (CADTH). Diagnosis and Monitoring of Liver Fibrosis in Patients with Chronic Hepatitis C: A Review of the Clinical Evidence and Cost Effectiveness. Rapid Response Report: Summary with Critical Appraisal. Ottawa, ON: CADTH; March 8, 2012.
41. American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA). Recommendations for testing, managing and treating hepatitis C. Alexandria, VA; AASLD; updated Mary 12, 2014. Available at: http://www.hcvguidelines.org/. Accessed Mary 24, 2014.

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ORIGINAL ARTICLE

Assessing Corneal Hysteresis Using the Ocular Response Analyzer

Charles W. McMonnies*

ABSTRACT

Purpose. An examination of studies that have assessed corneal biomechanical performance using the Ocular Response Analyzer (ORA: Reichert Ophthalmic Instruments, Depew, NY) raises some questions regarding the influence of measurement variables and the interpretation of the findings obtained with this instrument. This analysis of those questions describes additional factors which do or may contribute to the assessment of corneal hysteresis (CH).

Methods. Using key words CH and ORA, English language articles relevant to this analysis were selected after a PubMed search with the addition of some articles referenced in the selected publications.

Results. Corneal thickness, the level of edema, intraocular pressure, and corneal temperature as well as the area, location, rate, duration, and sequence of corneal unloading and loading may need to be considered as significant variables when assessing CH.

Conclusions. CH values may be specific to measurement method and conditions rather than representing an unequivocal corneal property. Consideration of the uncontrolled variables involved may help explain some of the findings obtained with the ORA. That a CH measurement might vary with the sequence of unloading and loading suggests that the ORA CH finding may not represent the CH, but instead represents a hysteresis value better described as central, applanation-derived hysteresis, which is based on a very short unloading/loading sequence. The potential for the ORA to contribute to improved clinical management appears to be considerable but so does the need for better understanding and further development of its functions and applications.

(Optom Vis Sci 2012;89:E343–E349)

Key Words: biomechanics, hysteresis, cornea, ocular response analyzer

For earlier forms of non-contact tonometry (NCT; Reichert Ophthalmic Instruments, Depew, New York), the instruments are calibrated to indicate intraocular pressure (IOP) according to the time taken to achieve applanation (1 to 3 ms).[1] The time taken to achieve applanation, in response to a non-linearly increasing air pressure, increases with baseline IOP and corneal rigidity[1] and the air pressure force required varies accordingly. The air pressure switches off soon after applanation but corneal indentation subsequent to applanation and then recovery to normal convexity are not relevant to measurement of IOP with earlier models of the non-contact tonometer. For measurements using an Ocular Response Analyzer (ORA) (Reichert Ophthalmic Instruments, Depew NY),[2] a modification of the NCT, milliseconds after the first applanation, the air pump generating the air pulse also shuts down and the air pressure applied to the eye decreases in an inverse-time, symmetrical fashion (Fig. 1).[2] However, before the decrease, the cornea is indented significantly as the air pressure reaches a peak about 3 ms after applanation (Fig. 1). As the pressure decreases from its peak, the cornea passes through a second applanated state while returning from indentation to its normal convex curvature.[2] The inward and outward applanation responses during the short stressor exposure to air puff pressure are monitored with a laser, which detects each corneal applanation as a peak in the reflected signal intensity (Fig. 1).[2]

In material science, a difference in the displacement history of a material during loading and unloading (adding stress followed by relief from that stress) is called hysteresis.[3] Hysteresis is the tendency for a device such as a shock absorber to rebound with less force than was required to deform it. An elastic bounce does not absorb energy but a viscoelastic bounce involves energy absorption, with a loss or dissipation of energy (as heat) and a slower rebound. Similarly, because of a dissipative or damping capacity in viscoelastic tissue, there is a delay in the response to and the recovery from a loading stressor.[3] Thus, energy adsorption during rapid corneal deformation delays the occurrence of the inward and outward

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Correlations between corneal hysteresis, intraocular pressure, and corneal central pachymetry

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PURPOSE: To analyze the correlation between corneal hysteresis (CH) measured with the Ocular Response Analyzer (ORA, Reichert) and ultrasonic corneal central thickness (CCT US) and intraocular pressure measured with Goldmann applanation tonometry (IOP GA).

SETTING: Bordeaux 2 University, Ophthalmology Department, Bordeaux, France.

METHODS: This study comprised 498 eyes of 258 patients. Corneal hysteresis, corneal resistance factor (CRF), and IOP corneal-compensated (IOPcc) were provided by the ORA device; CCT US and IOP GA were also measured in each eye. The study population was divided into 5 groups: normal (n = 122), glaucoma (n = 159), keratoconus (n = 88), laser in situ keratomileusis (LASIK) (n = 78), and photorefractive keratectomy (n = 39). The Pearson correlation was used for statistical analysis.

RESULTS: Corneal hysteresis was not strongly correlated with IOP or CCT US. The mean CH in the LASIK (8.87 mm Hg) and keratoconus (8.34 mm Hg) groups was lower than in the glaucoma (9.48 mm Hg) and normal (10.26 mm Hg) groups. The lower the CH, the lower its correlation with IOPcc and IOP GA. A CH higher than the CRF was significantly associated with the keratoconus and post-LASIK groups.

CONCLUSIONS: Corneal hysteresis, a new corneal parameter, had a moderate dependence on IOP and CCT US. Weaker corneas could be screened with ORA parameters, and low CH could be considered a risk factor for underestimation of IOP. The CCT US should continue to be considered a useful parameter.

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Knowledge of corneal biomechanical properties is important in the fields of intraocular pressure (IOP) measurement, corneal pathology, and corneal refractive surgery. The Ocular Response Analyzer (ORA, Reichert) is the first simple device able to provide an in vivo dynamic measurement of the corneal viscoelastic parameter called corneal hysteresis (CH).

Ophthalmologists know that central corneal thickness (CCT) is an important concern in estimating the true IOP with applanation tonometric systems.[1] Until recently, CCT was the only available parameter to correlate IOP measurements by applanation in different corneas.[2,3] However, it was recently theoretically shown that corneal properties, specifically the elastic modulus, have a greater impact on IOP measurement error in applanation tonometry than corneal thickness or curvature.[4] We hypothesize that viscoelastic properties are also important to evaluate in IOP measurement, as well as in corneal pathology and surgery. In addition, pachymetry may influence viscoelasticity. Corneal hydration, properties of corneal layers, and the biomechanical nature of the stroma are important to evaluate. Because of its dynamic time-measurement concept, the ORA may allow us to improve our understanding in this field.[5]

The aim of the current study was to define the relationships between different parameters: ORA data for CH, corneal resistance factor (CRF), corneal-compensated IOP (IOPcc), and Goldmann-correlated IOP (IOPg) as well as Goldmann applanation tonometry (IOP GA), ultrasonic CCT (CCT US), and age. The main objective was to evaluate CH and CRF as new corneal parameters and to clarify their relationship with IOP and CCT US.

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What Biomechanical Properties of the Cornea Are Relevant for the Clinician?

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Abstract. Although the effects of central corneal thickness and corneal curvature on intraocular pressure measurement are well known, it has only recently become possible to measure the biomechanical properties of the cornea in vivo. This article reviews the structural and material properties of the cornea and considers the effects of corneal parameters, including biomechanics, on IOP measurement. The role of corneal biomechanics as a potential indicator of the structural integrity of the globe will also be discussed. Current evidence suggests that the importance of corneal biomechanics to the glaucoma clinician rests primarily with its effects on IOP measurement. However, the possibility that corneal biomechanics may give an indication of the structural integrity of the optic nerve head cannot be completely excluded. Further population and longitudinal studies are needed to clarify whether current in vivo measures of corneal biomechanical properties, including corneal hysteresis, prove to be independent predictors of glaucoma susceptibility. (**Surv Ophthalmol 52**:S109–S114, 2007. © 2007 Elsevier Inc. All rights reserved.)

Key words. biomechanics • central corneal thickness • cornea • glaucoma • hysteresis

The effects of central corneal thickness (CCT) and corneal curvature (K) on intraocular pressure (IOP) measurement are well known, such that true IOP will be overestimated in eyes with thick or steep corneas and be underestimated in those with thin or flat corneas.[3] However, although the linear relationship between these corneal parameters and IOP measurement is statistically significant, the reported values of the coefficient of determination (the r^2 value) suggest that only a small fraction of the variance in IOP measurements is explained by variations of CCT or K. Although some of the variability in measured IOP will be due to inter-subject differences in true IOP, it is likely that corneal biomechanical properties other than CCT or K will have an important influence on the accuracy of IOP measurements.

The biomechanics of the cornea may also have a role as an indicator of overall globe biomechanics. Thus, an understanding of the differences in corneal biomechanics between individuals may explain why some optic nerve heads are more susceptible to damage by variations in IOP.

The purpose of this article is to review the current literature regarding the relevance of corneal biomechanics to the glaucoma clinician.

Corneal Structure

Collagen is the primary structural component of both the cornea and sclera. It has a high tensile strength and provides a resilient, protective coat to the globe. Corneal collagen is contained primarily in Bowman's layer and the stroma, the latter region constituting 90% of the total thickness of a hydrated cornea. The corneal stroma has a highly organized arrangement of collagen fibrils of small diameter that are braced apart by a proteoglycan matrix that maintains uniform, inter-fibrillar spacing.[21] These fibrils are further organized into flat bundles, or lamellae, that vary in number across the cornea, from approximately 300 centrally to 500 at the limbus.[16] Anterior stroma lamellae run obliquely and randomly across the cornea, often starting at

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Policy

1. Aetna considers the ocular blood flow tonometer, which has been used in the screening, diagnosis, and monitoring of glaucoma, experimental and investigational because there is a lack of evidence in the peer-reviewed published medical literature proving that measurement of pulsatile ocular blood flow significantly improves outcomes by enhancing the screening, diagnosis or management of glaucoma.

2. Aetna considers the ocular Blood Flow Analyzer (BFA) experimental and investigational for screening, diagnosis and monitoring of glaucoma and for all other indications because of insufficient evidence of its effectiveness.

3. Aetna considers measurement of corneal hysteresis experimental and investigational for the screening, diagnosis and monitoring of glaucoma and for all other indications because of insufficient evidence of its effectiveness.

4. Aetna considers continuous monitoring of intraocular pressure experimental and investigational for management of glaucoma and other indications because its effectiveness has not been established.

5. Aetna considers genotyping for the screening, diagnosis and monitoring of glaucoma experimental and investigational because of insufficient evidence of its effectiveness.

6. Aetna considers multi-focal visual evoked potential for the diagnosis and early detection of glaucomatous field defects experimental and investigational because of insufficient evidence of its effectiveness.

Background

Glaucoma is a leading cause of blindness, affecting over 60 million people worldwide. Open-angle glaucoma, the most common subtype of the disease, affects over 2.5 million people in the United States. On behalf of the American Academy of Ophthalmology,

Jampel et al (2011) reviewed the published literature to summarize and evaluate the effectiveness of visual function tests in diagnosing glaucoma and in monitoring progression. Literature searches of the PubMed and Cochrane Library databases were conducted last on May 7, 2010, and were restricted to citations published on or after January 1, 1994. The search yielded 1,063 unique citations. The first author reviewed the titles and abstracts of these articles and selected 185 of possible clinical relevance for further review. The panel members reviewed the full text of these articles and determined that 85 met inclusion criteria. They conducted data abstraction of the 85 studies, and the panel methodologist assigned a level of evidence to each of the selected articles. One study was rated as level I evidence. The remaining articles were classified broadly as providing level II evidence. Studies deemed to provide level III evidence were not included in the assessment. Standard white-on-white automated perimetry remains the most commonly performed test for assessing the visual field, with the Swedish interactive threshold algorithm (SITA) largely replacing full-threshold testing strategies. Frequency-doubling technology and its refinement into Matrix perimetry, as well as short-wavelength automated perimetry, now available with SITA, have been evaluated extensively. Machine learning classifiers seem to be ready for incorporation into software to help distinguish glaucomatous from non-glaucomatous fields. Other technologies, such as multi-focal visual-evoked potential and electro-retinography, which were designed as objective measures of visual function, provide testing free of patient input, but issues prevent their adoption for glaucoma management. The authors concluded that advances in technology and analytic tools over the past decade have provided us with more rapid and varied ways of assessing visual function in glaucoma, but they have yet to produce definitive guidance on the diagnosis of glaucoma or its progression over time. They stated that further research on an objective measure of visual function is needed.

An Agency for Healthcare Research and Quality review on "Screening for Glaucoma: Comparative Effectiveness" (Ervin et al, 2012) summarized evidence linking glaucoma screening to health outcomes. It found insufficient evidence to address whether glaucoma screening is effective in improving vision-related outcomes and concluded that more research is needed to address the association between screening and quality of life outcomes.

Ocular Blood Flow Tonometer:

The ocular blood flow (OBF) tonometer measures not only intra-ocular pressure (IOP) but also pulsatile OBF. According to the manufacturer, taking the IOP and OBF test results together increases the detection rate for glaucoma when compared to traditional tonometry, which measures only average IOP. In addition, the manufacturer claims that the OBF tonometer can be used to provide ongoing analysis of the effectiveness of glaucoma treatment. The manufacturer explains that the OBF applanation tonometer has a resolution of 0.01 mm Hg, and automatically takes 200 readings per second, continuously analyzing the pulsatile variations in IOP. These data are analyzed by a computer, which calculates the OBF. The OBF tonometer records the complete IOP waveform and prints out of the average, maximum, and minimum values in a group of chosen pulses.

To date, the OBF tonometer has been studied primarily as a research tool. The evidence published to date on the OBF tonometer provides comparisons of IOP measurements to standard tonometers. There are no prospective clinical studies, however, demonstrating that measurement of waveforms and calculation of pulsatile OBF improves the management of glaucoma patients or glaucoma suspects, such that clinical outcomes are improved.

Neither the American Academy of Ophthalmology's Preferred Practice Patterns on Glaucoma nor the American Optometric Association's Clinical Practice Guidelines on Glaucoma mention any role for the OBF tonometer in the evaluation and management of patients with glaucoma.

There is no adequate evidence that OBF tonometers offer any clinically significant benefits over conventional applanation or indentation tonometers for screening, diagnosing or monitoring glaucoma.

Bhan et al (2003) examined the repeatability of OBF pneumotonometry and its agreement with Goldmann tonometry. Intra-ocular pressure was measured by 1 experienced ophthalmologist in both eyes of 10 healthy female subjects on 10 different occasions at the same time of day. The 2 methods were performed by alternate allocation, and laterality was chosen by random order. The authors concluded that the repeatability of the OBF pneumotonometer was worse than that of the Goldmann tonometer. This casts doubt on the value of the OBF pneumotonometer as a tool for measuring IOP. The agreement plots indicate that the OBF pneumotonometer may produce significant numbers of false-positive results in screening programs.

Furthermore, pulsatile OBF assessment is used to measure the choroidal circulation and provides diagnostic value to certain ocular diseases such as glaucoma. This technique assumes a constant ocular rigidity and is influenced by axial length, diurnal variation, and age. Lam et al (2003) investigated the effect of age on pulsatile OBF, with consideration of the above factors (n = 118). Ocular blood supply in the ophthalmic artery was also determined using color Doppler ultrasonography. These investigators found that the reduction in pulsatile OBF with age was significant. Although aging affects scleral rigidity and systemic blood pressure, multiple regression analysis indicates that the most influential factor affecting pulsatile OBF is aging.

Also, Gunvant et al (2004) ascertained the effect of central corneal thickness and corneal curvature on IOP measurements using the pulsatile OBF tonograph and the Goldmann applanation tonometer, and assessed the agreement between the pulsatile OBF tonograph and the Goldmann applanation tonometer in IOP measurement (n = 479). The IOP measurements obtained with both the Goldmann applanation tonometer and the pulsatile OBF tonograph varied with central corneal thickness and mean keratometric reading. IOP measured using the Goldmann applanation tonometer increased by 0.027 mm Hg per micro m increase in central corneal thickness. IOP measured using the pulsatile OBF tonograph increased by 0.048 mm Hg per microm increase in central corneal thickness. For an increase of 1 mm of mean corneal curvature there was rise in IOP of 1.14 mm Hg measured by the Goldmann applanation tonometer and of 2.6 mm Hg measured by the pulsatile OBF. When compared to the Goldmann applanation tonometer, the pulsatile OBF tonograph under-estimated at low IOP and over-estimated at higher IOP. The authors concluded that central corneal thickness and corneal curvature affected measurements obtained with the pulsatile OBF tonograph more than they affected measurements obtained with the Goldmann applanation tonometer.

Tonnu et al (2005) compared the inter-method agreement in IOP measurements made with 4 different tonometric methods: (i) the Goldmann applanation tonometer (GAT), (ii) Tono-Pen XL, (iii) OBF tonograph, and (iv) Canon TX-10 non-contact tonometer (NCT) in a randomized order in 1 eye of each of 105 patients with ocular hypertension or glaucoma. A total of 3 measurements were made with each method, and by each of 2 independent GAT observers. GAT inter-observer and tonometer inter-method agreement was assessed by the Bland-Altman method. The outcome measures were 95 % limits of agreement for IOP measurements between GAT observers and between tonometric

methods, and 95 % confidence intervals for intra-session repeated measurements. The authors reported that there was good inter-observer agreement with the GAT and moderate agreement between the NCT and GAT. The differences between the GAT and OBF tonograph and between the GAT and Tono-Pen probably preclude the OBF tomography and Tono-Pen from routine clinical use as objective methods to measure IOP in normal adult eyes.

The ocular Blood Flow Analyzer (BFA) (Paradigm Medical Industries, Inc., Salt Lake City, UT) is an electronic pneumotonometer that measures IOP 200 times per second over a period of 5 to 15 seconds and automatically measures OBF. Ocular pressure rises and falls with each heartbeat and a pressure waveform is created when the bolus of blood from each heartbeat passes through the ocular choroid. The systolic increase and diastolic decrease in IOP caused by the pulsatile OBF is recorded by the BFA. The data are then analyzed by an on board computer in real time and a resultant OBF is calculated. Six test parameters are taken per eye with a calculated average mean value in microliters/second. Measurements for each pulse with a calculated average are given for IOP (tonometry) and pneumoplethysmographic vascular activity including: pressure, pulse amplitude, systole and diastole duration, pulse rate, and OBF rate. The BFA is fundamentally an OBF tonometer, using a pneumatic mode of operation.

Resch and colleagues (2011) stated that little information is available regarding the relationship between glaucomatous visual field defects, morphological changes of the optic disc and OBF. In this study, OBF parameters were correlated with parameters of optic nerve head (ONH) morphology and visual field performance in a cross-sectional study. A total of 103 patients with primary open angle glaucoma were included. Choroidal and ONH blood flow was assessed using laser Doppler flowmetry. Retinal blood velocities and retinal vessel diameters were measured with laser Doppler velocimetry and a Retinal Vessel Analyzer, respectively. To evaluate the ONH morphology, fundus photographs were taken and confocal laser scanning tomography was performed. Among all measured ocular hemodynamic parameters, the ONH blood flow was most strongly correlated to structural parameters of ONH damage and visual field loss. Reduced retinal vessel diameters were only slightly correlated with the degree of glaucomatous damage. The authors concluded that reduced blood flow in the ONH was associated with increasing amount of visual field defect and morphological changes of the ONH. Retinal vessel diameters were only marginally associated with glaucomatous optic nerve damage. Based on retinal vessel diameter determination alone, it is not possible to evaluate if reduced retinal blood flow is causative or secondary in glaucoma.

In a case-control study, Hwang et al (2012) examined the relationship among visual field, neural structural, and blood flow measurements in glaucoma. A total of 47 eyes of 42 patients with perimetric glaucoma were age-matched with 27 normal eyes of 27 patients. All patients underwent Doppler Fourier-domain optical coherence tomography to measure retinal blood flow and standard glaucoma evaluation with visual field testing and quantitative structural imaging. Linear regression analysis was performed to analyze the relationship among visual field, blood flow, and structure, after all variables were converted to logarithmic decibel scale. Retinal blood flow was reduced in glaucoma eyes compared to normal eyes ($p < 0.001$). Visual field loss was correlated with both reduced retinal blood flow and structural loss of rim area and retinal nerve fiber layer (RNFL). There was no correlation or paradoxical correlation between blood flow and structure. Multi-variate regression analysis revealed that reduced blood flow and structural loss are independent predictors of visual field loss. Each dB decrease in blood flow was associated with at least 1.62 dB loss in mean deviation (p â‰¤ 0.001), whereas each dB decrease in rim area and RNFL was associated with 1.15 dB and 2.56 dB loss in mean deviation, respectively (p â‰¤ 0.03). The authors concluded that there is a close link

between reduced retinal blood flow and visual field loss in glaucoma that is largely independent of structural loss. They stated that further studies are needed to elucidate the causes of the vascular dysfunction and potential avenues for therapeutic intervention. Blood flow measurement may be useful as an independent assessment of glaucoma severity.

Ocular Reponse Analyzer for Corneal Hysteresis:

Central corneal thickness has become an important biometric factor and is an essential part of the evaluation of glaucoma. Goldmann applanation tonometry is the most widely used method of measuring IOP, but it is well known that corneal parameters affect the accuracy of this instrument (Herndon, 2006). Corneal pachymetry is used to measure central corneal thickness, and is an adjunct to applanation tonometry for screening and diagnosis of glaucoma. In addition to central corneal thickness, there are probably further biomechanical properties that play a role in IOP measurement (Hager et al, 2007).

A new measure of corneal biomechanics, called corneal hysteresis, assesses corneal resistance to deformation. The Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY) is a new instrument that measures corneal hysteresis, the corneal biomechanical response to rapid indentation by an air jet (Kotecha et al, 2006). Corneal hysteresis is the difference in applanation pressures between the rising and falling phases of the air jet.

Current evidence for measurement of corneal hysteresis has focused on its potential use in glaucoma, and has focused on correlations between corneal hysteresis and IOP, corneal thickness, and other ocular measurements. There are no studies demonstrating that measurement of corneal hysteresis alters clinical management such that clinical outcomes are improved.

Kotecha (2007) stated that current evidence suggests that the importance of corneal biomechanics to the glaucoma clinician rests primarily with its effects on IOP measurement. However, the possibility that corneal biomechanics may give an indication of the structural integrity of the optic nerve head can not be completely excluded. The author noted that further population and longitudinal studies are needed to clarify whether current in vivo measures of corneal biomechanical properties, including corneal hysteresis, prove to be independent predictors of glaucoma susceptibility.

Genotyping for Glaucoma:

Gibson et al (2012) stated that primary open angle glaucoma (POAG) is a characteristic optic neuropathy which progresses to irreversible vision loss. Few genes have been detected that influence POAG susceptibility and other genes are therefore likely to be involved. These researchers analyzed carefully characterized POAG cases in a genome-wide association study (GWAS). They performed a GWAS in 387 POAG cases using public control data (WTCCC2). They also investigated the quantitative phenotypes, cup:disc ratio (CDR), central corneal thickness (CCT), and intra-ocular pressure (IOP). Promising single nucleotide polymorphisms (SNPs), based on various prioritization criteria, were genotyped in a cohort of 294 further POAG cases and controls. These investigators found 2 GWAS significant results in the discovery stage for association, one of which had multiple evidence in the gene neural precursor cell expressed, developmentally down-regulated 9' (NEDD9; rs11961171, p = 8.55E-13) and the second on chromosome 16 with no supporting evidence. Taking into account all the evidence from risk and quantitative trait ocular phenotypes these researchers chose 86 SNPs for replication in an independent sample. Their most significant SNP was not replicated (p =

0.59). They found 4 nominally significant results in the replication cohort, but none passed correction for multiple testing. Two of these, for phenotypes CDR (rs4385494, discovery p = 4.51x10-5, replication p = 0.029) and CCT (rs17128941, discovery p = 5.52x10-6, replication = 0.027), show the consistent direction of effects between the discovery and replication data. These investigators also assessed evidence for previously associated known genes and found evidence for the genes 'transmembrane and coiled-coil domains 1' (TMCO1) and 'cyclin-dependent kinase inhibitor 2B' (CDKN2B). The authors concluded that although they were unable to replicate any novel results for POAG risk, they did replicate 2 SNPs with consistent effects for CDR and CCT, though they do not withstand correction for multiple testing. There has been a range of publications in the last couple of years identifying POAG risk genes and genes involved in POAG related ocular traits. The authors found evidence for 3 known genes (TMCO1, CDKN2B, and S1 RNA binding domain 1 [SRBD1]) in this study. Novel rare variants, not detectable by GWAS, but by new methods such as exome sequencing (also known as targeted exome capture) may hold the key to unraveling the remaining contribution of genetics to complex diseases such as POAG.

Ulmer et al (2012) noted that central corneal thickness (CCT) is associated with POAG. Using SNP data from the GLAUGEN and NEIGHBOR consortia, these researchers investigated the effects of CCT-associated variants on POAG risk. They performed a replication analysis of previously reported CCT SNPs in their CCT dataset (n = 1,117) and tested these SNPs for association with POAG using the full dataset (n = 6,470). They performed a CCT GWAS, selected top SNPs from this analysis, and tested these for association with POAG. They generated cDNA libraries from fetal and adult brain and ocular tissue samples for candidate gene expression analysis. They replicated association with 1 of 20 previously published CCT SNPs: rs12447690, near the ZNF469 gene (p = 0.001; beta = -5.08 microns/allele). None of these SNPs was significantly associated with POAG. In the CCT GWAS, no SNPs reached genome-wide significance. After testing 50 candidate SNPs for association with POAG these investigators identified rs7481514 within the NTM gene that was significantly associated with POAG in a low tension subset (p = 0.00099; OR = 1.28). Additionally, SNPs in the CNTNAP4 gene showed suggestive association with POAG (top SNP = rs1428758; p = 0.018; OR = 0.84). They found evidence of NTM and CNTNAP4 gene expression in ocular tissues. The authors concluded that these findings suggested that previously reported CCT loci are not significantly associated with POAG susceptibility. By performing a quantitative analysis of CCT and a subsequent analysis of POAG, they identified SNPs in 2 cell adhesion molecules, NTM and CNTNAP4, which may increase POAG susceptibility in a subset of cases.

Gemenetzi et al (2012) noted that most of the molecular mechanisms leading to POAG development are still unknown. Gene mutations in various populations have been identified by genetic studies and a genetic basis for glaucoma pathogenesis has been established. Linkage analysis and association studies are genetic approaches in the investigation of the genetic basis of POAG. Genome-wide association studies are more powerful compared with linkage analysis in discovering genes of small effect that might contribute to the development of the disease. POAG links to at least 20 genetic loci, but only 2 genes identified in these loci, myocilin and optineurin, are considered as well-established glaucoma-causing genes, whereas the role of other loci, genes, and variants implicated in the development of POAG remains controversial. Gene mutations associated with POAG result in retinal ganglion cell death, which is the common outcome of pathogenetic mechanisms in glaucoma. The authors stated that if the sensitivity and specificity of genotyping increases, it may be possible to screen individuals routinely for disease susceptibility.

Guidelines from the Australian National Health and Medical Research Council (2010) state that mutations in transcription factor genes have been found to be responsible for developmental disorders associated with childhood glaucoma. The guidelines list the following genetic syndromes associated with childhood glaucoma: Nail Patella Syndrome with the LMX1B gene, Axenfeld Rieger Syndrome/Anterior segment dysgenesis with the PITX2 and FOXC1 genes and Aniridia with the PAX6 gene. Patients with these syndromes or mutations are usually followed closely for glaucoma. The guidelines note that there is some evidence that adult-onset POAG is linked to mutations in the same genes. The guidelines state that the situation is complex and it is likely that multiple mutations in more than one gene may be involved, given that POAG is likely to be inherited as a complex trait. The guidelines note that current research has identified more than 30 mutations of the myocilin gene alone, with connections to POAG in different ethnic groups. The guidelines conclude that there is "evolving evidence" for genetic screening for glaucoma.

Multi-Focal Visual Evoked Potential:

Multi-focal visual evoked potential (mfEVP) has been studied for the diagnosis and early detection of glaucomatous field defects. However, its effectiveness for this indication has not been established.

The National Collaborating Center for Acute Careâ€™s clinical guideline on â€œGlaucoma. Diagnosis and management of chronic open angle glaucoma and ocular hypertensionâ€ (2009) did not mention the use of mfVEP. The American Academy of Ophthalmologyâ€™s report on â€œAssessment of visual function in glaucomaâ€ (Jampel et al, 2011) stated that technologies, such as mfVEP and electro-retinography, which were designed as objective measures of visual function, provide testing free of patient input, but issues prevent their adoption for glaucoma management. The U.S. Preventive Services Task Forceâ€™s recommendation statement on â€œScreening for glaucomaâ€ (2013) listed tonometry, visual field measurement, dilated ophthalmoscopy and slit lamp examination as screening tests for (POAG). The USPSTF statement noted that â€œDiagnosis of POAG is based on a combination of tests showing characteristic degenerative changes in the optic disc and defects in visual fields (often loss in peripheral vision). Although increased IOP was previously considered an important part of the definition of this condition, it is now known that many persons with POAG do not have increased IOP and not all persons with increased IOP have or will develop glaucoma. Therefore, screening with tonometry alone may be inadequate to detect all cases of POAG. Measurement of visual fields can be difficult. The reliability of a single measurement may be low; several consistent measurements are needed to establish the presence of defects. Specialists use dilated ophthalmoscopy or slit lamp examination to evaluate changes in the optic disc; however, even experts have varying ability to detect glaucomatous progression of the optic disc. In addition, no single standard exists to define and measure progression of visual field defects. Most tests that are available in a primary care setting do not have acceptable accuracy to detect glaucomaâ€ .

Mousa et al (2104) stated that mfVEP is a newly introduced method used for objective visual field assessment. Several analysis protocols have been tested to identify early visual field losses in glaucoma patients using the mfVEP technique, some were successful in detection of field defects, which were comparable to the standard automated perimetry (SAP) visual field assessment, and others were not very informative and needed more adjustment and research work. These researchers implemented a novel analysis approach and evaluated its validity and whether it could be used effectively for early detection of visual field defects in glaucoma. Three groups were tested in this study: (i) normal controls (38 eyes), (ii) glaucoma patients (36 eyes) and (iii) glaucoma suspect patients (38

eyes). All subjects had a 2 standard Humphrey field analyzer (HFA) test 24-2 and a single mfVEP test undertaken in 1 session. Analysis of the mfVEP results was done using the new analysis protocol; the hemifield sector analysis (HSA) protocol. Analysis of the HFA was done using the standard grading system. Analysis of mfVEP results showed that there was a statistically significant difference between the 3 groups in the mean signal-to-noise ratio (ANOVA test, $p < 0.001$ with a 95 % confidence interval). The difference between superior and inferior hemispheres in all subjects were statistically significant in the glaucoma patient group in all 11 sectors (t-test, $p < 0.001$), partially significant in 5 / 11 (t-test, $p < 0.01$), and no statistical difference in most sectors of the normal group (1 / 11 sectors was significant, t-test, $p < 0.9$). Sensitivity and specificity of the HSA protocol in detecting glaucoma was 97 % and 86 %, respectively, and for glaucoma suspect patients the values were 89 % and 79 %, respectively. The authors concluded that the new HSA protocol used in the mfVEP testing can be applied to detect glaucomatous visual field defects in both glaucoma and glaucoma suspect patients. Using this protocol can provide information about focal visual field differences across the horizontal midline, which can be utilized to differentiate between glaucoma and normal subjects. They stated that sensitivity and specificity of the mfVEP test showed very promising results and correlated with other anatomical changes in glaucoma field loss. Well-designed studies are needed to establish the clinical value of mfVEP for the diagnosis of glaucoma.

Furthermore, UpToDate reviews on â€œOpen-angle glaucoma: Epidemiology, clinical presentation, and diagnosisâ€ (Jacobs, 2014) and â€œAngle-closure glaucomaâ€ (Weizer, 2014) do not mention the use of mfVEP as a diagnostic tool.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes not covered for indications listed in the CPB:

Multi-focal visual evoked potential:

No specific code

0181T	Corneal hysteresis determination, by air impulse stimulation, bilateral, with interpretation and report
0198T	Measurement of ocular blood flow by repetitive intraocular pressure sampling, with interpretation and report
0329T	Monitoring of intraocular pressure for 24 hours or longer, unilateral or bilateral, with interpretation and report

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

365.0 - 365.9	Glaucoma
V80.1	Special screening for glaucoma

The above policy is based on the following references:

1. American Academy of Ophthalmology (AAO). Primary angle closure. Preferred Practice Pattern. San Francisco, CA: AAO; October 2010.
2. American Academy of Ophthalmology (AAO). Primary open-angle glaucoma. Preferred Practice Pattern. San Francisco, CA: AAO; October 2010.
3. American Academy of Ophthalmology (AAO). Primary open-angle glaucoma

suspect. Preferred Practice Pattern. San Francisco, CA: AAO; October 2010.
4. American Optometric Association (AOA). Care of the patient with open angle glaucoma. St. Louis, MO: AOA; 2010.
5. National Collaborating Center for Acute Care. Glaucoma. Diagnosis and management of chronic open angle glaucoma and ocular hypertension. NICE Clinical Guideline 85. London, UK: National Institute for Health and Clinical Excellence (NICE); April 2009.
6. Veterans Health Administration. Screening for glaucoma in the primary care setting. Washington, DC: U.S. Department of Veterans Affairs; May 2000. Available at:http://www.oqp.med.va.gov/cpg/Glaucoma/GLA_Base.htm. Accessed May 22, 2002.
7. Rao VJ, Gnanaraj L, Mitchell KW, et al. Clinical comparison of ocular blood flow tonometer, Tonopen, and Goldmann Applanation Tonometer for measuring intraocular pressure in postkeratoplasty eyes. Cornea. 2001;20(8):834-838.
8. Bhan A, Browning AC, Shah S, et al. Effect of corneal thickness on intraocular pressure measurements with the pneumotonometer, Goldmann Applanation Tonometer, and Tono-Pen. Invest Ophthalmol Vis Sci. 2002;43(5):1389-1392.
9. Morgan A, Hosking S. Ocular blood flow tonometer reproducibility: The effect of operator experience and mode of application. Ophthalmic Physiol Opt. 2001;21(5):401-406.
10. Shaikh MH, Mars JS. The acute effect of pilocarpine on pulsatile ocular blood flow in ocular hypertension. Eye. 2001;15(Pt 1):63-66.
11. Bafa M, Lambrinakis I, Dayan M, et al. Clinical comparison of the measurement of the IOP with the ocular blood flow tonometer, the Tonopen XL and the Goldmann applanation tonometer. Acta Ophthalmol Scand. 2001;79(1):15-18.
12. Yang YC, Illango B, Cook A, et al. Intraocular pressure and pulse rate measurements by the OBF tonograph--comparison to reference instruments. Ophthalmic Physiol Opt. 2000;20(5):401-407.
13. Lam AK, Chan H, Fan W, et al. A preliminary study on the ocular blood flow (OBF) of Hong Kong Chinese. Ophthalmic Physiol Opt. 1999;19(6):512-517.
14. Esgin H, Alimgil ML, Erda S. Clinical comparison of the ocular blood flow tonograph and the Goldmann applanation tonometer. Eur J Ophthalmol. 1998;8(3):162-166.
15. Spraul CW, Lang GE, Ronzani M, et al. Reproducibility of measurements with a new slit lamp-mounted ocular blood flow tonograph. Graefes Arch Clin Exp Ophthalmol. 1998;236(4):274-279.
16. Silver DM, Farrell RA. Validity of pulsatile ocular blood flow measurements. Surv Ophthalmol. 1994;38 Suppl:S72-S80.
17. Gekkieva M, Orgul S, Gherghel D, et al. The influence of sex difference in measurements with the Langham Ocular Blood Flow System. Jpn J Ophthalmol. 2001;45(5):528-532.
18. Mori F, Konno S, Hikichi T, et al. Pulsatile ocular blood flow study: Decreases in exudative age related macular degeneration. Br J Ophthalmol. 2001;85(5):531-533.
19. Mori F, Konno S, Hikichi T, et al. Factors affecting pulsatile ocular blood flow in normal subjects. Br J Ophthalmol. 2001;85(5):529-530.
20. Esgin H, Alimgil ML, Erda S. The effect of systemic hypertension on pulsatile ocular blood flow in diabetic patients. Acta Ophthalmol Scand. 2001;79(2):160-162.
21. Centofanti M, Zarfati D, Manni GL, et al. The influence of oestrogen on the pulsatile ocular blood flow. Acta Ophthalmol Scand Suppl. 2000;78(232):38-39.
22. Geyer O, Man O, Weintraub M, et al. Acute effect of latanoprost on pulsatile ocular blood flow in normal eyes. Am J Ophthalmol. 2001;131(2):198-202.
23. Yang YC, Illango B, Cook A, et al. Intraocular pressure and pulse rate measurements by the OBF tonograph--comparison to reference instruments.

Ophthalmic Physiol Opt. 2000;20(5):401-407.
24. Geyer O, Neudorfer M, Snir T, et al. Pulsatile ocular blood flow in diabetic retinopathy. Acta Ophthalmol Scand. 1999;77(5):522-525.
25. McKibbin M, Cassidy L, Dabbs TR, et al. Intraocular pressure, pulse amplitude and pulsatile ocular blood flow measurement in premature infants screened for retinopathy of prematurity. Eye. 1999;13(Pt 2):266-267.
26. Rainer G, Kiss B, Dallinger S, et al. Effect of small incision cataract surgery on ocular blood flow in cataract patients. J Cataract Refract Surg. 1999;25(7):964-968.
27. Fontana L, Poinoosawmy D, Bunce CV, et al. Pulsatile ocular blood flow investigation in asymmetric normal tension glaucoma and normal subjects. Br J Ophthalmol. 1998;82(7):731-736.
28. Esgin H, Alimgil ML, Erda S. Clinical comparison of the ocular blood flow tonograph and the Goldmann applanation tonometer. Eur J Ophthalmol. 1998;8(3):162-166.
29. Kerr J, Nelson P, O'Brien C. A comparison of ocular blood flow in untreated primary open-angle glaucoma and ocular hypertension. Am J Ophthalmol. 1998;126(1):42-51.
30. Spraul CW, Lang GE, Ronzani M, et al. Reproducibility of measurements with a new slit lamp-mounted ocular blood flow tonograph. Graefes Arch Clin Exp Ophthalmol. 1998;236(4):274-279.
31. MacKinnon JR, O'Brien C, Swa K, et al. Pulsatile ocular blood flow in untreated diabetic retinopathy. Acta Ophthalmol Scand. 1997;75(6):661-664.
32. Yang YC, Hulbert MF, Batterbury M, et al. Pulsatile ocular blood flow measurements in healthy eyes: Reproducibility and reference values. J Glaucoma. 1997;6(3):175-179.
33. Ravalico G, Pastori G, Croce M, et al. Pulsatile ocular blood flow variations with axial length and refractive error. Ophthalmologica. 1997;211(5):271-273.
34. Bhan A, Bhargava J, Vernon SA, et al. Repeatability of ocular blood flow pneumotonometry. Ophthalmology. 2003;110(8):1551-1554.
35. Lam AK, Chan ST, Chan H, Chan B. The effect of age on ocular blood supply determined by pulsatile ocular blood flow and color Doppler ultrasonography. Optom Vis Sci. 2003;80(4):305-311.
36. Gunvant P, Baskaran M, Vijaya L, et al. Effect of corneal parameters on measurements using the pulsatile ocular blood flow tonograph and Goldmann applanation tonometer. Br J Ophthalmol. 2004;88(4):518-522.
37. Ko YC, Liu CJ, Hsu WM. Varying effects of corneal thickness on intraocular pressure measurements with different tonometers. Eye. 2005;19(3):327-332.
38. Tonnu PA, Ho T, Sharma K, et al. A comparison of four methods of tonometry: Method agreement and interobserver variability. Br J Ophthalmol. 2005;89(7):847-850.
39. Reichert, Inc. Ocular response analyzer from Reichert [website]. Depew, NY: Reichert Ophthalmic Instruments; 2007. Available at: http://www.ocularresponseanalyzer.com/. Accessed July 5, 2007.
40. U.S. Food and Drug Administration, Center for Devices and Radiologic Health (CDRH). Non-contact tonometers, ocular response analyzer. 510(k) No. K032799. Rockville, MD: FDA; January 20, 2004.
41. Hager A, Schroeder B, Sadeghi M, et al. The influence of corneal hysteresis and corneal resistance factor on the measurement of intraocular pressure. Ophthalmologe. 2007;104(6):484-489.
42. Kotecha A, Elsheikh A, Roberts CR, et al. Corneal thickness- and age-related biomechanical properties of the cornea measured with the ocular response analyzer. Invest Ophthalmol Vis Sci. 2006;47(12):5337-5347.
43. Herndon LW. Measuring intraocular pressure-adjustments for corneal thickness and new technologies. Curr Opin Ophthalmol. 2006;17(2):115-119.

44. Congdon NG, Broman AT, Bandeen-Roche K, et al. Central corneal thickness and corneal hysteresis associated with glaucoma damage. Am J Ophthalmol. 2006;141(5):868-875.
45. Shah S, Laiquzzaman M, Bhojwani R, et al. Assessment of the biomechanical properties of the cornea with the ocular response analyzer in normal and keratoconic eyes. Invest Ophthalmol Vis Sci. 2007;48(7):3026-3031.
46. Chui WS, Lam A, Chen D, Chiu R. The influence of corneal properties on rebound tonometry. Ophthalmology. 2008;115(1):80-84.
47. Pepose JS, Feigenbaum SK, Qazi MA, et al. Changes in corneal biomechanics and intraocular pressure following LASIK using static, dynamic, and noncontact tonometry. Am J Ophthalmol. 2007;143(1):39-47.
48. U.S. Food and Drug Administration (FDA) 510(k). Blood flow analyzer (BFA). Summary of Safety and Effectiveness. 510(k) No. K023245. Rockville, MD: FDA. October 21, 2002. Available at: http://www.fda.gov/cdrh/pdf2/K023245.pdf. Accessed on January 7, 2009.
49. Paradigm [website]. Ocular blood flow analyzer. Salt Lake City, UT. 2007. Available at: http://www.paradigm-medical.com/. Accessed January 7, 2009.
50. Kotecha A. What biomechanical properties of the cornea are relevant for the clinician? Surv Ophthalmol. 2007;52 Suppl 2:S109-S114.
51. Insull E, Nicholas S, Ang GS, et al. Optic disc area and correlation with central corneal thickness, corneal hysteresis and ocular pulse amplitude in glaucoma patients and controls. Clin Experiment Ophthalmol. 2010;38(9):839-844.
52. Detry-Morel M, Jamart J, Pourjavan S. Evaluation of corneal biomechanical properties with the Reichert Ocular Response Analyzer. Eur J Ophthalmol. 2011;21(2):138-148.
53. Resch H, Schmidl D, Hommer A, et al. Correlation of optic disc morphology and ocular perfusion parameters in patients with primary open angle glaucoma. Acta Ophthalmol. 2011;89(7):e544-e549.
54. National Health and Medical Research Council (NHMRC).) NHMRC guidelines for the screening, prognosis, diagnosis, management and prevention of glaucoma. NHMRC Reference Code: CP113. Canberra, ACT: NHMRC; 2010.
55. Jampel HD, Singh K, Lin SC, et al. Assessment of visual function in glaucoma: A report by the American Academy of Ophthalmology. Ophthalmology. 2011;118(5):986-1002.
56. Hwang JC, Konduru R, Zhang X, et al. Relationship among Visual Field, Blood Flow, and Neural Structure Measurements in Glaucoma. Invest Ophthalmol Vis Sci. 2012;53(6):3020-3026.
57. Ervin AM, Boland MV, Myrowitz EH, et al. Screening for Glaucoma: Comparative Effectiveness. Comparative Effectiveness Review No. 59. Prepared by the Johns Hopkins University Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ) under Contract No. 290-2007-10061. AHRQ Publication No. 12-EHC037-EF. Rockville, MD: AHRQ; April 2012. Available at: http://www.effectivehealthcare.ahrq.gov/ehc/products/182/1026/CER59_Glaucoma-Screening_Final-Report_20120524.pdf. Accessed June 18, 2012.
58. Gibson J, Griffiths H, De Salvo G, et al. Genome-wide association study of primary open angle glaucoma risk and quantitative traits. Mol Vis. 2012;18:1083-1092.
59. Ulmer M, Li J, Yaspan BL, et al. Genome-wide analysis of central corneal thickness in primary open-angle glaucoma cases in the NEIGHBOR and GLAUGEN consortia. Invest Ophthalmol Vis Sci. 2012;53(8):4468-4474.
60. Gemenetzi M, Yang Y, Lotery AJ. Current concepts on primary open-angle glaucoma genetics: A contribution to disease pathophysiology and future treatment. Eye (Lond). 2012;26(3):355-369.
61. U.S. Preventive Services Task Force. Screening for glaucoma: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2013;159(7):484-

489.
62. Mousa MF, Cubbidge RP, Al-Mansouri F, Bener A. Evaluation of hemifield sector analysis protocol in multifocal visual evoked potential objective perimetry for the diagnosis and early detection of glaucomatous field defects. Korean J Ophthalmol. 2014;28(1):49-65.
63. Jacobs DS. Open-angle glaucoma: Epidemiology, clinical presentation, and diagnosis. UpToDate Inc., Waltham, MA. Last reviewed April 2014.
64. Weizer JS. Angle-closure glaucoma. UpToDate Inc., Waltham, MA. Last reviewed April 2014.

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Technology Assessment

Technology
Assessment Program

Diagnosis and Treatment of Secondary Lymphedema

May 28, 2010

Prepared for:

Agency for Healthcare
Research and Quality
540 Gaither Road
Rockville, Maryland 20850

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SLNB group and 16 percent in the SLNB/ALND group.[6] The Royal Australian College of Surgeons conducted an international, multicenter, randomized controlled trial (RCT) that examined SLNB versus axillary dissection in women with breast cancer. The study found that women receiving SLNB had less lymphedema, less pain, and less arm dysfunction.[7]

Lymphedema is typically diagnosed by clinical history and physical examination.[2] When imaging tests are required to assist in diagnosis, lymphoscintigraphy is often the test of first choice.[3] When lymphoscintigraphy is not available, magnetic resonance imaging (MRI) and computed tomography (CT) can also be used.[3]

The U.S. Food and Drug Administration (FDA) regulates the marketing and use of medical devices in the United States. The FDA does not specifically mention the use of lymphoscintigraphy, MRI, ultrasound, or CT to diagnose lymphedema.

There are several nonpharmacological and nonsurgical treatments for lymphedema, including: compression techniques (e.g., multilayer bandaging techniques, self adherent wraps, compression garments at prescribed pressure gradients); intermittent pneumatic compression (IPC); decongestive therapy (also known as complex or complete decongestive therapy or complex decongestive therapy [CDT]); manual lymphatic drainage; exercise; laser treatment; ultrasound, and aquatherapy. No single treatment is considered usual care for lymphedema. Treatments are typically administered by physical or occupational therapists, though massage therapists, nurses, and physicians may also perform certain kinds of lymphedema treatment.

Methods

Literature Review

The following electronic databases were searched by exploding the subject heading 'lymphedema' and searching it as a textword (lymphedema or lymphoedema). Terms for complete decongestive therapy, manual lymphatic drainage, and intermittent pneumatic compression were included in the search. There were no language limitations for this search.

1. MEDLINE® (1990 – January 19, 2010);
2. EMBASE® (1990 – January 19, 2010);
3. Cochrane Central Register of Controlled Trials® (1990 – January 19, 2010);
4. AMED (1990 – January 19, 2010); and
5. CINAHL (1990 – January 19, 2010).

Further searches were conducted of reference lists of recently published review articles[2,8-11] and bibliographies of extracted articles.

Inclusion/exclusion criteria. For the diagnostic section, we included articles published in the English language that examined the sensitivity and specificity, or psychometric properties (e.g., reliability, validity, responsiveness) of diagnostic tests for lymphedema. Included articles had to contain an evaluation of the diagnostic test(s) on subjects with secondary lymphedema. For the treatment section, we included articles published in the English language, provided they were RCTs or observational studies with comparison groups (e.g., cohort, case control). We included studies of pediatric and adult patients who received any treatment for secondary lymphedema (except drug therapy or surgery) following any form of illness with the exception of filariasis infection.

Although rarely identified as gold standards, the frequency of use of different measures of limb volume or circumference would suggest that these measures are the de facto gold standards for diagnosing secondary lymphedema.

d) What is the sensitivity and specificity of tests used to diagnose lymphedema?

In the eight studies that contained examinations of the sensitivity and specificity of diagnostic tests for secondary lymphedema, sensitivities ranged from 5 to 100 percent (most were at least 40 or 50 percent or above) and specificities ranged from 71 to 100 percent.

e) What are the psychometric properties (reliability, validity, responsiveness) of these diagnostic methods?

Reliability. There is consistent evidence to indicate that lymphedema can be reliably measured using circumferential measures or volume displacement (although volume displacement calculated using Sitzia's method tended to produce the lowest intraclass correlation coefficients, which are measures of reliability). There is too little evidence to draw conclusions about the reliability of other tests such as tonometry, ultrasound, lymphoscintigraphy, or bioimpedance.

Validity. Based on consistently high correlation coefficients, there is strong evidence that limb volume and circumference are interchangeable among one another.

Responsiveness. Only two of the studies included in this report evaluated the responsiveness to change of diagnostic tests for secondary lymphedema. The dearth of evidence on this topic prohibits one from drawing firm conclusions about responsiveness.

f) How frequently and for how long should patients be measured for the development of lymphedema or its subclinical precursor? Does this vary with the diagnostic test method?

There is no evidence to answer these key questions as none of the included diagnostic studies were intended to address either question.

g) Does the diagnostic test method influence the choice of lymphedema treatment or patient outcome? What outcomes were measured in studies of diagnostic tests of lymphedema?

There is no evidence in the 41 diagnostic testing studies to answer either of these questions.

Treatment

Question 2. What were the patient selection criteria in the studies (inclusion and exclusion criteria)? Did they differ by treatment modality?

The major selection criterion in most of the 36 treatment studies was that persons had to have lymphedema secondary to breast cancer. Some studies contained specification that participants had to be in remission, have no relapse, or have no metastases. Various studies defined lymphedema as 'mild', 'chronic', or 'moderate to severe'; other definitions included categorization of lymphedema by excess volume in the affected limb, degree of swelling and excess volume, or degree of swelling alone. There was no evidence to suggest that patient selection criteria differed by treatment modality.

Question 7: Were there any treatments, combinations of treatment methods, or sequence of treatments shown to be more effective or ineffective for any type of lymphedema along the continuum, or patient characteristics (e.g., demographics or comorbidities)? Of particular interest: Is there evidence that the use of compression sleeves or low stretch bandaging is effective in maintaining reductions in lymphedema achieved through the use of other modalities (e.g., IPC, manual lymphatic drainage, exercise)?

There is no evidence to answer either part of this question. In no group of studies were the populations defined or the results reported in such a degree of detail that it was possible to identify groups of patients for whom these treatments are more, or less, effective. No studies were designed to examine the role of sleeve or bandaging in maintaining the benefits of initial treatment.

Question 8: What comparators were used in the studies? Are these comparators consistent with usual care for lymphedema?

Many treatments have been suggested to provide benefit for patients with lymphedema. Despite this, no single treatment has emerged as a gold standard in clinical trials. Due to this, there appears to be no agreement on a standard comparator for RCTs.

Question 9: What outcomes were measured in studies of lymphedema therapy? How effective were these treatment methods in reducing lymphedema?

Multiple outcomes were used in these reports (e.g., changes in limb volume or circumference, subjective symptoms [e.g., pain], range of joint motion, intra and extra cellular fluid levels through bioimpedance). Objective measurements, usually relating to some sort of assessment of limb volume, were the most frequently reported outcomes.

Question 10: Did any studies show that the time of treatment initiation (single modality or combination therapy) relative to symptom onset, any other lymphedema characteristics, or any patient characteristics influenced or predicted treatment outcome?

As few studies were sufficiently powered to detect a difference in the primary outcome (often defined as a reduction in lymphedema swelling over time), most trials were limited in their ability to detect differences in patient subgroups which were predictive for response. Few trials randomized patients with a stratification scheme or performed adjusted analyses to allow for detection of predictive factors.

Question 11: What was the length of followup in studies of lymphedema therapy? How long were the benefits of treatment maintained?

Considering the chronicity of lymphedema, very few trials performed long term followup in their study populations. Treatment benefits were shown to persist for up to 12 weeks in some studies with short term followup periods. Only eight of 36 studies reported outcomes at 6 months or more, with benefits shown to last for up to one year in some cases, provided there was use of maintenance therapy (i.e., elastic sleeve).

Question 12: What harms have been reported associated with the various treatments for lymphedema? Do any patient characteristics (e.g., demographics, comorbidities) or etiology of lymphedema increase the risk of these harms?

Conclusion

Although a great deal of research into the diagnosis and treatment of secondary lymphedema has already been undertaken, there is no evidence to suggest an optimal diagnostic testing protocol, an optimal frequency or duration of treatment, the most efficacious treatment combinations (including the use of maintenance therapy), the length of time for which persons should be tested or treated for lymphedema, and whether certain tests or treatments may benefit some types of patients more than others. The field of research into secondary lymphedema is ripe for advancement and the contents of this report may serve as a springboard to guide future scientific endeavors in this domain.

compression with calibrated gradient pressure) more effective in reducing lymphedema than another for any type of lymphedema along the continuum, or patient characteristic (e.g., demographics, comorbidities)?

b. Did the studies of IPC for lymphedema in patients with comorbidities such as wounds, arterial and/or venous insufficiency, diabetes, congestive heart failure, infection, etc., report the need to modify their treatment protocols? Did it affect treatment outcome?
c. Did the timing of an IPC application and/or the sequence of use of the various IPC device types (either alone or in combination with other therapies) influence outcomes either positively or negatively?

6. What protocols for single modality treatments resulted in the best outcomes of lymphedema therapy? Consider parameters such as usage schedules and characteristics of treatment such as intensity, duration, frequency and setting (self administered at home vs. professionally applied in a medical clinic), and if applicable pumping times/cycles and pressures.
7. Were there any treatments, combinations of treatment methods, or sequence of treatments shown to be more effective or ineffective for any type of lymphedema along the continuum, or patient characteristics (e.g., demographics, comorbidities)? Of particular interest: Is there evidence that the use of compression sleeves or low stretch bandaging is effective in maintaining reductions in lymphedema achieved through the use of other modalities (e.g., IPC, manual lymphatic drainage, exercise)?
8. What comparators were used in the studies? Are these comparators consistent with usual care for lymphedema?
9. What outcomes were measured in studies of lymphedema therapy? How effective were these treatment methods in reducing lymphedema?
10. Did any studies show that the time of treatment initiation (single modality or combination therapy) relative to symptom onset, any other lymphedema characteristics, or any patient characteristics influenced or predicted treatment outcome?
11. What was the length of followup in studies of lymphedema therapy? How long were the benefits of treatment maintained?
12. What harms have been reported associated with the various treatments for lymphedema? Do any patient characteristics (e.g., demographics, comorbidities) or etiology of lymphedema increase the risk of these harms?

Background

The human circulatory system is comprised of two interacting closed systems: the arterial-venous system and the lymphatic system. The lymphatic system is a network of vessels (lymphatics) which transport lymph. Lymph is a clear fluid that contains cells and proteins and originates as interstitial fluid (fluid that occupies space between cells). The lymphatic system drains lymph into the venous blood.[17]

Lymphedema is a pathological condition of the lymphatic system. The normal lymphatic system has three major functions, namely to transport lymph from the periphery of the body to the large veins of the chest and neck, to maintain homeostasis, and to regulate immunity.[18] Lymph flow occurs from peripheral lymphatics to the lymph nodes (distal to proximal). Peripheral lymphatics are dead ended and they originate in the distal-most tissues of the skin,

Secondary lymphedema. The exact pathophysiology of secondary lymphedema depends on its etiology. Globally, the most prevalent cause of secondary lymphedema is from infection with the nematode *Wusheria Bancrofti*, which leads to lymphatic filariasis. The filarial larvae enter the human host when a mosquito bites and then grow into adult worms that damage the lymphatic system, leading to a disruption of lymphatic flow. It has been estimated that more than 14 million people worldwide suffer from lymphedema and elephantiasis of the leg caused by lymphatic filariasis.[20] Filariasis is not endemic to the United States (U.S.) and thus incident cases of lymphatic filariasis are rare and occurrences can usually be traced back to a visit to an endemic country.

In the U.S., the most common cause of secondary lymphedema is malignancies and their related treatment (i.e., surgery, radiation). If a malignancy or tumor is present in the lymphatic system, then it can act as a physical block to lymph flow, thereby leading to lymphedema. When lymph nodes are removed during the treatment of cancer, scarring and adhesions may develop that decrease or block lymph flow. Radiation therapy over the lymph nodes can cause further damage and scarring, which may impair lymph flow and lead to lymphedema.

Less common causes of secondary lymphedema include trauma, chronic venous insufficiency, nonfilariasis infection, and obesity. Trauma can destroy lymphatic structures contained in the skin, resulting in impaired lymph flow (e.g., severe burns).[3] In chronic venous insufficiency, there is usually longstanding damage to the veins and their valves. Valve failure results in a continual backflow of blood in the veins, which increases pressure on the veins and damages the delicate surrounding lymphatic structures. When the lymphatic structures are damaged, lymphedema ensues.[3] Infection in the lymphatics from a variety of sources, including possibly venipuncture, can cause lymphedema. For this reason, patients recovering from cancer treatment must be vigilant about skin care and the prevention of infection.[2]

Obesity has also been shown to impede the flow of lymph, leading to the accumulation of protein rich fluid in the subcutaneous tissue.[1]

The Incidence of Secondary Lymphedema in Both the Upper and Lower Extremities in the United States

The incidence of secondary lymphedema for all diagnostic categories is generally poorly documented. There is great variability in the incidence rates, which results from the variety of measurement techniques and definitions used in studies that evaluate the rates of lymphedema, as well as a general lack of literature on the incidence of secondary lymphedema.[2]

Filariasis. The incidence of filariasis in the U.S. is essentially zero percent as filariasis is not endemic to the U.S. The rare cases that are recorded can be traced back to travel and exposure in an endemic country.[21]

Upper extremity lymphedema. Breast cancer accounts for the majority of upper extremity secondary lymphedema in the U.S.[2] Rates of lymphedema after mastectomy have been reported between 24 to 49 percent.[2] A 5 year, population based, prospective study of female U.S citizens with incident breast cancer documented a 42 percent cumulative incidence of lymphedema following treatment for breast cancer.[22] Axillary node clearance and radiation therapy to the axilla have been shown to increase the incidence of lymphedema after breast cancer treatment, especially when radiation therapy is used adjunctively.[2,20] Conversely, sentinel node biopsies have been shown to decrease the incidence of secondary lymphedema compared to axillary dissection.[2,6]

Contrast lymphography is rarely used today as it requires surgery and has been associated with complications such as wound infection and damage to the lymphatic vessels.[23] On the other hand, lymphoscintigraphy (isotope lymphography) involves the injection of a radioisotope labeled colloid into the interdigital region of the affected limb. A gamma camera is then used to track the flow of colloid as it moves towards the proximal lymph nodes. Lymphoscintigraphy is superior to contrast lymphography as it allows the practitioner to measure lymph flow and carries less risk of complications.[23] Though lymphoscintigraphy is often recommended as the test of first choice for the detection of lymph flow abnormalities,[3] the test lacks universal standards of application.[24,25] Thus further research is warranted to refine the standards of application. When lymphoscintigraphy is not available or desired, magnetic resonance imaging (MRI) and computed tomography (CT) can be used. Both MRI and CT image lymphedema as a subcutaneous honeycomb pattern, though MRI is seen as superior to CT because it also detects excess fluid.[3] Ultrasound can also be used for evaluation of lymphedema and has been used to correlate subcutaneous tissue thickness with lymphedema and fibrosis progression.[26]

During physical examination for lymphedema of the extremities, various methods of limb volume measurement may be employed to determine if a volume increase is present in the affected limb. These methods include limb circumference measurement, water displacement (volumetry), and perometry. Volume measurements are compared with the unaffected limb and lymphedema is often defined as a 2 cm or greater difference in girth, a 200 ml or greater difference in volume or a 10 percent or greater difference in limb volume.[27] Tonometry and tissue dielectric constant may also be used to assess whether lymphedema is present in the limb.

Limb circumference measurement is used to calculate limb volume. A flexible non elastic measuring tape is employed to measure limb circumference at various anatomical landmarks or at given distances from the fingertips or toes.[27-29] Limb volume is then calculated using the frustrum sign method (volume of a truncated cone) or the disk model method (summed truncated cones).[30] The volume of a truncated cone is calculated by taking the circumference of the limb at two different points and using the distance between the two points to calculate volume. The disk model method divides the arm into 10 disks, each with a height of 5 cm. The volume of each disk is then calculated and all 10 volumes summed.[30] On the upper limb, the typical points of measurement are at the hand, wrist and above and below the lateral epicondyle. The advantages to limb circumference measurement is that it is fairly easy to perform in a clinical setting, has a low cost and has good reliability.[30,31] A drawback to limb circumference is the inability to accurately measure the volume of the hand due to its irregular shape.[32]

Volumetry is used to calculate limb volume by having subjects submerge their swollen limb into a cylinder filled with a known amount of water. The amount of water that is displaced by the limb is equal to its volume. To measure the amount of displaced water, one can weigh the water or measure the volume. Water displacement is a reliable method of measuring limb volume[30,31] though its use is not very practical in a clinical setting because of water spillage and space considerations.

Perometry, also known as infrared optoelectric volumetry, uses infra red light to measure the volume of a limb. The limb is placed in a solid frame and the perometer scans the limb taking volume measurements at multiple segments. Limb volume is then calculated by summing the volumes of elliptical segments using a special computer program.[28,30] Perometry for the upper limb using Volometer® (Bosl Medizintechnik, Aschen Germany) was shown to have excellent intrarater and interrater reliability (ICC = 0.997).[30] Though shown to be reliable, perometry is expensive, which may limit its clinical application.

CT. CT scan is 510k cleared for medical use, though highly regulated by the FDA due to radiation risk. The FDA does not specifically mention the use of CT for the diagnosis of lymphedema.

Ultrasound. Ultrasound, which is sometimes used to help with the diagnosis of lymphedema, has been 510k cleared by the FDA for medical imaging. There is no specific mention of the use of ultrasound in the diagnosis of lymphedema.[38]

Bioimpedance devices. Certain bioelectrical impedance devices have 510k clearance from the FDA. Impedimed Imp SFB7 Body Composition Analyzer has been cleared by the FDA as has the Impedimed L-Dex U400 BIS extracellular fluid analysis. The L-Dex U400 BIS has been cleared specifically for lymphedema use.[39,40]

Perometer. No evidence has been found for whether perometry is considered a device or if it has been cleared, either in general or specifically for lymphedema.

Tonometer. A search of the devices product classification database yielded no results for whether tonometry is considered a device or if it has been cleared, either in general or specifically for lymphedema.

Tissue dielectric constant. A search of the devices product classification database yielded no results for whether tonometry is considered a device or if it has been cleared, either in general or specifically for lymphedema.

Nonpharmacologic/Nonsurgical Methods of Treatment for Lymphedema

Compression techniques (including multilayer bandaging techniques, self adherent wraps, and compression garments at prescribed pressure gradients). Compression techniques consist of bandaging and compression garments. Both act to restore hydrostatic pressure in the limb and improve lymph flow.[41] Bandaging is performed with low stretch bandages designed to maintain a constant pressure at rest and an increased pressure with exercise, thus assisting the muscle pump effect. High stretch bandages are not recommended because their application pressure may be difficult to control at rest, thereby increasing the potential for impaired circulation. During exercise, there may be decreases in the pressure exerted by high stretch bandages, thus preventing an increase in lymph flow via the muscle pump effect.[41,42]

Compression garments are fitted to the individual patient and constructed with the intent of exerting a prescribed pressure on the limb. They can be of use to patients who are unable to self wrap with bandages.

Intermittent pneumatic compression. Intermittent pneumatic compression (IPC) is used in the treatment of lymphedema, as well as arterial disease, DVT, and chronic venous insufficiency.[43]

IPC devices consist of pneumatic cuffs connected to a pump that, when applied to human limbs, mimics the muscle pump effect that naturally occurs when muscles contract around the peripheral lymphatics.[43] It is thought that compression may empty terminal lymphatics, thereby allowing drainage of fluid from the interstitium and possibly facilitate fluid flow from the interstitium to the lymphatics. It is still not known if IPC assists protein clearance from tissue.[43]

With IPC, a pumping action on the limb is created by an air filled bladder that fills and exerts pressure on the limb. Most pumps are electrically driven and the timing of the IPC application varies significantly between devices. Cycle time can be as short as 2 seconds or as long as 2

650-1000nm in a scanning or spot laser form.[10] It has been suggested that the mechanism of action of LLLT encourages formation of lymphatic vessels (lymphangiogenesis), promotes lymph flow and stimulates the immune system.[10,33] LLLT has also been shown to break down scar tissue.[49]

Ultrasound (US). At present there is very little literature examining the use of US for lymphedema management. It is thought that US promotes lymph flow by way of wave propagation at the cellular level, which modifies cell metabolism and microcirculation.[50] The efficacy and safety of US remains to be established.

Aquatherapy. Aquatherapy, which consists of slow water based exercises, has been tried as a therapy for lymphedema.[51] The physiological rationale for the use of aquatherapy is based on the concepts of hydrostatic pressure, water temperature, and water viscosity. Hydrostatic pressure increases with the depth of water and lymphedematous limbs are thought to benefit from this pressure gradient through the direction of interstitial fluid toward the trunk.[51] Aquatherapy is performed in warm water to prevent capillary vasodilatation and decreased flow that can occur at lower temperatures. Water viscosity provides resistance to movement, which is believed to assist lymph flow via the muscle pump effect and promotes muscle strengthening. At present there is very little literature examining the use or efficacy of aquatherapy for lymphedema management.

What Method(s) of Treatment is Considered Usual Care for Lymphedema Management?

No single treatment is considered usual care for lymphedema. At present CDT, which is a combination of therapies, is suggested as the main method of conservative care for lymphedema.[2,41,44] CDT includes MLD, application of compression low stretch bandages, exercise, and skin care. IPC devices are sometimes used to supplement CDT.[41,44]

Who are the Health Care Professionals That Administer These Treatments? Are any Training or Certification Standards Required?

Typically, physical or occupational therapists administer lymphedema treatments, though massage therapists, nurses and physicians may also perform certain kinds of lymphedema treatment.[52] Health care professionals do not require any specific training prior to administering lymphedema treatment other than a valid license to practice their profession. This being said, many practitioners seek out additional specialized training in lymphedema management. Several schools exist to offer specialized training in lymphedema care. The Lymphology Association of North America (LANA) is a non-profit corporation that offers certification exams for practitioners of lymphedema care in an attempt to regulate and improve lymphedema management.[52]

inclusion criteria were advanced to the next level. Citations were also advanced if there was insufficient information to determine whether the inclusion criteria were satisfied. The complete, published manuscript was retrieved for all citations that passed through title and abstract screening. Once retrieved, the complete manuscript was screened to determine if the inclusion criteria were met (level three – full text – screening). At this stage, the raters assigned the studies to the key question or questions to which they applied.

At every stage of screening, agreement was required from both raters for a study to be promoted to the next level. Discrepancies were resolved by consensus. If consensus could not be reached, then a neutral third party reviewed the study in question and made a final decision.

Studies that passed the full text screening phase proceeded to full data extraction. The following information was extracted from each diagnosis article: type of diagnostic test, study design, sample size, inclusion and exclusion criteria, sensitivity/specificity, psychometric properties of test, and outcomes. The following information was extracted from each treatment article: type of treatment, study design, sample size, inclusion and exclusion criteria, criteria used to start and stop therapy, time of treatment initiation, time of lymphedema onset, provider of treatment, comparators in study, parameters of treatment, outcomes, length of followup, and reporting of harms.

The authors of this report reviewed the extracted data to confirm the accuracy of the work.

Non English Language Studies

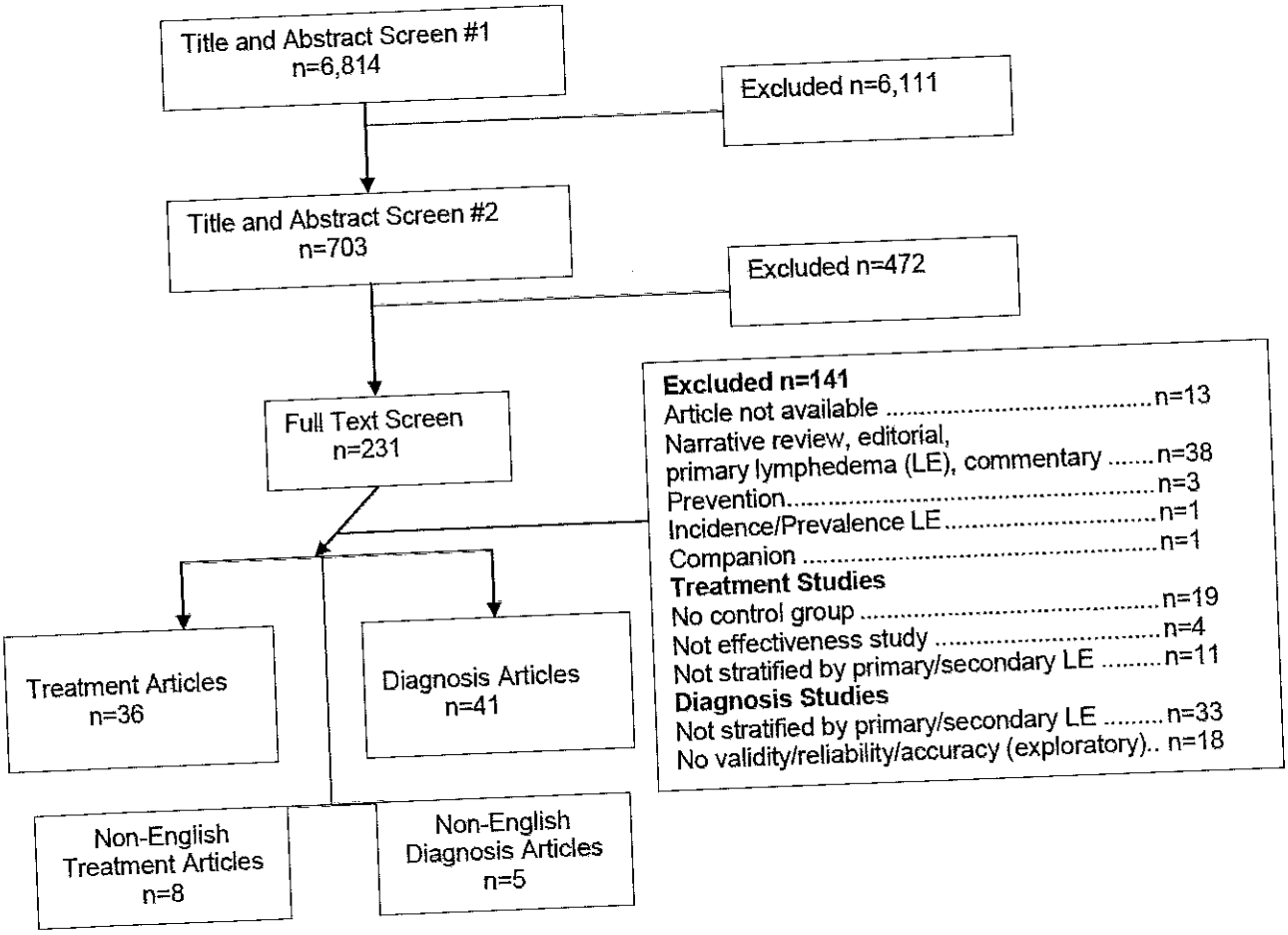
In response to peer review of the draft report, we reran the literature search a second time to identify non English language articles, which were screened at three levels as described above. The purpose was to examine whether the non English language literature was substantively different from the English language literature. We did not extract data from the non English language articles that survived the screening process. Rather, we provided a written summary of the main contents of these articles and discussed whether (and how) they differed from the English language literature.

Quality Assessment of Included Studies

Following data extraction of English language studies, two raters independently assessed the quality of these studies. Discrepancies were resolved by consensus or third party review. The quality of diagnostic studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) scale.[12] The QUADAS scale contains 14 questions that examine potential sources of bias in diagnostic studies. Response options are 'yes', 'no', or 'unclear'. The general domains covered by the questions include representativeness of subjects, clear selection criteria, and appropriateness of the reference standard test. Unlike many quality instruments, the QUADAS does not award points for answers that signify 'good quality', nor is there a summary score.

The quality of treatment studies was assessed using two scales, the modified Jadad scale for RCTs and the Newcastle-Ottawa Scale (NOS) for cohort and case control studies. The modified Jadad scale[13,14] contains six questions covering the following domains: randomization, double blinding, tracking of withdrawals and adverse effects, use of statistics, and inclusion and exclusion criteria. One point is awarded for each 'yes' response; zero points for 'no' responses.

Figure 1. Flow diagram showing the numbers of articles processed at each level



possibility exists that patient withdrawals were minimal or nonexistent in most of these studies due to the limited number of assessments (usually one or two conducted on the same day) and purpose of the assessments (to examine the utility of diagnostic tests rather than to administer a treatment). The reporting of intermediate results may in fact be irrelevant to most diagnostic studies because the intent was to compare the results of different tests, rather than to follow a cohort of persons over time. Given the intent of diagnostic accuracy studies, the authors may not have thought it necessary to use limited journal space to describe the selection and training of raters. Thus, many of the 'fair' studies may have been rated as such due to reporting or relevancy issues rather than due to fundamental flaws in the research. Certainly, one limitation of quality assessment is that reviewers essentially examine the quality of what was reported in the published article rather than what was actually done in the study.[102]

More problematic in terms of quality was the fact that four of nine reliability studies[28,67,92,100] did not contain reports of whether appropriate intervals were used between administrations of the tests of interest. While this may be a reporting rather than a quality issue, a fundamental aspect of any reliability study is to ensure that repeated administrations of the test occur in a timeframe where the underlying condition of interest has not changed, (e.g., the severity of a person's lymphedema remains constant). It will not be possible to assess test-retest or interrater reliability if the underlying condition changes between administrations of the diagnostic test. Authors of reliability studies should comment on the timeframe of their test administrations. In addition, none of the 30 validity studies reported whether the results of the index test were interpreted without knowledge of the results of the reference standard. To prevent the results of the first test from biasing the interpretation of results from the second test, different persons should assess the test results in a blinded fashion. Authors of validity studies should report whether the test results were interpreted in a blinded fashion.

Tables 2 to 4 contain a summary of the quality assessment of the diagnostic accuracy studies.

Treatment

Of the 36 studies that looked at treatments for secondary lymphedema, 30 were randomized controlled trials (RCTs)[33,47,48,103-129] and six were observational (cohort) studies.[50,130-134] The majority of the RCTs were of 'fair' quality and there was an even split between 'good' and 'fair' quality observational studies (Figure 3).

not specify how these conditions were defined or measured. Thus, there is no way to determine whether the ascertainment of adverse effects may have been biased in the extracted clinical trials.

Turning to the observational studies, the major quality issue was a lack of addressing the comparability of the exposed and unexposed groups in the design or analysis of the studies. In the absence of randomization, control of confounding in observational studies requires techniques such as matching, stratification, or use of multivariable regression analysis. Four of the six studies[130,131,133,134] did not contain reports of whether methods were used to control for confounding. The authors of the other two studies[50,132] indicated that the exposed and unexposed groups were matched on severity of lymphedema.

Tables 5 and 6 contain a summary of the quality assessment of the treatment studies.

Diagnosis Studies

Question 1. What is the performance of diagnostic tests for preclinical and/or clinically significant lymphedema?

a) What inclusion criteria (including patient demographics, signs, and symptoms) were used in studies evaluating the performance of diagnostic tests of lymphedema?

Of the 41 diagnosis studies contained in the review, 35 included persons who had breast cancer (Table 7).[27,28,30,31,36,67,68,70,71,73-79,81-87,89-99,101] Other disease related inclusion criteria were melanoma tumor removal,[69] AIDS and Kaposi's Sarcoma,[72] or lymphedema diagnosis.[66,73,77,79,80,88,90,99] Miscellaneous criteria included subjects who had a traumatic accident[79] or who lived within a certain radius of the study site.[68,74,95] For comparative purposes, many diagnostic studies also included nondiseased persons such as clinic staff,[28,91] healthy patients,[71,90,97] or medical students and surgical residents.[91,93] Most studies had liberal age requirements (e.g., 18 years or more,[27,71,77,78,97] less than 75 years[68,74]) and wide ranges of ages of included persons (e.g., 35 to 67 years,[82] 40 to 83 years[88]). Mean and median ages of included persons tended to lie above 50 years.[30,67,73,80,83,85,89,92,93,98] Time since diagnosis or treatment of the primary condition (e.g., cancer) was an inclusion criterion in three studies.[67,69,74] Timeframes in these studies were variable: six months or less,[74] more than six months,[69] and at least 12 months.[67] Three studies excluded persons with concomitant skin disease;[31,75,76] three studies excluded pregnant women.[97,98,100]

b) Is there any "gold standard" method to formally grade or measure the severity of lymphedema?

Only three articles pertaining to diagnostic testing for lymphedema included a measure of severity (Table 7). In a study comparing self reported lymphedema (i.e., patient questionnaire about whether limbs are a different size and whether the differences are noticeable) to physical therapists' measures of arm circumference, the severity of lymphedema was assessed by comparing the circumferential differences between the affected and unaffected arms.[83] Differences of ≤2 cm signified mild lymphedema, >2 or <5 cm indicated moderate lymphedema, and ≥5 cm or more suggested severe lymphedema. This severity scale was developed by the two physical therapists who were involved in the study. The authors did not provide any details about the validity of this classification. The authors also compared self-report on the questionnaire to a 'rule based' assessment of circumferential differences: ≤1 cm meant no lymphedema, >1 cm and ≤2 cm indicated mild lymphedema, >2 cm and <5 cm signified moderate lymphedema, and ≥5

bioimpedance. Sensitivity and specificity were 42 percent and 88 percent for arm circumference and 61 percent and 59 percent for self report, compared to bioimpedance.

Another study contained measures of whole limb volume perometry and arm circumference on persons diagnosed with lymphedema following melanoma.[69] Perometry changes of at least 15 percent and circumference changes of at least 7 percent signified lymphedema. The test of interest was patient self assessment of whether lymphedema was moderate or severe. Sensitivity and specificity were 56 percent and 95 percent for perometry and 50 percent and 100 percent for arm circumference, compared to self assessment. The authors of another study, conducted with persons suffering from lymphedema following breast cancer, compared an abbreviated number of circumferential measurements to a more extensive number of measurements.[78] For the abbreviated regimen, two measurements were taken, one above and one below the elbow. The comparator test involved measurements taken across the palm of the hand, at the wrist, at 10 cm intervals proximal to the wrist, and at the elbow. Sensitivity and specificity were 37 percent and 92 percent for the abbreviated measurement regimen when a 10 percent change in circumference versus the preoperative state was defined as lymphedema. When the threshold change was lowered to 5 percent, sensitivity was 80 percent and specificity was 71 percent.[78]

Persons with breast cancer were included in a study where the tests of interest involved differences in the sum of arm circumference between the treated and untreated arms.[74] Circumferential differences to diagnose lymphedema were established at $\geq$5 cm and $\geq$10 cm. A self report test was also evaluated in the study. Self report contained one question asking patients if they experienced swelling after the diagnosis of lymphedema (response: yes or no). The test of interest was multifrequency bioimpedance. For differences of $\geq$5 cm versus bioimpedance, sensitivity was 35 percent and specificity was 89 percent; for differences of $\geq$10 cm versus bioimpedance, sensitivity was 5 percent and specificity was 100 percent; for self report compared to bioimpedance, sensitivity was 65 percent and specificity was 77 percent.

Bioimpedance was again used diagnostically in a study of 102 persons with breast cancer.[95] Bioimpedeance measures were taken prior to surgery, one month postsurgery, and then at two month intervals until 24 months following surgery. Clinical diagnosis of secondary lymphedema was established through measures of limb volume. The sensitivity of bioimpedance compared to limb volume was 100 percent and the specificity was 98 percent.

A self report served as the test of interest in a study involving persons with breast cancer.[83] This self report contained two questions asking patients whether they noticed if and to what extent their limbs were a different size. The comparator was assessment by a physiotherapist, which was either rule based (i.e., measured changes in circumferential measurement) or clinical observation. Sensitivity comparisons to the rule based and clinical assessments ranged from 93 to 96 percent; specificity comparisons ranged from 69 to 75 percent.

In a study composed of persons with AIDS-related Kaposi's Sarcoma, the tests of interest were a ^{99m}Tc-hexakis-2-methoxy isobutyl isonitrate scan (scintigraphy) and an undefined clinical examination.[72] Forty persons were included in the study and 18 were diagnosed with lymphedema using the scan and 12 were diagnosed using the clinical examination.

The final study assessed sensitivity and specificity for multiple cut off points using volumetry and perometry taken together as a joined set of measurements.[70] The comparator test was unclear. Sensitivity ranged from 73 to 90 percent; specificity ranged from 69 to 78 percent.

A study without limb volume or circumference was a comparison of lymphoscintigraphy and clinical assessment (see Question 1b) to stage lymphedema on a five-point scale.[92] The weighted kappa of 0.77 indicated excellent agreement on staging between these methods. A 4-item questionnaire on truncal swelling was compared to caliper-based skinfold measures of truncal swelling in a study of 12 persons.[86] The questionnaire was developed by the authors and there was no report of whether it was validated prior to use in the study. The correlation between caliper measures of 'creep' (i.e., skin deformations over time) and the questionnaire score (score range is 0 to 8, with higher scores indicating more swelling) was 0.75. The final validity study without a measure of limb volume or circumference compared the torsional rigidity of normal versus lymphedema-affected skin in a group of persons with secondary lymphedema.[75] The authors found that the power to rotate normal skin exceeded the power to rotate diseased skin by 46.3 percent, although the difference was not statistically significant at the 5 percent level ($p = 0.13$).

Two validity studies compared perometer to tape measure. Correlation coefficients between measures were 0.98 for legs and 0.96 for arms in one study[66] and 0.99 for arms in the other study.[91]

Seven studies measured water displacement and made comparisons with limb circumference. In one of these studies, limb circumference was measured using frustrum calculation and tape measure.[79] In frustrum calculation, the limb is viewed as a geometric shape (usually a cone) and specialized formulae are used to measure circumference. In three other studies, limb circumference was calculated using tape measure alone[28,89] or an unexplained method.[90] Correlation coefficients ranged from 0.88 to 0.99. In the fifth study, inverse water volumetry was compared to limb circumference expressed as a ratio between the affected and unaffected limbs and the ICCs ranged from 0.89 to 0.91.[73] In the sixth study, water displacement was compared to Sitzia's method (a specific formula for frustrum calculation[137]) of measuring arm circumference (at 4 or 8 cm intervals) and ICCs ranged from 0.71 to 0.87.[77] Comparison of arm circumference measures at 4 cm with measures at 8 cm yielded ICCs of 0.80 for one rater and 0.92 for a second rater. The seventh study compared interstitial fluid pressure with arm volume ($r = 0.29$ after outlier removal).[88]

The focus of one study was entirely on different interval measures of arm circumference.[84] Intervals of 10 cm were compared to intervals of 3.81 cm (1.5 inches) and the correlation between measures was calculated to be 0.94 or greater.

One study was undertaken to compare two types of physiotherapists' assessments of arm circumference (see Question 1b) to a self report questionnaire.[83] The self report questionnaire asked respondents to indicate whether their affected and unaffected limbs were a different size and whether the differences were noticeable. Weighted kappa's ranged from 0.70 to 0.84, primarily depending on the type of assessment. The lowest kappa's were estimated when the rule-based assessment of arm circumference was compared to the questionnaire (kappa's of 0.70 and 0.76).

Five studies involved bioimpedance and a group of other tests: perometer alone,[80,98] tape measure alone,[85,87] or the combination of perometer, tape measure, and the Lymphedema Breast Cancer Questionnaire (LBCQ).[71] In three of these five studies,[71,80,87] correlation coefficients between all tests ranged from 0.61 to 0.99, with lows of 0.61 between bioimpedance and perometer[80] and 0.70 between bioimpedance and arm circumference assessed with tape measure.[87] Statistically significant correlations between symptoms on the LBCQ and other tests were limited to two domains, namely swelling and firmness/tightness (correlation coefficients

resistance.[31] Differences were 75 mL for water displacement, 0.46 to 1.02 cm for limb circumference measurement, and 0.32 to 1.01 mm for tissue resistance. In the second study, a standard error of the mean of less than or equal to 150 mL was found to be measurement error in an investigation of limb circumference measurement and water displacement.[28] Both studies recruited persons with breast cancer, although one study also included an undefined control group.[28]

f) How frequently and for how long should patients be measured for the development of lymphedema or its sub-clinical precursor? Does this vary with the diagnostic test method?

Fifteen of the 41 diagnostic studies included in this report involved a single assessment of patients (Table 7).[30,31,67,69,71,72,74,87-91,96,98,99] The authors of one of these studies reported that two of 40 patients received a repeat ^{99m}Tc-hexakis-2-methoxy isobutyl isonitrate scan,[72] but no reason was given for performing the second test. None of the authors provided a rationale for limiting their assessments to a single point in time.

The remaining 26 studies involved two or more assessments. In four studies, there were repeat assessments without a clear rationale to explain why.[66,68,78,136]

Seven of the 26 studies contained multiple assessments to permit the study of test-retest or interrater reliability.[28,73,77,79,82,83,100] These repeat assessments were typically performed two or three times, usually on the same day, 1 week apart, or 4 weeks apart.

In six studies, two assessments were conducted to assess the validity of various tests: lymphoscintigraphy versus clinical examination,[92] lymphoscintigraphy versus arm volume,[81] torsional rigidity on swollen and non-swollen arms,[75] ultrasound versus arm circumference,[76] LBCQ versus arm circumference,[36] and 10 cm versus 3.81 cm (1.5 inches) measures of arm volume.[84]

One study, on truncal lymphedema, contained two sets of two assessments to examine the test-retest reliability of skinfold caliper measurements and the validity of caliper measurements versus a 4-item questionnaire about truncal swelling.[86]

More than two assessments were done in seven of the 26 studies: a mean of two assessments to compare single frequency bioimpedance and bioimpedance spectroscopy;[97] four assessments to compare a single measure of tissue dielectric constant with the mean of three measures of tissue dielectric constant;[93,94] five quarterly assessments to examine the calculation of incidence of lymphedema over time using each of four methods (i.e., 200 mL difference in limb volume, 10 percent change in limb volume, 2 cm change in limb volume, or self reported limb swelling or heaviness);[27] five assessments (baseline, once weekly for 3 weeks, and 1 month post-baseline) to study the correlation of bioimpedance and perometry over time;[80] seven assessments of volumetry versus perometry at different cut off points;[70] and a maximum of 14 assessments to examine the diagnostic capability of bioimpedance.[95]

In the last of the 26 studies, measures of limb circumference and bioimpedance were taken daily for 4 weeks as part of the treatment protocol for a larger study being done to investigate a self-management program for lymphedema.[85]

In the 26 studies with multiple assessments, all except eight studies[75,86,92-94,97,99,136] included either limb volume or limb circumference as a diagnostic test. None of the 26 studies contained recommendations for the length of time that patients should be measured for the development of lymphedema, nor was there any evidence of variance based on type of test.

In the five observational studies included in the review, four were conducted in breast cancer survivors,[50,130,133,134] with the fifth in persons suffering from Kaposi's Sarcoma[132] and the sixth in persons with various cancers.[131] The observational studies generally had fewer inclusion and exclusion criteria than the RCTs (one observational study excepted[133]), but these criteria did not appreciably differ from the criteria used in the RCTs.

The inclusion and exclusion criteria were spread across the 36 studies. There was no grouping of similar criteria attached to any specific treatment modality.

Question 3. What were the criteria used to initiate treatment for lymphedema? When was treatment initiated compared to the time of onset of the lymphedema? What were the criteria used to stop therapy? Did these criteria vary with treatment modality?

In the 23 RCTs with Jadad scores between 4 and 8, the criterion used to initiate treatment was a diagnosis of secondary lymphedema. Details are shown in Table 11. This basic condition was specified as an inclusion criterion in all of the trials. In seven of these 23 RCTs, the authors specified general timeframes for recruitment compared to the time of onset of lymphedema: at least 3 months[104,118] or greater than 3 months,[114] a median of 9 to 10.5 months,[109] less than 1 year,[112] less than or equal to 2 years,[119] or 0 to 5 years.[113] Eleven studies contained reports of the time of recruitment following surgical or chemotherapy treatment for cancer: 3 to 6 weeks,[121] at least 3 months,[108] at least 4 months,[115,129] at least 6 months,[47,110] at least 12 months,[116,117,124] between 1 month and 1 year,[111] or at least 4 years.[120] In five RCTs, there was no report of when treatment was initiated compared to time of onset of lymphedema or treatment for cancer.[33,122,123,127,128] One study reported that subjects had a history of breast cancer 1 to 15 years before study entry.[48]

A single study reported that treatment was initiated for hospitalized patients only because a precondition of study entry was the failure of previous outpatient treatment for lymphedema.[124]

Only five studies contained criteria to stop therapy. Four of these studies were RCTs that scored in the 4 to 8 range on the Jadad scale. Two RCTs specified stoppage in the event of adverse effects.[47,112] Other stopping rules included a change of 25 percent change or more in the circumference of the lymphedema-affected arm versus the contralateral arm[110] or completion of the therapeutic regimen.[116] A single observational study contained criteria to stop therapy. Patients were not included in the second phase of treatment if there was less than a 10 percent volume difference between their abnormal and normal arm.[133]

The RCTs with Jadad scores between 0 and 3, as well as the six observational studies, did not exhibit characteristics that were vastly different from what was described above for the 18 RCTs with Jadad scores greater than 3. The exceptions were two RCTs in the Jadad 0 to 3 range that reported 'extreme' recruitment times of 2 days after surgery[106] or 5 years after surgery.[105]

Question 4. Who provided the treatments in the studies? What information was provided on their professional training or certification in lymphedema care?

Fifteen out of the 23 RCTs with a Jadad score between 4 and 8 reported the profession of the person who provided the lymphedema treatment. Some trials contained more than one type of professional.[47,48,113,116,117,123] The authors of nine trials reported that a physiotherapist provided treatment.[47,109,113-115,119,120,123,128] For four of these RCTs, the trial publication indicated that the physiotherapists had been trained in the Vodder technique for the provision of MLD.[109,114,115,123] In two other RCTs, the authors wrote that the person who delivered the treatment was trained in the Vodder technique, but they did not mention whether the person was a physiotherapist.[108,111]

randomized to one-to-one or three-to-one compression cycles, as well as to single-chamber or three-chamber sleeves, with no statistically significant differences found between groups when looking at the absolute extent of edema reduction.[125] The typical measure of efficacy was a change in arm volume or circumference.

None of the studies contained breakdowns of treatment efficacy by patient characteristics.

Question 5b. Did the studies of IPC for lymphedema in patients with comorbidities such as wounds, arterial and/or venous insufficiency, diabetes, congestive heart failure, infection, etc., report the need to modify their treatment protocols? Did it affect treatment outcome?

The need to modify protocols was not discussed by the authors of 10 of the 12 studies that included IPC therapy[50,103,104,108-113,128] (see Table 12). In one RCT, compression pressure was established for each patient based on the consistency of the edema: lower compression pressures for solid edema and higher pressures for soft edema. Pressures were always kept lower than diastolic blood pressure and ranged from 30 to 50mmHg.[125] The authors of one observational study wrote that lower levels of pressure were permitted in some patients treated with compression stockings, but they did not report the number of patients affected by the reductions, the mean decrease in pressure, nor how the reductions may have affected the comparisons with IPC.[130] The authors of the observational study indicated that the potential for pressure decreases was offered to participants as a means of increasing compliance, thereby suggesting a protocol modification. However, there was no discussion in either the RCT or the observational study of whether the compliance issue was related to comorbidity.

Question 5c. Did the timing of IPC application and/or the sequence of use of the various IPC device types (either alone or in combination with other therapies) influence outcomes either positively or negatively?

Six of the 12 studies involving IPC contained reports of the timing of the treatment.[104,108,109,111-113] IPC applied within 1 year of onset of lymphedema was not statistically significantly different from skin care and prophylaxis (cleaning wounds, gloves during gardening, avoidance of weight gain and venipuncture, prolonged sun exposure and carrying heavy weights),[112] but it was better as a supplement to MLD and compression garment when applied an average of 60 mths (3-480 mths) after lymphedema onset[111] (see Table 12). In two studies, IPC was applied within 5 years following lymphedema onset, showing better results versus massage as an adjunct to compression garment and showing no difference versus MLD.[109,113] One study had IPC applied at least 12 weeks following cancer treatment and results were better when IPC was added to MLD and compression bandaging versus MLD and bandaging alone.[108]When IPC was applied to patients who had arm lymphedema for at least 3 months, it performed statistically significantly worse than laser.[104]

Six studies did not report the precise timing of IPC application, with IPC performing statistically significantly better than massage (followed by elastic bandage),[103] no difference versus ultrasound,[50] no different when added to an elastic sleeve versus the sleeve alone,[110] no different when added to compression stockings versus the stockings alone,[130] and no different based on compression cycles or single versus triple sleeves, except for a relative edema reduction in the comparison of three-to-one compression cycles using single versus three-chamber sleeves ($p = 0.04$).[125] In the sixth study, IPC was given to both treatment groups as part of a multimodal therapy.[128]

receive any treatment. The difference in arm volume between groups, favoring the active treatment group, was 12.6 mL ($p = 0.04$).

One RCT ($n = 47$) compared laser to IPC over 12 weeks of followup.[104] Laser was delivered in three sessions per week for 4 weeks with a total of 1.5 Joules/cm^2 per session; IPC was given in five sessions per week for 4 weeks at 60mmHg per session. The change in arm circumference between affected and unaffected limbs was greater for laser than IPC over 12 weeks of followup ($p = 0.02$), but there were no differences on pain or grip strength measures.

A single RCT compared IPC to providing patients with guidelines on skin care for the affected limb.[112] IPC was delivered in two cycles lasting 2 weeks each, with a 5-week separation between the cycles. Each cycle consisted of five sessions per week at 60mmHg. Eighty patients were followed over 9 weeks, and no differences were shown in arm circumference between the groups.

The first exercise trial lasted 12 weeks and involved 32 persons.[47] Randomization was to a group that received 20 supervised, aerobic or resistance exercise sessions or to a group that was instructed to continue with habitual activities. No differences in reduction of lymphedema were found between the groups. The second exercise trial involved a semi-supervised weight-lifting program versus a fitness center membership with partial supervision. After 1 year, there were no statistically significant differences between groups in terms of the proportions of women with at least a 5% change in limb swelling.[48] The weight-lifting group reported reduced severity of lymphedema symptoms, improved upper- and lower-body strength, and fewer incidences of exacerbations of lymphedema.

An observational study examined the use of below-knee, custom-made stockings versus no treatment for lymphedema in a study of 65 persons that lasted for a mean of 5 to 6 months.[132] Differences in limb volume were highly significant between the groups and favored the stockings.

An RCT examining high (44 to 50mmHg) versus low (20 to 30mmHg) pressure bandages found statistically significant within group edema reductions after 24 hours, but no statistically significant between group differences.[124]

A trial comparing kinesiology taping versus MLD in persons who received limb-lengthening surgery reported that the taping was statistically significantly better than MLD.[126] However, the authors did not provide quantitative intergroup results (just intragroups results).

Question 7: Were there any treatments, combinations of treatment methods, or sequence of treatments shown to be more effective or ineffective for any type of lymphedema along the continuum, or patient characteristics (e.g., demographics, comorbidities)? Of particular interest: Is there evidence that the use of compression sleeves or low stretch bandaging is effective in maintaining reductions in lymphedema achieved through the use of other modalities (e.g., IPC, manual lymphatic drainage, exercise)?

There were 27 articles that addressed this question. Pneumatic compression was used as a study treatment in nine randomized trials. Six of those trials received a Jadad score of 4-8.[108-113] Three trials received a lower score.[103,104,125] Of the nine trials, IPC was shown superior to some form of massage-based treatment in three,[103,108,113] inferior to laser in one,[104] and equivalent to MLD with or without bandaging,[109,111] elastic sleeve,[110] and skin care.[112]

In one IPC study, a three-chamber sleeve was more efficacious than a single-chamber sleeve, using a three-to-one compression cycle, in reducing the relative extent of edema in women who had breast cancer.[125]

demonstrated long term volume reduction without the use of maintenance therapy. None of these studies was designed to examine the role of sleeve or bandaging in maintaining benefits of the initial treatment. No trials were identified which compared sleeve or bandage to no treatment in the volume reduction phase of a study.

Question 8: What comparators were used in the studies? Are these comparators consistent with usual care for lymphedema?

In five non-randomized studies, which included a comparator group, all five used different treatments as their comparator. These comparator treatments were pneumatic compression, bandaging, elastic compression garment, stocking and MLD, or no active treatment.[50,130,132-134]

Several RCTs did not clearly identify the treatments that were considered experimental or control. For purposes of this report, it was assumed that the more conservative therapy was the comparator.

There appeared to be little difference between the comparators chosen for higher or lower quality RCTs. The most common comparator used in nine randomized trials was a group of strategies loosely defined as either "usual care", sham treatment or no treatment.[33,47,107,112,116-118,121,130] The most commonly reported active therapies used as a comparator were some form of decongestive therapy,[105,108,111] or elastic sleeve.[110,115,130] Less commonly used study comparators were self-massage,[113] bandaging alone,[123,124] therapies reported as "simple lymphatic drainage".[114,122] Comparators used in some trials included IPC,[104] manual lymphatic drainage,[120,126] and physiotherapy.[106] In two randomized trials involving sequential pneumatic compression vs. MLD[103,109] it was difficult to interpret which of the two treatments was intended as experimental and which was the control. In one RCT, the comparator was a 1-year fitness membership with partially supervised instruction.[48] See Table 11 for details.

Question 9: What outcomes were measured in studies of lymphedema therapy? How effective were these treatment methods in reducing lymphedema?

A multiplicity of outcomes was used to detect benefit in the trials. The vast majority included some form of measurement related to volume of the affected area, although a few simply recorded changes in limb circumferences without reporting volumes.[103,104,110,112,118,121] Other outcomes included subjective symptoms such as pain, heaviness or tension,[104,105,109,118,120,121,133] range of motion in joints (usually shoulder),[33,108,109,115,118,120,129] grip strength,[104,109] measurements of intra- and extra-cellular fluid levels through bioimpedance,[33,47] skin-fold thickness,[116,117] and skin tonicity using tonometry.[33,108,111] Finally, several studies attempted to correlate results of lymphedema treatment with changes in quality of life.[107,113]

The six observational studies examined a mixed group of patients and treatments. One study reported on the use of ultrasound and pneumatic pressure therapy in breast cancer patients. For reasons that are not clear only 96 of 150 study patients contributed data to the final analysis. The authors found that both groups experienced a reduction in arm circumference over baseline values, but that there was no difference between the two treatment groups.[50] Another study of breast cancer patients included some patients with active disease. The reduction in lymphedema volume was 22 percent regardless of disease status but p values were not reported.[131] A study in breast cancer patients looked at the value of adding group talks and exercise sessions to a regimen including MLD and compression stocking. Outcomes in this study were measured using 'psychic well-being' and 'physical complaints' scales, with better psychic well-being shown in the talk/exercise group versus the comparator group ($p<0.05$). No between-group difference was

Therapy with a Deep Oscillation machine in addition to MLD was found to provide initial benefit to women who experienced swelling of the breast following surgery but this was not apparent at study end. Shoulder mobility was improved in the control group. This was the only study of breast, as opposed to arm, lymphedema in women with breast cancer.[120]

Lower quality trials were more likely to suggest benefit in the study group. Two involving pneumatic compression reported significantly more reduction in arm circumferences when compared to MLD,[103] but less than seen following laser.[104] A study of bone marrow stromal cell (BMSC) transplantation versus decongestive therapy reported greater reductions in excess arm volumes with transplant (81% vs. 55%; $p<0.001$) by study end. Both groups experienced a reduction in pain. The hypothesis for the transplant study was that BMSCs would promote the regeneration and reconstitution of lymphatic vessels.[105] A study of adding exercise as a method of reducing arm volumes did not suggest any improvement.[107] A further study examined the use of manual drainage in addition to physiotherapy in patients who had recently undergone ankle surgery.[106] The authors reported improved lymphedema volume loss with the addition of MLD (6.4%) over physiotherapy alone (0.1%; $p = 0.01$).

Question 10: Did any studies show that the time of treatment initiation (single modality or combination therapy) relative to symptom onset, any other lymphedema characteristics, or any patient characteristics influenced or predicted treatment outcome?

A minority of publications (8/36) commented on factors predictive for response to therapy.[48,112,113,115,119,123,131,132] With only two exceptions,[131,132] studies reporting on predictive factors were RCTs. No RCTs with a low quality score commented on predictive factors.

Pretreatment lymphedema volume was the most commonly reported factor, with contradictory findings. One study of massage and bandaging suggested a greater percentage response in those patients with mild, as opposed to moderate, cases.[123] Another similar study, however, suggested opposite results but did not provide any supporting statistics.[119] A third study suggested that pretreatment volumes were "predictive of treatment response" but did not report the direction of this association.[113] A fourth study examining pneumatic compression reported no influence of lymphedema severity on response.[112] One study also reported a non-significant trend toward better responses in those patients who had been diagnosed with lymphedema for less than 1 year.[123] Another reported no such difference with respect to duration.[112]

One study of MLD suggested that compliance with the use of elastic compression sleeves predicts for a better treatment response.[115]

Two non-randomized studies reported predictive factors on very specific patient populations in which active disease was allowed in the study groups. One report found no difference in the response to decongestive therapy, regardless of the presence or absence of active disease.[131] A further study found that those patients with leg lymphedema from Kaposi's sarcoma had a similar response to elastic stockings, regardless of chemotherapy use.[132]

Across all studies, several factors were not found to predict treatment response, including a history of prior radiation, prior chemo therapy, type of previous surgery, a history of prior infection, age, body mass index (BMI), and gender. The authors of an RCT where weight-lifting was the active treatment adjusted their results for cancer stage, number of cancer nodes, race, physical activity, diet, and BMI, and found no effects on the unadjusted results.[48]

visits, personal reasons, or refusal of therapy. Overall, 36 of 616 patients (6.0%) were reported as not receiving therapy as intended.[116,117 33,104,106,112,118,122,123] Unfortunately, it was not possible to discern whether refusal of therapy was due to adverse events in these situations.

Other adverse events more specifically addressed were much rarer. Because the majority of trials addressed lymphedema in patients with cancer, more specifically breast cancer, the most common finding reported was recurrence of malignant disease. Overall, 11 patients (2%) were found to have recurrent disease during or shortly after the study period.[33,47,112,116,117,119] Adverse events which may have been specific to therapy were less common, occurring in less than 1 percent of patients, such as infection, "skin reaction"/ dermatitis,[33,123] arm thrombosis,[33,117] headache with elevated blood pressure,[108] and arm pain.[123]

In a trial evaluating bandages, subjects getting high pressure bandages reported more pain and discomfort than subjects receiving low pressure bandages, although the assessment was done using an author developed scale.[124] A similar scale was used in an RCT comparing kinesiology tape with short stretch bandaging: subjects reported greater wound development from usage of the tape relative to the bandage ($p = 0.013$).[128]

Table 2. Quality of sensitivity and specificity studies using QUADAS (continued)

	Hayes[68] 2008 Australia	Spillane[69] 2008 Australia	Peer[72] 2007 Canada	Hayes[74] 2005 Australia	Bland[78] 2003 U.S.	Norman[83] 2001 U.S.	Cornish[85] 2001 Australia	Godoy[70] 2007 Brazil
11. Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13. Were uninterruptable/ intermediate test results reported?	No	No	Yes	Yes	Unclear	Unclear	No	Unclear
14. Were withdrawals from the study explained?	No	No	No	No	Yes	No	Yes	Yes
Quality Rating	Poor	Fair	Fair	Good	Fair	Fair	Fair	Fair

Table 4. Quality assessment of validity studies using modified QUADAS

Study	Were study patients representative of the patients who will receive the test(s) in practice?	Were selection criteria for patients clearly described?	Were the index test and comparator described in sufficient detail to permit replication in another study?	Were withdrawals from the study explained?	Were intermediate results/incomplete data reported?	Did assessors have adequate professional training to perform test/measurement?	Is the comparator test likely to correctly classify the condition?	Were the correct statistical tests used to measure validity?	Was the time period between the application of the index test and the comparator test short enough to ensure the condition did Not change between tests?	Did all patients who received the index test also receive the comparator test?	Were the index and comparator tests performed independently of one another?	Were the results of the index test interpreted without knowledge of the comparator test?	Quality rating (Good/Fair/Poor)
Armer[27] 2005 U.S.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	No	Fair
Armer[36] 2003 U.S.	Yes	Yes	No	No	No	Yes	Yes	Yes	Unclear	Yes	Unclear	Unclear	Fair
Bates[85] 1992 U.K	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Unclear	Yes	Yes	Unclear	Fair
Cornish[85] 1996 Australia	Yes	Yes	Yes	No	No	Unclear	Yes	Unclear	Yes	Yes	Yes	No	Fair
Czerniec[100] 2010 Australia	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear	Good
Damstra[73] 2006 Netherlands	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Good
Gebousky[92] 2008 Czech Republic	Yes	Yes	Yes	No	No	Yes	Unclear	Unclear	Unclear	Yes	Unclear	No	Fair

Table 4. Quality assessment of validity studies using modified QUADAS (continued)

Study	Were study patients representative of the patients who will receive the test(s) in practice?	Were selection criteria for patients clearly described?	Were the index test and comparator described in sufficient detail to permit replication in another study?	Were withdrawals from the study explained?	Were intermediate results/incomplete data reported?	Did assessors have adequate professional training to perform test/measurement?	Is the comparator test likely to correctly classify the condition?	Were the correct statistical tests used to measure validity?	Was the time period between the application of the index test and the comparator test short enough to ensure the condition did Not change between tests?	Did all patients who received the index test also receive the comparator test?	Were the index and comparator tests performed independently of one another?	Were the results of the index test interpreted without knowledge of the comparator test?	Quality rating (Good/Fair/Poor)
Meijer[77] 2004 Netherlands	Yes	Yes	Yes	No	No	Unclear	Yes	Yes	Yes	Yes	Yes	No	Fair
Mellor[78] 2004 U.K.	Yes	Yes	Yes	No	No	Unclear	Unclear	Yes	Unclear	Yes	Yes	Unclear	Fair
Mimajafi[75] 2004 Australia	Yes	Yes	Yes	Yes	No	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Fair
Moseley[80] 2002 Australia	Yes	Yes	Yes	No	No	Unclear	Yes	Yes	Yes	Yes	Yes	No	Fair
Norman[55] 2001 U.S.	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Unclear	Yes	Yes	Unclear	Fair
Ridner[71] 2007 U.S.	Yes	Yes	Yes	No	No	Yes	Yes	Unclear	Yes	Yes	Yes	Unclear	Fair
Ridner[97] 2009 U.S	Yes	Yes	No	Yes	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Unclear	Fair

Table 4. Quality assessment of validity studies using modified QUADAS (continued)

Study	Were study patients representative of the patients who will receive the test(s) in practice?	Were selection criteria for patients clearly described?	Were the index test and comparator described in sufficient detail to permit replication in another study?	Were withdrawals from the study explained?	Were intermediate results/incomplete data reported?	Did assessors have adequate professional training to perform test/measurement?	Is the comparator test likely to correctly classify the condition?	Were the correct statistical tests used to measure validity?	Was the time period between the application of the index test and the comparator test short enough to ensure the condition did Not change between tests?	Did all patients who received the index test also receive the comparator test?	Were the index and comparator tests performed independently of one another?	Were the results of the index test interpreted without knowledge of the comparator test?	Quality rating (Good/Fair/Poor)
Ward[88] 2009 Australia	Yes	Yes	Yes	No	Un-clear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Unclear	Fair
York[89] 2009 Australia	Yes	Yes	Yes	No	Un-clear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Unclear	Fair

Table 5. Quality assessment of RCT's with Jadad Scale (continued)

Study	Jadad Score	Quality Rating
Szuba[111] 2002 U.K.	4	Fair
Shaw[116] 2000 U.K.	5	Fair
Shaw[117] 2007 U.K.	5	Fair
Kaviani[118] 2007 Iran	5	Fair
Didem[119] 2005 Turkey	5	Fair
Dini[112] 1998 Italy	5	Fair
Jahr[120] 2008 Germany	5	Fair
Maiya[121] 2008 Singapore	5	Fair
Sitzia[122] 2002 U.K.	5	Fair
Irdesel[123] 2007 Turkey	5	Fair
Damstra[124] 2009 Netherlands	6	Good
Lau[127] 2009 China	6	Good

Table 6. Quality assessment of observational studies using Newcastle-Ottawa Scale (NOS)

Study	Type of Study	NOS Star Rating	Quality Assessment
Johansson[133] 1999 Sweden	Cohort	6	Fair
Berlin[130] 1999 Sweden	Cohort	6	Fair
Frischenschlager[134] 1991 Austria	Cohort	6	Fair
Pinell[131] 2007 U.S.	Cohort	7	Good
Brambilla[132] 2006 Italy	Cohort	8	Good
Balzarini[60] 1993 Italy	Cohort	8	Good

Table 7. Basic data diagnostic studies (continued)

Author	Study Type (reliability, validity, Sensitivity/ Specificity)	Sample Size	Question #1a	Question #1b	Question #1f	Question #1g
			Inclusion/ Exclusion Criteria	Measure of Severity of LE	Frequency of Assessment for LE	Outcomes
Chen[31] 2008 Taiwan	Reliability	Total n=31 Trial 1: Water displacement and circumference n=14 Trial 2: Tonometry n=17	Pts who developed LE after breast carcinoma surgery Those with skin problems or wounds around measurement areas	NR	Single assessment	NR
Cornish[85] 2001 Australia	Sensitivity and Specificity	n=102 LE patients n=60 healthy control	102 pts with BCa from 25 to 82 years old, living within 50 km of Brisbane 60 female volunteers	NR	Maximum of 14 assessments to examine Dx capability of BIS	NR
Cornish[85] 1996 Australia	Validity	LE patients n=20 Control n=20	Pts with ≥Grade II unilateral LE of upper limb after surgery and/or radiotherapy for BCa. Mean age 60 yrs (32-78) Controls volunteers from clinic and staff	NR	Daily measurements for four weeks as part of treatment protocol	NR
Czerniec[100] 2010 Australia	Validity and reliability	n=33 LE patients n=18 controls	Women with unilateral arm LE following treatment for BCa (mean age 58±10.0 years); healthy controls (mean age 52 ±7 years)	NR	Two assessments four weeks apart	NR
Damstra[73] 2006 Netherlands	Validity	n=25	Females suffering from LE age range 47-82 years (mean ± SD: 61.7±9.5); complete and partial mastectomy following BCa surgery axillary node dissection No signs of metastasis	NR	Multiple assessments to permit study of test-retest or interrater reliability	NR

Table 7. Basic data diagnostic studies (continued)

Author	Study Type (reliability, validity, Sensitivity/ Specificity)	Sample Size	Question #1a	Question #1b	Question #1f	Question #1g
			Inclusion/ Exclusion Criteria	Measure of Severity of LE	Frequency of Assessment for LE	Outcomes
Halaska[90] 2006 Czech Republic	Validity	Total n=101 Group A n=60 (circumference & MFBIA): subgroups A1 n=7 (circumference & MFBIA 1-100kHz & water displacement), A2 n=20 (& MFBIA 200kHZ) Group B n=5 (circumference & MFBIA); Group C n=36 (circumference & MFBIA)	Group A: healthy women as control, mean age 40.20 years (22-75yrs) Group B: pronounced LE, mean age 63.3 years (55-78 yrs) Group C: undergoing BCa surgery, mean age 60.0 years (37-76yrs)	NR	Single assessment	NR
Hayes[74] 2005 Australia	Sensitivity and Specificity	Total n=294 clinical component n=218 data complete n=176	Women diagnosed unilateral BCa ≤6 months Age: ≤75 years, residing within 100 km of Brisbane	NR	Single assessment	NR
Hayes[68] 2008 Australia	Sensitivity and Specificity	n=287	Women with unilateral breast cancer (BCa) with or without LE after Rx age: <75 years, (aged 54±10 years on average) residing within 100 km of Brisbane	NR	5 assessments at 3 mo intervals 6 to 18 mo post surgery	NR

Table 7. Basic data diagnostic studies (continued)

Author	Study Type (reliability, validity, Sensitivity/ Specificity)	Sample Size	Question #1a	Question #1b	Question #1f	Question #1g
			Inclusion/ Exclusion Criteria	Measure of Severity of LE	Frequency of Assessment for LE	Outcomes
Megens[62] 2001 Canada	Reliability	n=25	Women at risk for LE who had undergone axillary lymph node dissection surgery for BCa age range 35-67 years	NR	Multiple assessments to permit study of test-retest or interrater reliability	NR
Meijer[77] 2004 Netherlands	Reliability and Validity	n=18 right upper extremity n=12 left upper extremity	BCa Rx-related LE of upper extremity Age: ≥18 years (mean 56.4 ± 11.6 SD) Co-morbidity, recent operations on the upper extremity, inability to elevate the upper extremity 90 degrees in the shoulder girdle, inability to extend the elbow	NR	Multiple assessments to permit study of test-retest or interrater reliability	NR
Mellor[76] 2004 U.K.	Validity	n=10	Ten women (mean 59 +/- SD 9) with LE subsequent to unilateral BCa surgery or radiation Rx Skin disease or skin trauma	NR	2 assessments to measure validity	NR
Mirnajafi[75] 2004 Australia	Validity	n=17	Seventeen women with unilateral arm LE secondary to axillary clearance and RT Skin comorbidities	NR	2 assessments to measure validity	NR

Table 7. Basic data diagnostic studies (continued)

Author	Study Type (reliability, validity, Sensitivity/ Specificity)	Sample Size	Question #1a	Question #1b	Question #1f	Question #1g
			Inclusion/ Exclusion Criteria	Measure of Severity of LE	Frequency of Assessment for LE	Outcomes
Ridner[71] 2007 U.S.	Validity	Study completers n=31 Data included n=25 Healthy volunteers (HV) n=14 LE patients n=11	HV group: ≥18 years with no self reported LE or BCa LE group: ≥18 years with BCa Rx LE in one arm only no swelling or primary LE before BCa Rx, no medical contraindications; no pregnant women; no metal implants or pacemakers that would interfere with impedance measurements	NR	Single assessment	NR
Ridner[97] 2009 U.S	Validity	n=98 BCa LE n=75 BCa no LE n=60 controls	Women with arm LE after BCa	NR	Single assessment	NR
Roberts[86] 1995 U.K.	Validity and Reliability	n=15	14 subjects with LE 1 healthy subject	NR	Two sets of 2 assessments to measure test-retest reliability and validity	NR
Sagen[96] 2009 Norway	Validity	n=23	Women with LE following surgery for BCa Mean age 64±11years	NR	Single assessment	NR

Table 7. Basic data diagnostic studies (continued)

Author	Study Type (reliability, validity, Sensitivity/ Specificity)	Sample Size	Question #1a Inclusion/ Exclusion Criteria	Question #1b Measure of Severity of LE	Question #1f Frequency of Assessment for LE	Question #1g Outcomes
Ward[87] 1992 Australia	Validity	LE n=15 Control n=15	Women with LE following BCa treatment; average age 53 years; duration of LE 1.5 months (average) Female controls from clinical staff/investigative team	Grade 2 LE (on 1-6 scale)	Single assessment	NR
Ward[98] 2009 Australia	Validity	LE n=45 Control n=21	Women clinically diagnosed with unilateral arm LE after BCa. Healthy control group, no history LE or BCa	NR	2 assessments each device to measure validity	NR
York[99] 2009 Australia	Validity	Arm LE n=28 Leg LE n=16 Healthy controls n=28	Women with arm LE post BCa Women with leg LE secondary to Ca Controls, no history of LE or surgery to axilla	NR	Single assessment	NR

Table 8. Psychometric properties of diagnostic studies (continued)

Author	Test(s)	Sample Size	Reliability	Validity	Responsiveness
Chen[31] 2008 Taiwan	Water displacement Circumference measurement Tonometry	Total n=31 Trial 1: Water displacement and circumference n=14 Trial 2: Tonometry n=17	ICCs for test-retest and interrater reliability ranged from 0.69 to 0.88	NR	Defined as smallest difference detectable 75 ml for water displacement 0.46 to 1.02 cm for limb circumference measurement 0.32 to 1.01mm for tissue resistance
Cornish[85] 2001 Australia	Index Test: MFBIA Reference Test: Limb circumference	n=102 LE patients n=60 healthy control	NR	NR	NR
Cornish[85] 1996 Australia	Bioimpedance daily measurements vs. circumferential measurements taken daily throughout 4 weeks of lymphedema treatment	n=20 LE Patients n=20 Control	NR	Bias scores decreased from 31% to 15% between 1 and 26 days of followup. Lower bias scores indicate better agreement	NR
Czerniec[100] 2010 Australia	Limb circumference, perometry, bioimpedance spectroscopy (BIS) and self report of LE	n=33 LE patients n=18 control	ICCs for intrarater reliability 0.95 to 1.00 for the three physical measures and 0.50 for visual analogue scale; interrater reliability, calculated for the physical measures only, ranged from 0.98 to 1.00	High agreement between limb circumference, perometry and BIS (r = 0.89- 0.99) Moderate agreement between physical measures and self-report (r= 0.65-0.71)	NR
Damstra[73] 2006 Netherlands	Inverse water volumetry vs. circumferential measurements (Herpertz method)	n=25	NR	ICCs ranged from 0.89 to 0.91	NR

Table 8. Psychometric properties of diagnostic studies (continued)

Author	Test(s)	Sample Size	Reliability	Validity	Responsiveness
Halaska[90] 2006 Prague	Multifrequency bioelectrical impedance, circumferential measurements, subgroup with water displacement	Total n=101 Group A n=60 (circumference & MFBIA):subgroups A1 n=7 (circumference & MFBIA 1-100kHz & water displacement), A2 n=20 (& MFBIA 200kHZ) Group B n=5 (circumference & MFBIA); Group C n=36 (circumference & MFBIA)	NR	Correlation between circumferential measurements and water displacement was 0.94	NR
Hayes[74] 2005 Australia	Index Test: Multifrequency bioelectrical impedance Reference Test: Sum of arm circumference and self report	Total n=294 Clinical component n=218 Data complete n=176	NR	NR	NR
Hayes[68] 2008 Australia	Index Test: Bioimpedance spectroscopy (BIS) Reference Test: Comparator: Sum of arm circumference and self report	n=287	NR	NR	NR
Karges[79] 2003 U.S.	Volumetric measurements taken with a volumeter minus fingers (UE-F) circumferential measures taken with a tape measure, calculated volume formula using truncated cone formula	n=14	NR	Correlation coefficient for volumetric measurements and tape measure was 0.98	NR

Table 8. Psychometric properties of diagnostic studies (continued)

Author	Test(s)	Sample Size	Reliability	Validity	Responsiveness
Meijer[77] 2004 Netherlands	Indirect volume determination (Sitzia's method) vs. water displacement	n=30	Intrarater reliability for water displacement ranged from 0.95 to 0.98 Intrarater reliability for Sitzia's method ranged from .90 to .99 with one low ICC of .62	Comparing Sitzia's method to water displacement: ICCs ranged from 0.71 to 0.87 Comparison of arm circumference measures at 4 cm with measures at 8 cm ICCs of 0.80 for one rater and 0.92 for a second rater	NR
Mellor[76] 2004 U.K.	Dermascan ultrasound	n=10	NR	Ultrasound strongly correlated with arm circumference, r=0.95	NR
Mirnajafi[75] 2004 Australia	Torsional rigidity of skin	n=17	NR	Power to rotate normal skin exceeded power to rotate diseased skin by 46.3%. Not significant (p=0.13)	NR
Moseley[67] 2002 Australia	Bioimpedance vs. Tonometry	n=12 healthy volunteers n=12 LE patients	Covariance for bioimpedance ranged from 0.002 to 0.0086 Covariance for tonometry ranged from 0.0129 to 0.0325	NR	NR

Table 8. Psychometric properties of diagnostic studies (continued)

Author	Test(s)	Sample Size	Reliability	Validity	Responsiveness
Roberts[86] 1995 U.K.	Modified Harpenden Skinfold Calipers Arm volume measurements	n=14 LE patients n=1 Healthy subject	Coefficient of standard variation of 5%	Correlation between caliper measures and questionnaire scores was 0.75	NR
Sagen[90] 2009 Norway	Simplified water displacement instrument (SWDI) vs. Cross sectional area (CSA) by computed tomography	n= 23	NR	Correlation of SWDI and total CSA of upper arm was r=0.904; correlation of SWDI and CSA of subcutaneous tissue r=0.867; correlation of SWDI and CSA of muscle tissue r=0.725	NR
Spillane[89] 2008 Australia	Index Test: Infrared Opto-electronic perometer technique Reference Test: circumference measurements, brief questionnaire	n=66	NR	NR	NR
Stanton[91] 1997 U.K	Opto-electronic limb volumeter (Perometer) and limb circumference	n=12	NR	Correlation of 0.988 between Perometer and limb circumference in patients with LE	NR
Szuba[61] 2002 U.S.	Quantitative radionuclide lymphoscintigraphy	n=19	NR	Correlation of outcome of therapy with pre-therapeutic axillary radioactivity level r=0.5	NR

Table 9. Sensitivity and specificity studies

Author	Study Design	Sample Size	Inclusion/Exclusion Criteria	Index Test	Reference Test	Sensitivity/Specificity
Bland[78] 2003 U.S.	Sensitivity/Specificity	n=90	≥18 years, male or female eligible. New diagnosed resectable BCa. Scheduled for mastectomy or lumpectomy, with lymph node sampling, dissection, or sentinel node biopsy, or breast conservation therapy followed by radiation therapy. Participants average age was 53.7 years, all female, half of patients had RT	Percent change in circumferential measurements above and below the elbow	10% change or more in volume. 1 cm change in circumference at any site	Sensitivity 37% and Specificity 92% for a 10% change in circumference above and below elbow Sensitivity 80% and Specificity 71% for a 5% change in circumference above and below elbow
Cornish[95] 2001 Australia	Sensitivity/Specificity	n=102 n=60 control	Dx BCa, living within 50 km of Brisbane pathological confirmation of tumor malignancy Axillary dissection	Bioimpedance (BI)	Limb volume	Sensitivity BI vs. limb Volume=100% Specificity BI=98%
Godoy[70] 2007 Brazil	Sensitivity/Specificity	n= 90	Women with LE following surgery for BCa Mean age 54.8 ± 11.7 yrs	Perometry	Volumetry	Comparator test was unclear; Sensitivity ranged from 73 to 90%; specificity ranged from 69 to 78 percent

Abbreviations: AIDS-KS=Acquired Immune Deficiency Syndrome-Karposi's Sarcoma, BCa=Breast Cancer, BI=Bioimpedance, BIS=Bioimpedance Spectroscopy, Dx=Diagnosis LE=Lymphedema, MFBIA=Multifrequency Bioelectrical Impedance, Rx=treatment, SOAC=Sum of Arm Circumference

Table 9. Sensitivity and specificity studies (continued)

Author	Study Design	Sample Size	Inclusion/Exclusion Criteria	Index Test	Reference Test	Sensitivity/Specificity
Spillane[69] 2008 Australia	Sensitivity/Specificity	n=66	Patients who had previously undergone an inguinal or ilio-inguinal dissection for melanoma >6 months previous, 31 male, 35 female, median age 44.2 (20-95) years range (20-95 years), 9 received RT	Infrared Opto-electronic perometer technique	Arm circumference Self assessment questionnaire	Sensitivity 56% and Specificity 95% for perometry vs. self assessment Sensitivity 50% and Specificity 100% for perometry vs. arm circumference

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
RCT							
Carati[33] 2003 Australia	RCT crossover plus within group comparison of one cycle vs. two cycle	LLLT one cycle vs. LLLT two cycles	n=37 n=27	LE secondary to BCa treatment	>200 ml difference between arms or ≥2 cm difference in arm circumference	NR	Inclusion: - female Exclusion: - presence of co-morbidities - significant change to the arm in past 3 months - inability to abduct arm for measurement - presence of primary LE of lower limbs
Damstra[124] 2009 Netherlands	RCT	Compression therapy: low vs. high pressure bandaging	n=18 low pressure n=18 high pressure	LE following BCa	Patients with moderate to severe LE as defined by ISL	NR	Inclusion: -female ->18 years of age -12 months post BCa Rx without signs of reoccurrence Exclusion: -allergy to materials -systemic diseases -arterial or venous disease

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
RCT							
Hayes[47] 2009 Australia	RCT	Mixed exercise program (aerobic and resistance)	n=16 Exercise n=16 Control	LE secondary to BCa treatment	Upper limb LE diagnosed by a health professional	NR	Inclusion: - women <76 years with completed Rx for unilateral BCa ≥6 months prior - able to travel to clinic for exercise for 12 weeks
Hou[105] 2008 China	RCT	Bone Marrow Stromal Cell Transplantation or Complex Decongestive Therapy	n=15 BMSC n=35 CDT	Lymphedema secondary to BCa	NR	NR	Exclusion: - radiotherapy
Irdesel[129] 2007 Turkey	RCT	Compression garment and exercise	n=10 exercise n=11 exercise + compression	LE secondary BCa	NR	NR	Exclusion: -BCa operation <4 months ago -recurrence or bilateral BCa -elephantiasis -congestive heart failure -deep vein thrombosis -acute infection -stage 4 BCa

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
RCT							
Johansson[109] 1998 Sweden	RCT	MLD vs. sequential pneumatic compression	n=14 MLD n=14 SPC	Unilateral LE after BCa surgery with axillary nodal dissection	>10% difference in LE affected arm vs. unaffected arm	NR	Exclusion: - previous contralateral breast disease - intercurrent disease affecting the swollen arm - difficulty participating for reasons such as dementia - treatment within the last 6 mths (except for wearing compression sleeve) - resolution of LE during initial use compression sleeve by all participants
Kaviani [118] 2006 Iran	RCT	LLLT	n=4 LLLT n=4 Control	LE secondary to BCa treatment	≥2 cm swelling in affected arm	NR	Inclusion: - no contraindications to laser therapy Exclusion: - metastatic disease
Kessler[106] 2003 Switzerland	RCT	Standard physiotherapy plus manual lymphatic drainage	n=11 SP and MLD n=12 SP	LE following hindfoot surgery	Clinically diagnosed post-operative swelling	NR	- Age: 18-75 yrs - good physical condition - no contraindications for lymph drainage

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
RCT							
Lau[127] 2009 China	RCT	LLLT vs. no Rx	n=11 LLLT n=10 control	LE secondary to BCa treatment	Arm volume difference of more 200 ml	NR	Inclusion: -18+ years -unilateral mastectomy + chemo and/or radiation Exclusion: -metastases -Hx of severe trauma to arm -kidney, heart or lung disorder -medications that alter body fluids -primary LE of lower limb -decrease shoulder ROM -cellulitis past 3 months
Maiya[121] 2008 India	RCT	LLLT and exercise	n=10 LLLT + exercise n=10 control (compression + exercise)	LE secondary to BCa treatment	circumference of UE 2 cm at any 2 points compared to normal limb	NR	Inclusion: - mastectomy for BCa - completion of RT Exclusion: - primary LE - infection of the limb
McKenzie[107] 2003 Canada	RCT	Exercise (resistance training plus arm ergometer)	n=7 Exercise n=7 Control	LE secondary to BCa	LE >2 cm and <8 cm at 1 measurement point	NR	Exclusion: - stage III LE - bilateral disease - medication that effects swelling

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
RCT							
Schmitz[48] 2009 U.S *companion Schmitz[138]	RCT	Weight lifting vs. no weight lifting	n= 141 intervention n=71 control n=70	LE secondary to BCa	Difference in volume or circumference of 10% or more affected vs. unaffected arm	NR	Inclusion: -1-15 years since BCa Dx -unilateral LE -BMI less 50 -not actively trying to lose weight -no evidence of cancer -no medical conditions that would limit exercise -no history of weight lifting in past year -at least one lymph node removed
Shaw [116] 2007 U.K.	RCT	Weight reduction program along with conventional treatment with compression hosiery	n=21 intervention n=11 control n=10	Arm LE following surgery for BCa	Affected arm volume ≥15% larger than unaffected	May or may not have been receiving hormone treatment	Inclusion: - remission from Ca - BMI ≥25 kg/m²
Shaw [117] 2007 U.K.	RCT	Diet intervention plus multilayer bandaging then compression hosiery or hosiery alone	n=19 weight reduction n=17 low fat diet n=15 control	Arm LE secondary to BCa treatment	≥20% greater volume than unaffected arm	NR	Inclusion: - Ca remission

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
RCT							
Tsai[128] 2009 China	RCT	DLT + IPC with short stretch bandages vs. DLT + IPC with kinesiotape	n=21 DLT n=20 DLT with kinesiotape	LE secondary BCa	Moderate or severe LE (2 cm + difference between arms)	NR	Inclusion: -unilateral LE 3+ months Exclusion: -active Ca -diuretics or other lymphedema influencing drugs -port catheter -skin disease -irremovable bracelet/ring -decrease ROM UE
Wilburn[113] 2006 U.S.	RCT crossover trial with 30 day washout period	IPC Maintenance Therapy Flexitouch™ vs. standard care (self-administered message plus elastic compression garment	n=5 Flexitouch n=5 control	Unilateral, BCa associated LE	≥10% volume increase over normal arm	NR	Exclusion: - bilateral LE of upper extremity - active Ca - active infection - clinical evidence of venous obstruction or active thrombophlebitis - pulmonary edema - congestive heart failure - history of pulmonary embolism - contraindications to the Rx used in study

Table 10. Treatment basic study data (continued)

Study	Study Design	Type of Treatment	Sample Size	Cause of Lymphedema	Definition of Lymphedema	Co-morbidities	Other Inclusion/Exclusion Criteria
Observational Studies							
Frischenschlager[134] Austria	Cohort	MLD + compression stockings + exercise + psychosocial support vs. above treatment without psychosocial	Total n=30 Psychosocial n=15 Control n=15	LE secondary to BCa surgery	NR	NR	Inclusion: -female
Johansson[133] 1999 Sweden	Cohort	CB vs. CB + MLD	n=18 CB group n=20 CB + MLD	Unilateral arm LE after BCa surgery with axillary nodal dissection	≥10% difference in volume between abnormal and normal arm	NR	Exclusion: - contralateral breast disease - intercurrent disease affecting the swollen arm - difficulty participating for reasons such as dementia - Rx within the last 6 mths (except for wearing compression sleeve)

Table 11. Key questions treatment

Study	Question #3 Time of LE Onset Time of Rx initiation Criteria to Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Andersen[115] 2000 Denmark	LE onset: After surgery Time Rx start: ≥4 months from BCa Rx Criteria to start Rx: Unilateral LE of arm after early treatment of breast cancer Criteria to stop Rx: NR	"an experienced and certified lymphothera-pist according to the Vodder school of practice"	Intervention Group: standard care + MLD and training in self-massage. Standard care=custom-made sleeve and glove garment providing 32-40 mmHg compression; educational information and recommendations; instruction in physical exercises; education in skin care. MLD=8 1hr session over 2 wk period Control Group: Standard care as described above (control group was allowed to crossover to treatment group after 3 mths)	Comparator was standard care Usual care	Change in volume of affected arm patient-reported symptoms related to LE No significant difference in arm volume or patient-reported symptoms between the 2 groups	Length of study: 2 weeks Length of followup: 12 months	NR

Abbreviations: AROM=Active Range of Motion; BCa=Breast Cancer; BMI=Body Mass Index; BMSC=Bone Marrow Stomal Cell Transplantation; CB=Low stretch compression bandaging; CDP=Complex Decongestive Physiotherapy; CDT=Complex Decongestive Therapy; DASH=Disability of Arm Shoulder and Hand; DLT=Decongestive Lymphatic Therapy DOT®=Deep Oscillation; ESLD=Electronically Stimulated Lymphatic Drainage; HRQOL=health related quality of life; KS=Karposi's Sarcoma; LC=Limb Circumference; LE=Lymphedema; LLLT=Low-level Laser Therapy; LS=Lymphedema Specialist Nurse; MLD=Manual Lymph Drainage; MPT=mechanical pressure therapy; NR=Not Reported; NS=No Significance; PC=Pneumatic Compression; PCEV=Percentage Change in Excess limb Volume; PML=Post Mastectomy Lymphedema; PT=physical therapist; pts=patients; RT=Radiation Therapy; QoL=Quality of Life; RCT=Randomized Control Trial; ROM=Range of Motion; Rx=Treatment; SEPC=Sequential External Pneumatic Compression; SF-36=short form 36; SLD=Simple Lymphatic Drainage; SP=Standard Physiotherapy; SPC=Sequential Pneumatic Compression; UST=Ultrasound therapy; VAS=visual analogue scale; wk=week; wks=weeks

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Bialoszewski[1] [26] 2009 Poland	LE onset: post leg lengthening surgery Time Rx start: post surgery Criteria to start Rx: LE lower extremity Criteria to stop Rx:NR	NR	Intervention group: Kinesiotaping for 10 days (tape remained on skin 10 days) Control group: lymphatic drainage 1x/day x 10days Both groups had standard physiotherapy (not described)	Kinesiotape vs. lymphatic drainage Consistent with usual care	Limb circumference Kinesiotape resulted in a significant reduction in limb circumference	Length of study: 10 days Length of followup: NR	NR
Carati[33] 2003 Australia	LE onset: NR Time Rx start: NR Criteria to start Rx: Diagnosis of postmastectomy LE Criteria to stop Rx: NR	NR	Intervention Group: LLLT 1 Block (9 sessions, 17 minutes each, 3x/ week x 3 weeks), 8 weeks rest followed by a repeat block of laser Control Group: Sham First Block (9 sessions, 3x week x 3 weeks), 8 weeks rest followed by a block of LLLT	LLLT vs. sham laser treatment Not consistent with usual care	Groups matched at baseline 2 LLLT sessions: 31% of pts had reduction in 2-3 mths time (>200 mls); 1 LLLT session and sham session showed NS Measured by perometry, bioimpedance, tonometry and goniometer	Length of study: 24 months Length of followup: 3 months	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Didem [119] 2005 Turkey	LE onset: LE onset >1 year after surgery Time Rx start: Rx started average of 3 years after surgery Criteria to start Rx: Diagnosis of LE (mild to moderate) Criteria to stop Rx: NR	Physiotherapist	Therapy sessions: 3x/wk x 4 wks Intervention group: CDP (MLD, compression, exercise & skin care) Control group: PT (bandage, elevation, exercises) Both groups: trained for home program of compression bandage, exercise, self message, skin care, and walking	Complex decongestive therapy vs. physiotherapy Consistent with usual care	Circumference volume Range of motion (goniometry) and shoulder function CDP decrease >PT ($p<0.05$). No significant difference between groups ROM	Length of study: 4 weeks Length of followup 3,6,12,&24 months to be reported later	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Hayes[47] 2009 Australia	LE onset: NR Time Rx start:≥6 months after BCa Rx Criteria to start Rx: Finished BCa treatment 6 months prior and have LE Criteria to stop Rx: Occurrence of adverse event	Exercise physiologist Physiotherapist No details provided about qualifications	12 weeks of moderate intensity, aerobic and resistance exercise (supervised) 20-45 min per session (progressed) 3-4x/week (progressed)	NR	Bioimpedance Perometry No significant change between groups	Length of study: 12 weeks Length of followup: 3 months	One person had significant increase in swelling throughout study. Diagnosed with recurrent cancer 6 months after end of study
Hou[105] 2008 China	LE onset: NR Time Rx start: 5 years after surgery Criteria to start Rx: Diagnosis of secondary LE Criteria to stop Rx: NR	Provider qualifications not stated	Intervention group: BMSC one time operation followed by custom garment Control group: CDT (MLD, compression therapy, remedial exercises and deep breathing) details not reported	Stromal cell transplant vs. decongestive therapy BMSC not usual care	Volume (disk model), pain (self report scale) Both groups reduction in volume and pain; BMSC group had better longterm results	Length of study: NR Length of followup: 52 weeks	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Johansson[109] 1998 Sweden	LE onset: Median of 9-10.5 months Time Rx start: Median of 9-10.5 months Criteria to start Rx: Unilateral arm lymphedema Criteria to stop Rx: NR	MLD provided by physiotherapist trained in Vodder technique	Both groups wore a compression sleeve for 2 wks then the MLD group had MLD treatments (Vodder technique) lasting 45 min/day 5 day/wk for 2 wks SPC group were treated with Lympha-Press pump for 2 hrs/day, 5 days/wk for 2 wks	MLD vs. SPC Consistent with usual care	Arm volume body weight passive mobility isometric muscle strength subjective assessment MLD or SPC when applied in conjunction with a compression sleeve resulted in a notable reduction of lymphedema but no significant difference between the two treatment regimes	Length of study: 2.5 years Length of followup: NR	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Kozanoglu[104] 2009 Turkey	LE onset: History of arm LE >3 mo Time Rx start: LE >3 mo Criteria to start Rx: LE defined as difference of ≥2 cm at least 3/7 points Criteria to stop Rx: NR	Physician performed assessments No details of who performed treatment No other details provided	IPC: 2 hrs at 60 mmHg x 20 sessions over 4 wks Laser: 20 min/3x wk x 4 wks (2800Hz, 1.5J/cm2) with a Ga-As 904nm laser device (Electronica Pagani IR27/4) 12 sessions total Both groups daily limb exercises, hygiene and skin care	Pneumatic compression and laser therapy Could be seen as usual care	Limb circumference Visual Analogue Scale Grip strength Significant difference LC and VAS from pretreatment to 12 month followup	Length of study: 4 weeks Length of followup: 12 months	Withdrawals but not mention of adverse events

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Maiya[121] 2008 India	LE onset: NR Time Rx start: 3-6 weeks following mastectomy Criteria to start Rx: Lymphedema defined by 2 cm difference at 2 or more points on upper extremity Criteria to stop Rx: NR	NR	LLLT: (He-Ne Laser-632.8nm and Diode Laser 850nm) at different points in axillary region. 2.4J/cm2 of laser energy per point was given for total of 34 min/day for 10 days After laser, patients performed exercise program for upper extremity (no details given) Control Group: Upper extremity exercises and compression garments for 10 days (no other details provided) Both groups advised to continue their regular daily activity	Upper extremity exercise + compression garments Consistent with usual care	Pain Limb Circumference at 4 points Significant difference mean pain score between groups Significant difference mean circumference at 10 cm and 15 cm LE between groups	Length of study: 10 days Length of followup: NR	"All patients completed the 10 days of treatment without any adverse reactions"

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
McNeely[123] 2004 Canada	LE onset: NR Time Rx start: NR Criteria to start Rx: Diagnosis unilateral LE, mild, moderate or severe, both early and chronic Criteria to stop Rx: NR	MLD provided by Physical Therapist trained in the Vodder method Bandaging by physical therapist assistant	Intervention Group: 45 minutes of daily MLD 5 days/week x 4 weeks + bandaging each day Control Group: short stretch bandaging 5 days/week x 4 weeks Both groups educated on proper arm and skin care	Vodder MLD + short stretch bandaging vs. short stretch bandaging alone Consistent with usual care	Volume (volumetry) circumference significant reduction in both groups; but between groups: NS largest reduction in MLD/CB group with early, mild LE	Length of study: 4 weeks Length of followup: None	1 pt withdrew due to skin reaction, 1pt due to discomfort of bandages
Pilch[125] 2009 Poland	LE onset: NR Time Rx start: NR Criteria to start Rx: LE following BCa Criteria to stop Rx: NR	NR	All groups had 1 hour Rx, 5x/week for 5 weeks Group 1: single chamber, 90sec on: 90 sec off compression Group 2: 3 chamber, 90sec on: 90 sec off Group 3: single chamber, 45 sec on: 15 sec off Group 4: 3 chamber, 45 sec on: 15 sec off	Single chamber vs. multichamber IPC and timing of pressure application Consistent with usual care	Change in arm volume (volumetry) All four groups had significant decrease in LE volume No significant difference between groups	Length of study: 5 weeks Length of followup: none	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Schmitz[48] 2009 U.S *companion Schmitz[138]	LE onset: NR Time Rx start: 1-15 years after BCa Criteria to start Rx: unilateral BCa with nodes removed and LE Criteria to stop Rx: LE exacerbation or Ca recurrence	Certified lymphedema therapists Fitness trainers with knowledge of lymphedema	Intervention group: 13 weeks supervised weight lifting, 90 min x 2/week; then 39 weeks unsupervised weight lifting; patients wore compression garment during exercise Control group: No prescribed exercise	Weight lifting vs. no weight lifting Consistent with usual care 1 yr fitness membership	Change in arm and hand volume at one year; LE exacerbation; muscle strength No difference between groups limb swelling Exercise group had increased strength, less LE exacerbations	Length of study: 12 months Length of followup: 1 year	No serious adverse events
Shaw [116] 2007 U.K.	LE onset: NR Time Rx start: Rx onset ≥12 months after CT or RT Criteria to start Rx: Diagnosis of arm LE Criteria to stop Rx: Completion of regimen	Dietary intervention provided by registered dietitian conventional therapy provided by trained LE nurses	Intervention Group: Individualized dietary advice given on weight reduction diet; dietary compliance assessed at week 4 and week 8 visits Control Group: Healthy eating booklet with no specific dietary intervention	Dietary intervention consistent with usual care	Changes in arm volume (measured manually) Significant change in lymphedema arm (7% ± 6%) vs. normal arm (3% ± 6%) in dietary group	Length of study: 12 weeks Length of followup: NR	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Sitzia [122] 2002 U.K.	LE onset: NR Time Rx start: NR Criteria to start Rx: Secondary arm LE after BCa surgery Criteria to stop Rx: NR	Lymphedema specialist Nurse (LS) MLD training (2 year diploma) in specialist management of chronic edema	Intervention Group: MLD: 40 - 80 minutes 5 x week x 2 weeks Control Group: SLD: 20 minutes 5x week x2 weeks	MLD vs. SLD Consistent with usual care	Change in excess limb volume (PCEV) MLD: 33.8%, SLD: 22.0% (mean difference 11.8%)	Length of study: 2 weeks Length of followup: NR	NR
Szuba[111] 2002 U.S.	Study 2: LE onset: NR Time Rx start: between 1 month and 1 yr post intensive decongestive lymphatic therapy (DLT) Criteria to start Rx: diagnosis of secondary LE Criteria to stop Rx: NR	MLD performed according to Vodder School technique (no details on Rx providers)	Study 2 (2 month-groups switched treatment after 1 month): Group 1: daily self-administered MLD and use of Class II compression garment Group 2: same + 1 hr daily IPC (40-50 mm Hg)	Study 2: DLT alone (regular care)	Study 2: arm volume, skin elasticity (tissue tonometry) joint range of motion (goniometry) IPC was effective as adjunct therapy; there was no impact on skin elasticity or joint ROM	Length of study: 2 months Length of followup: 6 months	Study 2: No adverse responses

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators In Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Tsai[126] 2009 China	LE onset: 3+ months post BCa treatment Time Rx start: after 4 weeks control period Criteria to start: LE moderate-severe Criteria to stop: NR	Physical therapists	Both groups received treatment 2h/day, 5x/week for 4 weeks: education on skin care; 30 min MLD, IPC x 1hour at 40mmHg, 20 min exercise and bandaging Intervention group: bandaging was done with kinesiotape Control group: bandaging with short stretch bandages	Kinesiotape and short stretch bandages Consistent with usual care	Limb volume (volumetry) and arm circumference; water content of limb; EORTC questionnaire; time bandages worn daily No significant difference in arm volume or circumference between two groups; kinesiotape better accepted and longer wear time	Length of study: 8 weeks Length of followup: 3 months	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
RCT's							
Williams[114] 2002 U.K.	LE onset: LE >3 mths Time Rx start: LE >3 mths Criteria to start Rx: Diagnosis of secondary LE Criteria to stop Rx: NR	Therapists qualified in Vodder method of MLD SLD performed by participants (self) after training	Group A: 3 wks (5x wk) daily 45 min MLD treatment followed by 6 wks no treatment followed by 3 wks daily 20 min SLD treatment Group B: 3 wks daily 20 min SLD treatment followed by 6 wks no treatment followed by 3 wks (5x wk) daily 45 min MLD treatment	Both groups same treatment but in reverse order; SLD was comparator treatment Usual Care	Limb volume, caliper creep, dermal thickness, QoL, altered symptoms/ sensations MLD reduced volume, dermal thickness, improved some QoL measures and some symptoms/ sensations	Length of study: 12 weeks Length of followup: NR	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
Observational Studies							
Berlin[130] 1999 Sweden	LE onset: NR Time Rx start: NR Criteria to start Rx: Diagnosis of secondary LE Criteria to stop Rx: NR	NR	Group 1: Arm compression stockings only for minimum of 4 wks compression used varied between 25 and 50 mmHg Group 2: Intermittent compression with Flowtron used at least 20 min/day minimum 4 wks Group 3: Pneumatic compression with Lympha Press–pressure 90-120 mmHg for 20-30 min 2x/day 5 day/wk Patients also received compression stockings	1)compression with sleeves 2) intermittent compression with Flowtron 3) intermittent compression Lympha-Press + compression sleeves Not consistent with usual care	Group 1: Arm compression stockings only for minimum of 4 wks. Compression used varied between 25 and 50 mmHg Group 2: Intermittent compression with Flowtron used at least 20 min/day minimum 4wk Group 3: Pneumatic compression with Lympha Press-pressure 90-120 mmHg for 20-30 min 2x/day 5 day/wk Patients also received compression stockings	Length of study: 5 years Length of followup: NR	NR

Table 11. Key questions treatment (continued)

Study	Question #3 Time of LE Onset Time of Rx Initiation Criteria To Start/stop Rx	Question #4 Provider of Treatment and Qualifications	Question #6 Treatment Parameters	Question #8 Comparators in Study Consistent With Usual Care	Question #9 Patient Outcomes Results	Question #11 Length of Study Length of Followup	Question #12 Did any Harms (adverse events) Occur From Rx?
Observational Studies							
Johansson[133] 1999 Sweden	LE onset: NR Time Rx start: NR Criteria to start Rx: Diagnosis of secondary unilateral LE Criteria to stop Rx: Therapy stopped with resolution of arm swelling	Physiotherapist trained in bandaging and in the Vodder MLD technique	Part 1: Both groups received 2 weeks of CB (bandage changed every 2nd day) Part 2: During the 3rd week both groups continued CB but one group also received MLD 45 min/day x 5 days	CB alone vs. CB + MLD Consistent with usual care	Arm volume body weight subjective assessment CB + MLD group had significant difference % volume reduction	Length of study: 19 days Length of followup: NR	NR
Pinell[131] 2007 U.S.	LE onset: NR Time Rx start: NR Criteria to start Rx: Secondary LE with or without axillary or inguinal disease Criteria to stop Rx: NR	Certified therapist	Intervention group: Skin and nail care, multilayer compression bandages worn at all times and decongestive exercise, MLD 60 - 90 minute/day. Modified MLD technique for patients with axillary or inguinal disease at time of therapy Control group: Same as intervention group without modification	Manipulative therapy (MLD) plus compression bandages Consistent with usual care	Interval measurements of girth along affected limb and computation of volume	Length of study: 39 months Length of followup: NR	NR

Table 12. IPC treatment (continued)

Study	Question #5a		Question #5b		Question #5c
	Type of IPC Device	Pumping Time/Cycles and Pressures	Co-morbidities of Patients	Modification of Protocol for Co-morbidities	Timing of IPC Application
RCT's					
Pilch[125] 2009 Poland	Flowtron Plus Flowtron Flowpac Plus	Flowtron Plus/Flowtron Flowpac Plus Pump Time: 1 hour x 5 days week for 5 weeks Cycle: 1 cycle over 5 weeks Pressure: 30-50 mmHg (single chamber or multi chamber) Flowtron Plus 90sec on, 90 sec off Flowtron Flowpac Plus 45 sec: 15 sec	NR	NR	NR
Radakovic[103] 1998 Yugoslavia	Sleeve with 12 overlapping compression chambers	Pump Time: 60 min/day Cycle: 1/day x 10 days Pressure: 60 - 65 mbar (gradually activated over 7 min)	NR	NR	NR
Szuba[111] 2002 U.S.	Sequential Circulator 2004: (BioCompression Systems Inc)	Pump Time: 60 min/day Cycle: 1 month Pressure: 40-50 mmHg (4 chamber, sequential)	NR	NR	Average duration LE 5 yrs
Szuba[108] 2002 U.S.	Sequential Circulator 2004: (BioCompression Systems Inc)	Pump Time: 30 min/day Cycle: Daily: 10 days Pressure: 40-50 mmHg (4 chamber, sequential)	NR	NR	12 weeks following cancer treatment
Wilburn[113] 2006 U.S.	Flexitouch™ Type: Lightweight portable device for home use consisting of up to 32 separate chambers	Pump Time: 1 hour/day Cycle: 1/day x 14 days Pressure:1-3 seconds of mild pressure	NR	NR	5 years of LE onset

diagnose lymphedema. However, few of the extracted studies included ultrasound or lymphoscintigraphy; studies that included these imaging tests did not consider them to be gold standards. Rather, these tests were evaluated as index tests (the tests under investigation).

d) What is the sensitivity and specificity of tests used to diagnose lymphedema?

In the eight studies that contained examinations of the sensitivity and specificity of diagnostic tests for secondary lymphedema, sensitivities ranged from 5 to 100 percent and specificities ranged from 71 to 100 percent. However, it was not possible to rank order the tests in terms of performance because there were too few studies from which to permit generalization, persons with three different underlying conditions were the subject of the studies (breast cancer, melanoma, Kaposi's Sarcoma), a mix of different tests were used (changes in circumference with different cut points, self reports, imaging), and several different reference standards were also used. Researchers must use a common reference standard as a first step to providing a clearer picture of the sensitivity and specificity of tests in persons with secondary lymphedema.

e) What are the psychometric properties (reliability, validity, responsiveness) of these diagnostic methods?

Reliability. There is consistent evidence to indicate that lymphedema can be reliably measured using circumferential measures or volume displacement. It should be noted that these studies were conducted in breast cancer patients with secondary lymphedema primarily in the upper extremities. One study pertained to the trunk of the body.[86] The excellent reliability of these measures might not be transferable to situations where lymphedema occurs in other parts of the body, or to cases where lymphedema occurs secondarily to other diseases besides breast cancer.

There is too little evidence to draw conclusions about the reliability of other tests such as tonometry, ultrasound, lymphoscintigraphy, or bioimpedance.

Validity. Twenty-three of the 30 validity studies involved some use of volume displacement or limb circumference as a diagnostic test for secondary lymphedema. Based on consistently high correlation coefficients, there is strong evidence that displacement and circumference are interchangeable amongst one another in terms of results. This interchangeability applies despite the various means of measuring displacement or circumference.

Tests involving bioimpedance show good validity when compared to tape measured circumference or perometry, although the correlation coefficients were not as high as the coefficients in the displacement-circumference comparisons. Self reported symptoms on the Lymphedema and Breast Cancer Questionnaire (LBCQ) also show good validity in comparison to bioimpedance, perometer, and tape measure, although the evidence is limited to a single study and correlation coefficients were also lower than the ones calculated for the displacement-circumference comparisons.[71]

The validity of ultrasound, lymphoscinitgraphy, CT scan, or MRI was evaluated in four studies. There is little evidence for the validity of these tests owing to the limited number of studies, small sample sizes, a questionable reference standard in one study,[92] and questionable means of scoring lymphoscinitgraphy in two studies.[81,92]

Given the limited extent to which the LBCQ has been examined as a diagnostic tool for secondary lymphedema, and the lower correlation coefficients found in the bioimpedance studies, the evidence suggests that volume displacement or limb circumference are the most valid means of diagnosing secondary lymphedema. Most of the validity studies included breast cancer

Treatment

Question 2. What were the patient selection criteria in the studies (inclusion and exclusion criteria)? Did they differ by treatment modality?

There were multiple inclusion and exclusion criteria spread across the 36 studies. There was no grouping of criteria attached to any specific treatment modality. Consequently, there is no evidence to suggest that patient selection criteria differed by treatment modality.

Question 3. What were the criteria used to initiate treatment for lymphedema? When was treatment initiated compared to the time of onset of the lymphedema? What were the criteria used to stop therapy? Did these criteria vary with treatment modality?

In all 36 treatment studies extracted for this report, a diagnosis of lymphedema was the only specific criteria used to initiate treatment. Only seven studies reported the approximate time of recruitment following onset of lymphedema. All except one of these studies began recruitment within 1 year of onset, but there was a wide range of recruiting times within this 1 year period. It should be noted that these are recruitment times following onset of lymphedema, not the times to initiation of treatment. Although it is likely that lymphedema treatment was initiated soon after recruitment, the time frame between recruitment and initiation was not reported in any article. Therefore, no evidence exists to provide a clear answer to the question about time of treatment initiation.

Only five studies reported specific criteria to stop treatment.[47,110,112,116,133] This number is too small to assess whether stopping criteria varied with treatment modality in the 36 studies that were extracted for this report.

Question 4. Who provided the treatments in the studies? What information was provided on their professional training or certification in lymphedema care?

The authors of 17 of the 36 treatment studies did not detail who provided the lymphedema treatment. Except in the case of patient self-massage, the provision of lymphedema treatment requires a trained professional such as a physiotherapist or a technician familiar with the operation of an IPC device. To enhance reporting, as well as to facilitate judgments about the generalizability of published studies, authors of future studies in the domain of lymphedema treatment should report on the professional status and qualifications of the persons delivering lymphedema therapy.

Question 5a. Was one type of pneumatic compression device and sleeve (e.g., non-segmented compression device, sequential segmented compression, or segmented compression with calibrated gradient pressure) more effective in reducing lymphedema than another for any type of lymphedema along the continuum, or patient characteristic (e.g., demographics, comorbidities)?

Twelve studies included IPC treatment.[50,103,104,108-113,130] There was no evidence from which to determine whether one type of IPC device and sleeve were more effective than others were across the continuum. The lack of evidence was partly a result of the fact that there were simply too few studies from which to conduct meaningful comparisons. Comparison was mainly inhibited by the degree of heterogeneity between articles: nine different types of IPC were investigated against multiple comparators. Comparators included MLD,[108,109,111] compression

There is no evidence to address whether the timing of the IPC application might have influenced the study outcomes. Six of the studies did not contain reports on timing.[50,103,104,110,125,128,130] In the other six studies, the treatment regimens were too heterogeneous to allow for the isolation of any potential effect of timing. For sequence of use, the conclusion is the same: the mix of different treatments does not permit the isolation of the effect of sequence. Hence, there is no evidence for sequence as well. Additionally, there are simply too few studies from which to establish definitive patterns about timing or sequence.

Question 6. What protocols for single modality treatments resulted in the best outcomes of lymphedema therapy? Consider parameters such as usage schedules and characteristics of treatment such as intensity, duration, frequency and setting (self-administered at home versus professionally administered applied in a medical clinic), and if applicable pumping times/cycles and pressures.

There were 12 studies that examined single modality treatments for lymphedema. This reflects the fact that most lymphedema treatment is delivered as some form of combination therapy. Most of the studies adopted unrealistic comparators to maintain the 'single modality' distinction. For example, it is unlikely that persons with secondary lymphedema would be treated in standard clinical practice with only a booklet on healthy eating[116] or instructions to continue with usual activities,[47,117] or no treatment whatsoever.[127,132] By the same token, the use of sham laser[33,118] is questionable because there is no actual treatment given to patients. Ideally, the comparator should be the standard, medically-accepted treatment for lymphedema in the locality where researchers are conducting the study.

Notwithstanding the above, there was no evidence from which to ascertain whether certain treatment protocols would lead to better outcomes. Certainly this was the case for exercise[47,48,129] and elastic stockings,[132] where too few studies of each treatment negated any ability to compare protocols. For the other treatments (diet, laser, IPC, bandage, tape), the number of studies of each treatment was also not sufficient to investigate the effect of protocol differences on outcomes.

To address the effect of protocol on outcome, a series of studies with nearly identical samples, lengths of followup, comparator therapies, and outcomes would need to be constructed, with the only difference being the protocol used to deliver the treatment of interest. In the diet, laser, and IPC studies, there was little standardization in most of these areas (with the exception of the definition of outcome). Additionally, epidemiologic and statistical issues such as bias and power would have to be addressed in the design and analysis of the studies to increase the confidence in results. In one RCT, placebo patients were allowed to cross over to the active treatment group and analyses were conducted with these 'crossovers' included in both groups. Also, all of the studies except one[48] had fewer than 100 participants. From a methodological perspective, there is simply too much noise from which to tease out the signal of a protocol effect on outcome.

Question 7: Were there any treatments, combinations of treatment methods, or sequence of treatments shown to be more effective or ineffective for any type of lymphedema along the continuum, or patient characteristics (e.g., demographics, comorbidities)? Of particular interest: Is there evidence that the use of compression sleeves or low stretch bandaging is effective in maintaining reductions in lymphedema achieved through the use of other modalities (e.g., IPC, manual lymphatic drainage, exercise)?

Question 9: What outcomes were measured in studies of lymphedema therapy? How effective were these treatment methods in reducing lymphedema?

Multiple outcomes were used in these reports. Objective measurements, usually relating to volume were most reported.

Many studies reported that treatment brought about a reduction in lymphedema volume. However, relative benefit is difficult to appreciate in that despite studies including comparator groups, some provided pre- and post-treatment assessments of each group but did not provide between group comparisons.[111,131] Other studies provided p values for comparisons but did not report point estimates in differences of benefit[110,112,130,132] leaving clinicians to question the clinical benefit of treatment. Even in those studies that did, report point estimates of benefit, the specific outcomes reported varied such that cross comparisons were difficult to make. For instance, arm measurements may have resulted in reporting of circumference differences,[103] percent volume loss,[122] absolute volume loss,[109] or proportion of patients achieving a pre-specified degree of benefit.[48,110]

Question 10: Did any studies show that the *time* of treatment initiation (single modality or combination therapy) relative to symptom onset, any other lymphedema characteristics, or any patient characteristics influenced or predicted treatment outcome?

Since few studies were sufficiently powered to detect a difference in the primary outcome, most trials were did not have power to detect differences in patient subgroups which were predictive for response. Few trials randomized patients with a stratification scheme or performed adjusted analyses to allow for detection of predictive factors. One RCT with 141 subjects specifically looked at differences according to cancer severity, race, physical activity, diet, and BMI and found no effect.[48]

Question 11: What was the length of followup in studies of lymphedema therapy? How long were the benefits of treatment maintained?

Considering the chronicity of lymphedema, very few trials performed long term followup in their study population. Only eight trials reported outcomes at 6 months or beyond.[48,50,104,105,110,111,115,132] One study reported outcomes at week 30 of the study, but this was only 12 weeks from the last treatment.[33] There was no consistent evidence regarding the length to which treatment benefits could be maintained.

Question 12: What harms have been reported associated with the various treatments for lymphedema? Do any patient characteristics (e.g., demographics, comorbidities) or etiology of lymphedema increase the risk of these harms?

Due to the nature of these studies, it was not always possible to delineate which patients were in which treatment group, preventing readers from drawing conclusions about the relative harms of various treatments. The majority of withdrawals and adverse events were related to treatment scheduling or disease recurrence, neither of which would be the direct result of therapy. Adverse events likely related to study therapy were all rare.

Even if all adverse events were in the treatment groups, their infrequency would be unlikely to result in statistical significance if formally tested. No studies reported on factors which may increase the risk of harms associated with treatment. There was no evidence in the extracted studies to answer this question.

Overall, the 5 non English diagnosis studies did not contribute substantive new information to the TA. The studies were conducted in the familiar population of breast cancer patients and the tests had all been evaluated in English language publications. The correlation between ultrasound and arm circumference in one non English study[142] was lower than many of the reported correlations in the English literature, but this could be a function of the specifics of one study rather than an indication of a new and important finding. Another non English study[141] addressed two key questions that were not covered in the English literature; however no general conclusions can be drawn from a single observational study of only two diagnostic tests.

Treatment

The 8 treatment studies were observational, with sample sizes ranging from 30[144,145] to 440.[146] Lengths of followup, in the studies where authors reported such information, ranged from 28 days[144] to 10 years.[146] All 8 studies involved breast cancer survivors with upper limb lymphedema. The authors of only one report specified the type of individual who provided therapy, i.e., a 'kinesiology therapist'.[147]

Three studies examined single modality treatments. The first study compared an education program for self administered MLD against an unspecified control treatment over 6 weeks of followup. Arm function, measured using a vaguely described questionnaire, was better in the treated group over the entire followup period ($p<0.05$).[148] In the second study, three groups were compared over 28 days: MLD via the 'Asdonk standard' for 1 hour/day; non Asdonk standard MLD for 1.5 hours/day; non Asdonk standard MLD for 1 hour/day. Although the author describes the Asdonk standard method, there are no references to this method given in the published manuscript.[144] The author reports a greater reduction in arm volume in the Asdonk group versus the other groups over the course of followup, but does not provide p-values or other quantitative statistics. The third study of single modality treatments compared single chamber IPC using a Jobst machine with multi chamber IPC using a Lymph-a-mat machine. Lymphedema severity decreased within each group over an unspecified followup time (described as 'beginning' and 'end' in the published figures); however, there was no statistically significant difference between groups at the 5% level.[149]

Three studies investigated multi modal treatments. The first study compared multi layer bandaging and MLD to simplified bandaging and MLD. Simplified bandaging, which was considered the active treatment, was 2 bandages juxtaposed one over the other rather than a more complex method of wrapping multiple bandages. After 12 to 16 sessions over 4 weeks, the group treated with simplified bandaging in addition to MLD showed larger decreases in edema relative to the comparison group ($p = 0.04$).[147] The second study involved a combination treatment of MLD, IPC, and exercise in two groups, with one of the groups given additional treatment in the form of bandage. The authors provide intragroup comparisons over time, but do not provide intergroup comparisons.[145] The third study involved a 10 year followup of 440 persons assigned nonrandomly to one of four groups. Each group received IPC at 40 to 110mmHg for 15 sessions lasting 1 hour each. Treatment was limited to IPC in one group, but treatment was IPC plus electrostimulation of muscles in a second group, IPC plus magnetic therapy in a third group, and IPC plus both electrostimulation and magnetic therapy in a fourth group. Percent changes in limb volume were highest in the fourth group, with all results statistically significantly different at the 5% level.[146]

ROC curve, perhaps due to the lack of agreement on a gold standard means of diagnosing lymphedema.

There was no evidence to suggest an adequate diagnostic testing protocol. The extracted studies failed to provide an indication of suitable frequencies of testing or time spans within which testing should be done. Additionally, there was no information to suggest whether the type of diagnostic test would have an effect on the choice of treatment or on patient outcomes.

Regarding treatment for secondary lymphedema, there was no evidence concerning the optimal criteria to initiate or stop treatment. While the studies suggested that most treatments did reduce the size of the lymphatic limb, there was too much heterogeneity in terms of therapy, inclusion, and exclusion criteria, and treatment protocols to suggest the optimality of one type of treatment over another. Despite the multiplicity of inclusion and exclusion criteria, almost no studies contained reports of treatment benefits in any subgroup of patients. In fact, most studies were not designed to look for treatment benefits in subgroups.

Adverse effects were only reported in a small number of studies. The adverse effects that were reported were generally rare and mild, and unlikely to be a major clinical issue.

The methodological quality of the extracted diagnosis and treatment studies was generally 'fair'. Many quality issues may have been related to a lack of adequate reporting rather than to methodological shortcomings in the conduct of the research. However, the authors of some studies omitted the reporting of fundamental aspects of their research. For example, there were reliability articles that did not contain mention of the intervals between administrations of the tests of interest, none of the validity studies indicated whether index test results were interpreted without knowledge of reference test results, and the majority of RCTs did not include comment on whether outcome assessors were blinded. While reporting oversight may be one reason for these omissions, the fundamental nature of the omitted elements suggests a certain degree of caution should be exercised when interpreting study results. This suggestion reflects a degree of healthy skepticism in the assessment of scientific research, i.e., to assume inadequate quality unless the study authors present evidence to the contrary.[102]

Although the quality of the extracted articles suggests the need for a guarded approach to interpreting results, quality did not appear to play a major role the answers to the key questions. The articles were far too heterogeneous in terms of test, treatment, and outcome to ascertain whether studies of a certain quality tended to group around any particular test, treatment, or outcome. Indeed, most of the studies were of 'fair' quality anyway, which suggests that quality was not a major factor in the response or interpretation of the key questions.

In looking at the extracted articles as a whole, it can be concluded that there is no evidence in the literature to suggest an optimal diagnostic testing protocol, an optimal frequency or duration of treatment, the most efficacious treatment combinations (including the use of maintenance therapy), and the length of time for which persons should be tested or treated for lymphedema.

Recommendations for Future Research

Diagnostic Testing. Prior to the initiation of further research into diagnostic testing, clinicians and researchers in the field of secondary lymphedema need to agree on a uniform, gold standard, diagnostic test. Existing work suggests that limb volume or circumference has already emerged as the de facto gold standard, but a set means of measuring volume or circumference should be adopted by the clinical and research communities. Ideally, this set means should be accessible by clinical and research centers globally to promote uniformity. If the strong validity

Most of the studies extracted for this report involved lymphedema to the upper extremities. Lower limb lymphedema was not well represented in the studies, despite its high incidence from cancer treatment.[20] More RCTs should be conducted in patients with secondary lymphedema in the lower limbs.

Although a great deal of research into the diagnosis and treatment of secondary lymphedema has already been undertaken, there is no evidence to suggest the optimal diagnostic test or treatment. Similarly, there is no evidence to suggest whether certain tests or treatments may benefit some types of patients more than others may. The field of research into secondary lymphedema is ripe for advancement and the contents of this report may serve as a springboard to guide future scientific endeavors in this domain.

22. Norman SA, Localio AR, Potashnik SL, et al. Lymphedema in breast cancer survivors: incidence, degree, time course, treatment, and symptoms. J Clin Oncol 2009;27(3):390-7. PMID:19064976
23. Witte CL, Witte MH, Unger EC, et al. Advances in imaging of lymph flow disorders. [Review] [34 refs]. Radiographics 2000;20(6):1697-719. PMID:11112825
24. Dabrowski J, Merkert R, Kusmierek J. Optimized lymphoscintigraphy and diagnostics of lymphatic oedema of the lower extremities. Nucl Med Rev 2008;11(1):26-9. PMID:19173185
25. O'Mahony S, Rose SL, Chilvers AJ, et al. Finding an optimal method for imaging lymphatic vessels of the upper limb. Eur J Nucl Med Mol Imaging 2004;31(4):555-63. PMID:14722673
26. Balzarini A, Milella M, Civelli E, et al. Ultrasonography of arm edema after axillary dissection for breast cancer: a preliminary study. Lymphology 2001;34:152-5. PMID:11783592
27. Armer JM, Stewart BR. A comparison of four diagnostic criteria for lymphedema in a post-breast cancer population. Lymphat Res Biol 2005;3(4):208-17. PMID:16379589
28. Taylor R, Jayasinghe UW, Koelmeyer L, et al. Reliability and validity of arm volume measurements for assessment of lymphedema. Phys Ther 2006;86(2):205-14. PMID:16445334
29. Kato S, Shimoda H, Ji RC, et al. Lymphangiogenesis and expression of specific molecules as lymphatic endothelial cell markers. [Review] [124 refs]. Anatomical Science International 2006;81(2):71-83. PMID:16800291
30. Deltombe T, Jamart J, Recloux S, et al. Reliability and limits of agreement of circumferential, water displacement, and optoelectronic volumetry in the measurement of upper limb lymphedema. Lymphology 2007;40(1):26-34. PMID:17539462
31. Chen Y, Tsai H, Hung H, et al. Reliability study of measurements for lymphedema in breast cancer patients. Am J Phys Med Rehabil 2008;87(1):33-8. Publisher URL: www.cinahl.com/cgi-bin/refsvc?jid=113&accno=2009858783;http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=2009858783&site=ehost-live
32. Gerber LH. A review of measures of lymphedema. [Review] [12 refs]. Cancer 1998;83(12:Suppl American):2803-4. PMID:9874401
33. Carati CJ, Anderson SN, Gannon BJ, et al. Treatment of postmastectomy lymphedema with low-level laser therapy: a double blind, placebo-controlled trial.[erratum appears in Cancer. 2003 Dec 15;98(12):2742]. Cancer 2003;98(6):1114-22. PMID:12973834
34. Nuutinen J, Ikaheimo R, Lahtinen T. Validation of a new dielectric device to assess changes of tissue water in skin and subcutaneous fat. Physiol Meas 2004;25(2):447-54. ISI:000221075000006
35. Cornish B. Bioimpedance analysis: scientific background. Lymphat Res Biol 2006;4(1):47-50. PMID:16569208
36. Armer JM, Radina ME, Porock D, et al. Predicting breast cancer-related lymphedema using self-reported symptoms. Nurs Res 2003;52(6):370-9. PMID:14639083
37. U.S Food and Drug Administration. Product Classification:System, Nuclear Magnetic Resonance Imaging. Last Accessed July 16, 2009. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPCD/classification.cfm?ID=4624
38. U.S Food and Drug Administration. FDA 510k premarket notification: ultrasound. Last Accessed July 16, 2009. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPCD/classification.cfm?ID=4564
39. U.S Food and Drug Administration. FDA 510K premarket notification: Analyzer Body Composition. Last Accessed July 16, 2009. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=19276
40. U.S Food and Drug Administration. FDA 510k premarket notification: monitor extracellular fluid, lymphedema, extremity. Last Accessed July 16, 2009. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=27442
41. Morgan CL. Medical Management of Lymphedema (Chapter 5). In: Lymphedema: Diagnosis and Treatment, London: Springer-Verlag London Limited; 2008. 5 p. 43-53.
42. Cheville AL, McGarvey CL, Petrek JA, et al. Lymphedema management. [Review] [107 refs]. Semin Radiat Oncol 2003;13(3):290-301. PMID:12903017
43. Morris RJ. Intermittent pneumatic compression - systems and applications. [Review] [98 refs]. J Med Eng Technol 2008;32(3):179-88. PMID:18432465
44. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema. Consensus document of the International Society of Lymphology. [Review] [5 refs]. Lymphology 2003;36(2):84-91. PMID:12926833
45. Bernas M, Witte M, Kriederman B, et al. Massage therapy in the treatment of lymphedema. Rationale, results, and applications. [Review] [76 refs]. IEEE Eng Med Biol Mag 2005;24(2):58-68. PMID:15825847
46. Zuther, J. Traditional massage therapy in the treatment and management of lymphedema.

72. Peer FI, Pui MH, Mosam A, et al. 99mTc-MIBI imaging of cutaneous AIDS-associated Kaposi's sarcoma. Int J Dermatol 2007;46(2):166-71. PMID:17269969
73. Damstra RJ, Glazenburg EJ, Hop WC. Validation of the inverse water volumetry method: A new gold standard for arm volume measurements. Breast Cancer Res Treat 2006;99(3):267-73. PMID:16752072
74. Hayes S, Cornish B, Newman B. Comparison of methods to diagnose lymphoedema among breast cancer survivors: 6-month follow-up. Breast Cancer Res Treat 2005;89(3):221-6. PMID:15754119
75. Mirnajafi A, Moseley A, Piller N. A new technique for measuring skin changes of patients with chronic postmastectomy lymphedema. Lymphat Res Biol 2004;2(2):82-5. PMID:15615489
76. Mellor RH, Bush NL, Stanton AW, et al. Dual-frequency ultrasound examination of skin and subcutis thickness in breast cancer-related lymphedema. Breast J 2004;10(6):496-503. PMID:15569205
77. Meijer RS, Rietman JS, Geertzen JH, et al. Validity and intra- and interobserver reliability of an indirect volume measurements in patients with upper extremity lymphedema. Lymphology 2004;37(3):127-33. PMID:15560108
78. Bland KL, Perczyk R, Du W, et al. Can a practicing surgeon detect early lymphedema reliably? Am J Surg 2003;186(5):509-13. PMID:14599616
79. Karges JR, Mark BE, Stikeleather SJ, et al. Concurrent validity of upper-extremity volume estimates: comparison of calculated volume derived from girth measurements and water displacement volume. Physical Therapy 2003;83(2):134-45. PMID:12564949
80. Moseley A, Piller N, Carati C. Combined opto-electronic perometry and bioimpedance to measure objectively the effectiveness of a new treatment intervention for chronic secondary leg lymphedema. Lymphology 2002;35(4):136-43. PMID:12570322
81. Szuba A, Strauss W, Sirsikar SP, et al. Quantitative radionuclide lymphoscintigraphy predicts outcome of manual lymphatic therapy in breast cancer-related lymphedema of the upper extremity. Nucl Med Commun 2002;23(12):1171-5. PMID:12464781
82. Megens AM, Harris SR, Kim-Sing C, et al. Measurement of upper extremity volume in women after axillary dissection for breast cancer. Arch Phys Med Rehabil 2001;82(12):1639-44. PMID:11733875
83. Norman SA, Miller LT, Erikson HB, et al. Development and validation of a telephone questionnaire to characterize lymphedema in women treated for breast cancer. Phys Ther 2001;81(6):1192-205. PMID:11380275
84. Latchford S, Casley-Smith JR. Estimating limb volumes and alterations in peripheral edema from circumferences measured at different intervals. Lymphology 1997;30(4):161-4. PMID:9476247
85. Cornish BH, Bunce IH, Ward LC, et al. Bioelectrical impedance for monitoring the efficacy of lymphoedema treatment programmes. Breast Cancer Res Treat 1996;38(2):169-76. PMID:8861835
86. Roberts CC, Levick JR, Stanton AW, et al. Assessment of truncal edema following breast cancer treatment using modified Harpenden skinfold calipers. Lymphology 1995;28(2):78-88. PMID:7564495
87. Ward LC, Bunce IH, Cornish BH, et al. Multi-frequency bioelectrical impedance augments the diagnosis and management of lymphoedema in post-mastectomy patients. Eur J Clin Invest 1992;22(11):751-4. PMID:1478244
88. Bates DO, Levick JR, Mortimer PS. Subcutaneous interstitial fluid pressure and arm volume in lymphoedema. International Journal of Microcirculation: Clinical & Experimental 1992;11(4):359-73. PMID:1459796
89. Tewari N, Gill PG, Bochner MA, et al. Comparison of volume displacement versus circumferential arm measurements for lymphoedema: Implications for the SNAC trial. ANZ J Surg 2008;78(10):889-93. PMID:18959643
90. Halaska MJ, Komarek V, Mala I, et al. A method for the detection of post-operative lymphoedema after operation for breast cancer: Multifrequency bioelectrical impedance analysis. J Appl Biomed 2006;4(4):179-85.
91. Stanton AWB, Northfield JW, Holroyd B, et al. Validation of an opeoelectronic lomb volumeter (perometer). Lymphology 1997;30(2):77-97.
92. Gebousky P, Karny M, Krizova H, et al. Staging of upper limb lymphedema from routine lymphoscintigraphic examinations. Comput Biol Med 2009;39(1):1-7. PMID:19041964
93. Mayrovitz HN, Davey S, Shapiro E. Local tissue water changes assessed by tissue dielectric constant: Single measurements versus averaging of multiple measurements. Lymphology 2008;41(4):186-8. PMID:19306665
94. Mayrovitz HN, Davey S, Shapiro E. Suitability of single tissue dielectric constant measurements to assess local tissue water in normal and lymphedematous skin. Clin Physiol Funct Imaging 2009;29(2):123-7. PMID:19076729
95. Cornish BH, Chapman C, Hirst C, et al. Early diagnosis of lymphoedema using multiple

postmastectomy lymphedema. Lasers Med Sci 2006;21(2):90-4. PMID:16673054
119. Didem K, Ufuk YS, Serdar S, et al. The comparison of two different physiotherapy methods in treatment of lymphedema after breast surgery. Breast Cancer Res Treat 2005;93(1):49-54. PMID:16184458
120. Jahr S, Schoppe B, Reisshauer A. Effect of treatment with low-intensity and extremely low-frequency electrostatic fields (deep oscillation) on breast tissue and pain in patients with secondary breast lymphoedema. J Rehabil Med 2008;40(8):645-50. PMID:19020698
121. Maiya AG, Olivia ED, Dibya A. Effect of low energy laser therapy in the management of post-mastectomy lymphoedema. Physiotherapy Singapore 2008;11(1):2-5.
122. Sitzia J, Sobrido L, Harlow W. Manual lymphatic drainage compared with simple lymphatic drainage in the treatment of post-mastectomy lymphoedema. Physiotherapy 2002;88(2):99-107.
123. McNeely ML, Magee DJ, Lees AW, et al. The addition of manual lymph drainage to compression therapy for breast cancer related lymphedema: a randomized controlled trial. Breast Cancer Res Treat 2004;86(2):95-106. PMID:15319562
124. Damstra RJ, Partsch H. Compression therapy in breast cancer-related lymphedema: A randomized, controlled comparative study of relation between volume and interface pressure changes. J Vasc Surg 2009;49(5):1256-63. PMID:19394553
125. Pilch U, Wozniewski M, Szuba A. Influence of compression cycle time and number of sleeve chambers on upper extremity lymphedema volume reduction during intermittent pneumatic compression. Lymphology 2009;42(1):26-35.
126. Bialoszewski D, Wozniak W, Zarek S. Clinical efficacy of kinesiology taping in reducing edema of the lower limbs in patients treated with the ilizarov method--preliminary report. Ortopedia Traumatologia Rehabilitacja 2009;11(1):46-54. PMID:19240683
127. Lau RW, Cheing GL. Managing postmastectomy lymphedema with low-level laser therapy. Photomedicine and Laser Surgery 2009;27(5):763-9. PMID:19878027
128. Tsai HJ, Hung HC, Yang JL, et al. Could Kinesio tape replace the bandage in decongestive lymphatic therapy for breast-cancer-related lymphedema? A pilot study. Support Care Cancer 2009;17(11):1353-60. PMID:19199105
129. Irdesel J, Kahraman CS. [Effectiveness of exercise and compression garments in the treatment of breast cancer related lymphedema]. Turkiye Fiziksel Tip ve Rehabilitasyon Dergisi 2007;53(1):16-21.
130. Berlin E, Gjores JE, Ivarsson C, et al. Postmastectomy lymphoedema. Treatment and a five-year follow-up study. Int Angiol 1999;18(4):294-8. PMID:10811517
131. Pinell XA, Kirkpatrick SH, Hawkins K, et al. Manipulative therapy of secondary lymphedema in the presence of locoregional tumors. Cancer 2008;112(4):950-4. PMID:18085587
132. Brambilla L, Tourlaki A, Ferrucci S, et al. Treatment of classic Kaposi's sarcoma-associated lymphedema with elastic stockings. J Dermatol 2006;33(7):451-6. PMID:16848816
133. Johansson K, Albertsson M, Ingvar C, et al. Effects of compression bandaging with or without manual lymph drainage treatment in patients with postoperative arm lymphedema. Lymphology 1999;32(3):103-10. PMID:10494522
134. Frischenschlager O, Fialka V, Schindt L. Comparison of two rehabilitation programmes for lymphedema following ablatio mammae: emotional well-being. Eur J Phys Med Rehabil 1991;1(5):123-5.
135. Tretbar LL, Morgan CL, Lee BB, et al. Lymphedema: Diagnosis and Treatment. London: Springer-Verlag London Limited; 2008.
136. Mayrovitz HN. Assessing lymphedema by tissue indentation force and local tissue water. Lymphology 2009;42(2):88-98. PMID:19725274
137. Sitzia J. Volume measurement in lymphoedema treatment: examination of formulae. European Journal of Cancer Care 1995;4(1):11-6. PMID:7620649
138. Schmitz KH, Troxel AB, Cheville A, et al. Physical activity and lymphedema (the PAL trial): Assessing the safety of progressive strength training in breast cancer survivors. Contemporary Clinical Trials 2009;30(3):233-45.
139. Halaska M, Strnad P, Chod J, et al. [Detection of postoperative lymphoedema in patients with breast cancer]. [Czech]. Ceska Gynekol 2007;72(4):299-304. PMID:17966613
140. Schuchhardt C. Volume measurement of extremities comparing optoelectronic and plethysmographic methods. [German]. Lymphologie in Forschung und Praxis 2003;7(1):22-4.
141. Campisi C, Boccardo F, Zilli A, et al. [Lymphedema secondary to breast cancer treatment: possibility of diagnostic and therapeutic prevention]. [Italian]. Ann Ital Chir 2002;73(5):493-8. PMID:12704989
142. Hansdorfer-Korzon R, Bakula S, Nowakowski M. The role of B-MODE ultrasound examination compared to limb circumference measurement in the diagnosis of early stages of

Ovid AMED (Allied and Complementary Medicine)
Search Strategy:

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1 lymphedema/
2 lymph?edema.tw.
3 elephantiasis.tw.
4 (comple* adj (lymph?edema or lymphatic or decongestive) adj (therapy or physiotherapy or physical therapy)).tw.
5 manual lymphatic drainage.tw.
6 foldi.tw.
7 vodder.tw.
8 casley-smith.tw.
9 pneumatic compression/
10 intermittent pneumatic compression.tw.
11 or/1-10
12 limit 11 to yr="1990 -Current"

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations
Search Strategy:

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1 lymphedema/ or elephantiasis/
2 lymph?edema.tw.
3 elephantiasis.tw. not (elephantiasis, filarial/ or filarial.tw.)
4 (comple* adj (lymph?edema or lymphatic or decongestive) adj (therapy or physiotherapy or physical therapy)).tw.
5 manual lymphatic drainage.tw.
6 foldi.tw.
7 vodder.tw
8 casley-smith.tw.
9 Intermittent Pneumatic Compression Devices/ not exp *thrombosis/
10 intermittent pneumatic compression.tw. not exp *thrombosis/
11 or/1-10

EBM Reviews: Cochrane Central Register of Controlled Trials
Search Strategy
1. lymphedema/ or elephantiasis/
2. lymph?edema.tw.
3. elephantiasis.tw. not (elephantiasis, filarial/ or filarial.tw.)
4. (comple* adj (lymph?edema or lymphatic or decongestive) adj (therapy or physiotherapy or physical therapy)).tw.
5. manual lymphatic drainage.tw.
6. foldi.tw.
7. vodder.tw.
8. casley-smith.tw.
9. Intermittent Pneumatic Compression Devices/ not exp *thrombosis/
10. intermittent pneumatic compression.tw. not exp *thrombosis/

Appendix B – Forms

Full Text Screening Questions

Ref ID:_____ Name of Screener:_____

1. Is this study a narrative review, a case study (n=1), a commentary, an editorial, a study of primary lymphedema/filariasis/drug treatments for lymphedema/surgical treatments ALONE for lymphedema?

○ Yes (STOP)

2. Is this study: (Check all that apply)

□ A qualitative study (STOP)

□ A Quality of Life (QOL) assessment of subjects with lymphedema (that does not examine efficacy of a Rx intervention) (STOP)

□ An incidence/prevalence study of lymphedema following surgery for cancer (STOP)

□A study of prevention for lymphedema (ie all subjects do not have lymphedema) (STOP)

□ An investigation study of lymph flow/lymphatic system with no treatment of diagnosis of lymphedema included (STOP)

□ None of the above (continue)

3. All or some of the patients have secondary LE or suspected secondary LE and if primary and secondary patients used, results are stratified by primary and secondary LE?

○ Yes (continue)

○ No (exclude)

4. Is this study:

○ Primarily an investigative/exploratory study of a diagnostic method(s) for lymphedema? (STOP)

○ Article evaluates the Sensitivity/Specificity, Reliability, Validity or Responsiveness of a diagnostic test for LE OR gives data to calculate 2x2 table for test (STOP) (Include)

○ Not a diagnosis study (continue)

5. Is this a study focusing on the efficacy of a non-surgical/non pharmacological treatment for secondary lymphedema?

○ Yes (continue)

○ No (STOP)

6. Is this study a:

○ RCT (include)

○ A non-RCT WITH a Control group (include)

○ No control group/subjects act as their own controls (exclude)

DATA ABSTRACTION FORM FOR TREATMENT STUDIES

Author: _____ RefID: _____ Data Abstractor: _____

Type of Treatment
Study Design
Sample Size Intervention: Control:
Inclusion/Exclusion criteria
Criteria used to start treatment
Time of treatment initiation
Time of lymphedema onset
Criteria used to stop therapy
Provider of treatment Details of qualifications/professional training
Comparators in study Are they consistent with usual care?

REFID _____ 1st AUTHOR _____ EXTRACTOR _____

QUALITY SCORE FOR JADAD SCALE AND FOR MODIFIED JADAD SCALE

CRITERIA	RESULT	SCORING	SCORE
Reported as randomized	☐ YES ☐ NO	1 point for YES	
Randomization is appropriate	☐ YES ☐ NO ☐ NOT DESCRIBED	1 point for YES -1 point for NO	
Double blinding is reported	☐ YES ☐ NO	1 point for YES	
Double blinding is appropriate	☐ YES ☐ NO ☐ NOT DESCRIBED	1 point for YES -1 point for NO	
Withdrawals are reported by number and reason per arm	☐ YES ☐ NO	1 point for YES	
JADAD SCORE			/5
Method used to assess adverse events is described	☐ YES ☐ NO	1 point for YES	
Methods of statistical analysis are described	☐ YES ☐ NO	1 point for YES	
Inclusion criteria reported	☐ YES ☐ NO	1 point for YES in at least one of two criteria	
Exclusion criteria reported	☐ YES ☐ NO		
MODIFIED JADAD SCORE			/8

COHORT STUDIES

Selection

10. Representativeness of the exposed cohort

- □ Truly representative of the average secondary lymphedema patient in the community
- □ Somewhat representative of the average secondary lymphedema patient in the community
- □ Selected group of users e.g. nurses, volunteers
- □ No description of the derivation of the cohort

11. Selection of the non exposed cohort

- □ Drawn from the same community as the exposed cohort
- □ Drawn from a different source
- □ No description of the derivation of the non exposed cohort

12. Ascertainment of exposure

- □ Secure record (e.g. surgical records/clinical records)
- □ Structured interview
- □ Written self report
- □ No description

13. Demonstration that outcome of interest was not present at start of study

- □ Yes
- □ No

Comparability

14. Comparability of cohorts on the basis of the design or analysis

- □ Study controls for stage of lymphedema
- □ Study controls for time of onset of lymphedema

Outcome

15. Assessment of outcome

- □ Independent blind assessment
- □ Record linkage (some other objective measure not encompassed by "independent blind assignment" see above)
- □ Self report
- □ No description

16. Was follow-up long enough for outcomes to occur

- □ Yes (6 weeks +)
- □ No (less than 6 weeks)

17. Adequacy of follow up of cohorts

- □ Complete follow up – all subjects accounted for
- □ Subjects lost to follow up unlikely to introduce bias – small number lost (> 80%follow up), or description provided of those lost
- □ Follow up rate < 80% and no description of those lost
- □ No statement

QUADAS – Quality Assessment Tool for Reliability Diagnosis papers

	Yes	No	Unclear
1. Were study patients representative of the patients who will receive the test(s) in practice?			
2. Were selection criteria for patients clearly described?			
3. Were correct statistical measures used?			
4. Was execution of test and comparator described in sufficient detail to permit replication in another study?			
5. Were withdrawals from the study explained?			
6. Were intermediate results/incomplete data reported?			
7. Did assessors have adequate professional training to perform test/measurement?			
8. How were raters selected?			
9. Was interval between test-retest appropriate?			
10. Did independent ratings take place within a time frame that would ensure the condition did not change?			
Comments:			

[1]Appendix C – Excluded Studies

[1] Includes studies excluded at full text level of screening only

Adam DJ, Naik J, Hartshorne T, et al. The diagnosis and management of 689 chronic leg ulcers in a single-visit assessment clinic. Eur J Vasc Endovasc Surg 2003;25(5):462-8. OVID-Embase.
Exclude: Not a study of efficacy of secondary LE treatment

Adamian AA, Gordeev VF, Zolatarevskii VI, et al. [Radionuclide diagnosis of lymphodynamic disorders in the upper limb after radical mastectomy]. Sov Med 1990;(5):108-10. (Rus). PMID:2389191 OVID-Medline.
Exclude: exploratory study

Albert U-S, Seifart U, Hoffmann M, et al. Feasibility test: Recommendations for the diagnosis of lymphedema after breast cancer in long-term follow-up and rehabilitation. Geburtshilfe Frauenheilkd 2007;67(5):468-74. (Ger). OVID-Embase.
Exclude: no control

American Cancer Society. Lymphedema patient page. CA Cancer Journal for Clinicians 2009;59(1):25-6. OVID-Embase.
Exclude: narrative, primary LE, editorial, conference etc.

Andersen JS. Lymfodem - nye behandlingsprincipper. Danske Fysioter 2000;17:6-11. OVID-AMED.
Exclude: not able to retrieve

Anderson L, Hojris I, Anderson J. Treatment of breast ancer related lymphedema with or without manual lymphatic drainage: A randomized study. Eur J Cancer 1993;35(Suppl 4):S30-S31 OVID-CCTR.
Exclude: Narrative, primary LE, editorial, conference etc.

Armer JM, Heuggeler MH, Brooks CW, et al. The health deviation of post-breast cancer lymphedema: symptom assessment and impact on self-care agency. Self-Care, Dependent-Care & Nursing 2008;16(1):14-21. Publisher URL: www.cinahl.com/cgi-bin/refsvc?jid=2476&accno=2009798231; http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=2009798231&site=ehost-live EBSCO-CINAHL.
Exclude: narrative, primary LE, editorial, conference etc.

Badger CM, Peacock JL, Mortimer PS. A randomized, controlled, parallel-group clinical trial comparing multilayer bandaging followed by hosiery versus hosiery alone in the treatment of patients with lymphedema of the limb. Cancer 2000;88(12):2832-7. PMID:10870068 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (treatment)

Bak M. Analysis of changes in perimeters of upper limb among women after mastectomy participating in motor rehabilitation. Postepy Rehabilitacji 2008;22(2):15-21. (Pol). OVID-Embase.
Exclude: no control

Balzarini A, Milella M, Civelli E, et al. Ultrasonography of arm edema after axillary dissection for breast cancer: a preliminary study. Lymphology 2001;34:152-5. PMID:11783592
Exclude: Diagnostic Exploratory Study

Barclay J, Vestey J, Lambert A, et al. Reducing the symptoms of lymphoedema: is there a role for aromatherapy? Eur J Oncol Nurs 2006;10(2):140-9. PMID:16563861 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (treatment)

Berard A, Zuccarelli F. Test-retest reliability study of a new improved Leg-O-meter, the Leg-O-meter II, in patients suffering from venous insufficiency of the lower limbs. Angiology 2000;51(9):711-7. PMID:10999611 OVID-Medline.
Exclude: not stratified by primary/secondary LE or no LE (diagnosis)

Bergan JJ, Sparks S, Angle N. A comparison of compression pumps in the treatment of lymphedema. Vasc Surg 1998;32(5):455-62. OVID-Embase.
Exclude: Primary/Secondary not stratified or patients did not have LE (treatment)

Bertelli G, Venturini M, Forno G, et al. Pneumatic compression in postmastectomy lymphedema: a phase II study. Ann-Oncol 1990;1(Suppl):30 OVID-CCTR.
Exclude: Narrative, primary LE, editorial, conference etc

Boccardo FM, Ansaldi F, Bellini C, et al. Prospective evaluation of a prevention protocol for lymphedema following surgery for breast cancer. Lymphology 2009;42(1):1-9. OVID-Embase.
Exclude: prevention

Bolcal C, Iyem H, Sargin M, et al. Primary and secondary lymphoedema in male patients with oedema in lower limbs. Phlebology 2006;21(3):127-31. OVID-Embase.
Exclude: Diagnostic exploratory study

Boris M, Weindorf S, Lasinski BB. The risk of genital edema after external pump compression for lower limb lymphedema. Lymphology 1998;31(1):15-20. PMID:9561507 OVID-Medline.
Exclude: No Control Group-Treatment

Cornish BH, Chapman M, Thomas BJ, et al. Early diagnosis of lymphedema in postsurgery breast cancer patients. Ann N Y Acad Sci 2000;904:571-5. PMID:10865807 OVID-Medline.
Exclude: Diagnostic Exploratory Study

Cornish BH, Thomas BJ, Ward LC, et al. A new technique for the quantification of peripheral edema with application in both unilateral and bilateral cases. Angiology 2002;53(1):41-7. PMID:11863308 OVID-Medline.
Exclude: Diagnostic Exploratory Study

Cuello V, Guerola S, Lopez R. Clinical and therapeutic profile of post-mastectomy lymphedema. Rehabilitacion 2003;37(1):22-32. OVID-AMED.
Exclude: no control

Damstra RJ, Brouwer ER, Partsch H. Controlled, comparative study of relation between volume changes and interface pressure under short-stretch bandages in leg lymphedema patients. Dermatologic Surgery 2008;34(6):773-8. PMID:18336577 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (treatment)

Dimakakos E, Koureas A, Koutoulidis V, et al. Interstitial magnetic resonance lymphography: is it a new method for the diagnosis of lymphedema? Int Angiol 2007;26(4):367-71. PMID:18091705 OVID-Medline.
Exclude: Diagnostic Exploratory Study

Dimakakos E, Koureas A, Koutoulidis V, et al. Interstitial magnetic resonance lymphography: the clinical effectiveness of a new method. Lymphology 2008;41(3):116-25. PMID:19013879 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Dimakakos PB, Stefanopoulos T, Antoniades P, et al. MRI and ultrasonographic findings in the investigation of lymphedema and lipedema. Int Surg 1997;82(4):411-6. PMID:9412843 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Drinan KJ, Wolfson PM, Steinitz D, et al. Duplex imaging in lymphedema. J Vasc Technol 1993;17(1):23-6. OVID-Embase.
Exclude: Diagnostic Exploratory Study

Duman I, Ozdemir A, Tan AK, et al. The efficacy of manual lymphatic drainage therapy in the management of limb edema secondary to reflex sympathetic dystrophy. Rheumatol Int 2009;29(7):759-63. PMID:19030864 OVID-Medline.
Exclude: not effectiveness of LE

Durand A, Thibaut G. Microcirculation variations during lymphedema. European Journal of Lymphology and Related Problems 2003;10(37-38):12-4. OVID-Embase.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Ferrandez J-C, Bourassin A, Debeauquesne A, et al. Prospective study on an out-patient basis of the arm after breast cancer (with reference to 76 cases). Oncologie 2005;7(4):316-22. (Fre). OVID-Embase.
Exclude: no control

Fiaschi E, Francesconi G, Fiumicelli S, et al. Manual lymphatic drainage for chronic post-mastectomy lymphoedema treatment. Panminerva Med 1998;40(1):48-50. PMID:9573754 OVID-Medline.
Exclude: No Control Group-Treatment

Florez-Garcia MT, Valverde-Carrillo MD. Effectiveness of nonpharmacological interventions in the management of lymphedema postmastectomy. Rehabilitacion 2007;41(3):126-34. (Span). EBSCO-CINAHL.
Exclude: narrative, primary LE, editorial, conference etc

Froldi M, Piana M, De Luca S, et al. Combined salty-bromoiodic hydromassage and intermittent pneumatic compression in the treatment of lower limbs lymphedema. Medicina Clinica e Termale 2002;14(50-51):365-73. (Ital). OVID-Embase.
Exclude: no control

Garfein ES, Borud LJ, Warren AG, et al. Learning from a lymphedema clinic: an algorithm for the management of localized swelling. Plastic & Reconstructive Surgery 2008;121(2):521-8. PMID:18300971 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Giraldi E, Dalla PF, Spreafico G, et al. Lymphoscintigraphy in the diagnosis of the lower extremities lymphedema. Acta Chir Ital 1995;51(2):143-51. (Ital). OVID-Embase.
Exclude: narrative, primary LE, editorial, conference etc

Goffin V, Pierard-Franchimont C, Pierard GE. [Dermometric evaluation of edema of the lower limbs]. Rev Med Liege 1993;48(12):681-5. (Fre). PMID:8310202 OVID-Medline.
Exclude: narrative, primary LE, editorial, conference etc.

Gonzalez V, Condon H, Lecuona N, et al. Efectividad del tratamiento del linfedema de extremidad superior mediante presoterapia neumatica secuencial multicompartimental. Rehabilitacion 1998;32(4):234-40. OVID-AMED.
Exclude: no control

Gothard L. Phase II Randomized Study of Hyperbaric Oxygen Therapy Versus Standard Management in Women With Chronic Arm Lymphedema After Radiotherapy for Early Breast Cancer. Physician Data Query (PDQ) 2004;2004(PDQ): OVID-CCTR.
Exclude: Narrative, primary LE, editorial, conference etc.

lymphedema in the extremities]. Nippon Geka Gakkai Zasshi 1999;100(9):551-6. (Jap). PMID:10516971 OVID-Medline.
Exclude: narrative, primary LE, editorial, conference etc

Larcos G, Foster DR. Interpretation of lymphoscintigrams in suspected lymphoedema: contribution of delayed images. Nucl Med Commun 1995;16(8):683-6. PMID:7491181 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Lefevre F. Special report: comparative efficacy of different types of pneumatic compression pumps for the treatment of lymphedema. BlueCross and BlueShield Technology Evaluation Center (TEC) Assessment 1998;13(2):42-3. PMID:10183361 OVID-Medline.
Exclude: Narrative, primary LE, editorial, conference etc.

Letellier M, Towers A, Cohen R. Aqualymphatic exercise as an alternative therapy for lympedema management following breast cancer: a randomized controlled pilot study... 17th International Congress on Palliative Care, September 23-26, 2008/Palais Des Congres, Montreal, Canada. Journal of Palliative Care 2008;24(3):215 http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=2010064750&site=ehost-live; Publisher URL: www.cinahl.com/cgi-bin/refsvc?jid=549&accno=2010064750 EBSCO-CINAHL.
Exclude: narrative, primary LE, editorial, conference etc.

Lette J. A simple and innovative device to measure arm volume at home for patients with lymphedema after breast cancer. Journal of Clinical Oncology 2006;24(34):5434-40. PMID:17135645 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Li W-M, Chen W-A, Rao G-H, et al. Diagnostic value of radionuclide lymphoscintigraphy in extremity lymphedema. Chinese Journal of Medical Imaging Technology 2008;24(9):1462-4. (Chin). OVID-Embase.
Exclude: not stratified by primary/secondary LE or no LE

Li X-J, Wang L. Radioisotope scanning evaluation on the improvement of post-mastectomy upper extremity lymphedema in breast cancer patients following compression therapy. Journal of Clinical Rehabilitative Tissue Engineering Research 2007;11(22):4329-32. (Chin). OVID-Embase.
Exclude: No control group

Liu N, Wang C, Sun M. Noncontrast three-dimensional magnetic resonance imaging vs lymphoscintigraphy in the evaluation of lymph circulation disorders: A comparative study. J Vasc Surg 2005;41(1):69-75. PMID:15696047 OVID-Medline.
Exclude: not stratified by primary/secondary LE or no LE (diagnosis)

Lohrmann C, Kartz O, Speck O, et al. Chronic lymphedema: Detected with high-resolution magnetic resonance lymphangiography. J Comput Assist Tomogr 2006;30(4):688 PMID:16845303 OVID-Medline.
Exclude: Narrative, primary LE, editorial, conference etc.

Marotel M, Cluzan R, Pascot M, et al. CT staging in 150 cases of lower limb lymphedema. J Radiol 1998;79(11): 1373-8. (Fre). OVID-Embase.
Exclude: exploratory study

Marshall M, Schwahn-Schreiber C. Lymphedema, lipedema and venous edema. Differential diagnostic clarification using high-resolution duplex sonography. Gefasschirurgie 2008;13(3):204-12. (Ger). OVID-Embase.
Exclude: narrative, primary LE, editorial, conference etc

Matthews KL, Smith JG. Effectiveness of modified complex physical therapy for lymphoedema treatment. Australian Journal of Physiotherapy 1996;42(4):323-8. OVID-Embase.
Exclude: Primary/Secondary not stratified or patients did not have LE (treatment)

Mayrovitz HN, Sims N, Litwin B, et al. Foot volume estimates based on a geometric algorithm in comparison to water displacement. Lymphology 2005;38(1):20-7. PMID:15856683 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Mayrovitz HN, Sims N, Brown-Cross D, et al. Transcutaneous oxygen tension in arms of women with unilateral postmastectomy lymphedema. Lymphology 2005;38(2):81-6. PMID:16184817 OVID-Medline.
Exclude: No Control Group-Treatment

Mayrovitz HN, Sims N, Hill CJ, et al. Hand volume estimates based on a geometric algorithm in comparison to water displacement. Lymphology 2006;39(2):95-103. PMID:16910100 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Mayrovitz HN. Assessing local tissue edema in postmastectomy lymphedema. Lymphology 2007;40(2):87-94. PMID:17853619 OVID-Medline.
Exclude: Diagnostic Exploratory Study

Mayrovitz HN, Weingrad DN, Davey S. Local tissue water in at-risk and contralateral forearms of women with and without breast cancer treatment-related lymphedema. Lymphatic Research & Biology 2009;7(3):153-8. PMID:19778203 OVID-Medline.
Exclude: not stratified by primary/secondary LE or no secondary LE

McLaughlin SA, Van Zee KJ. Lymphedema 5 years after SLNB or ALND for breast cancer: objective measurements, patient perceptions, and precautionary behaviors. American

lymphoedema. Lymphology 1996;29 (suppl 1):297-300.
Exclude: no control group

Pohjola RT, Pekanmaki K, Kolari PJ. Intermittent pneumatic compression of lymphoedema: Evaluation of two clinical methods. Eur J Lymphology Relat Probl 1995;5(19):87-90. OVID-Embase.
Exclude: Not stratified by primary/secondary LE or no LE (treatment)

Preisler VK, Hagen R, Hoppe F. Pros and cons of the manual lymphdrainage treatment for secondary lymphedema of the head and neck. Laryngorhinootologie 1998;77(4):207-12. (Ger). OVID-Embase.
Exclude: not effectiveness of LE treatment

Proby CM, Gane JN, Joseph AE, et al. Investigation of the swollen limb with isotope lymphography. Br J Dermatol 1990;123(1):29-37. PMID:2390494 OVID-Medline.
Exclude: Diagnostic exploratory study

Richards TB, McBiles M, Collins PS. An easy method for diagnosis of lymphedema. Ann Vasc Surg 1990;4(3):255-9. PMID:2340247 OVID-Medline.
Exclude: Diagnostic exploratory study

Rijke AM, Croft BY, Johnson RA, et al. Lymphoscintigraphy and lymphedema of the lower extremities. J Nucl Med 1990;31(6):990-8. PMID:2348245 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Rudat CH. Die Lymphe - die reinigende Flussigkeit unseres Korpers. Natur und Heilen 1999;76(9):548-52. (Ger.) OVID-AMED.
Exclude: narrative review

Sagen A, Karesen R, Risberg MA. The reliability of a simplified water displacement instrument: a method for measuring arm volume. Archives of Physical Medicine & Rehabilitation 2005;86(1):86-9. Publisher URL: www.cinahl.com/cgi-bin/refsvc?jid=123&accno=2005084545; http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=2005084545&site=ehost-live EBSCO-CINAHL.
Exclude: not stratified by primary/secondary LE or no LE

Sander AP, Hajer NM, Hemenway K, et al. Upper-extremity volume measurements in women with lymphedema: a comparison of measurements obtained via water displacement with geometrically determined volume. Physical Therapy 2002;82(12):1201-12. PMID:12444879 OVID-Medline.
Exclude: Primary/Secondary not stratified or patients did not have LE (diagnosis)

Sawan S, Mugnai R, Lopes AB, et al. Lower-limb lymphedema and vulval cancer: feasibility of prophylactic compression garments and validation of leg volume measurement. International Journal of Gynecological Cancer 2009;19(9):1649-54. PMID:19955953 OVID-Medline In Process.
Exclude: prevention

Schmitz KH, Troxel AB, Cheville A, et al. Physical activity and lymphedema (the PAL trial): Assessing the safety of progressive strength training in breast cancer survivors. Contemporary Clinical Trials 2009;30(3):233-45. OVID-Embase.
Exclude: companion to 15036

Seifart U, Albert US, Heim ME, et al. [Lymphedema in patients with breast cancer--a consensus regarding diagnostics and therapy in patients with postoperative lymphedema after primary breast cancer]. Rehabilitation (Stuttg) 2007;46(6):340-8. (Ger). PMID:18188805 OVID-Medline.
Exclude: narrative, primary LE, editorial, conference etc.

Sitzia J, Sobrido L. Measurement of health-related quality of life of patients receiving conservative treatment for limb lymphoedema using the Nottingham Health Profile. Qual Life Res 1997;6(5):373-84. PMID:9290304 OVID-Medline.
Exclude: No Control Group-Treatment

Smirnov AS, Bubnova NA, Blokhina MV, et al. [The sanatorium-health resort treatment of patients with lymphedema of the legs]. Vopr Kurortol Fizioter Lech Fiz Kult 1992;(1):38-40. (Rus). PMID:1533476 OVID-Medline.
Exclude: not stratified by primary/secondary LE or no LE

Stoberl C, Partsch H, Wruhs M. [Diagnostic value and criteria for evaluating indirect lymphography in lymphedema]. Vasa 1990;19(3):212-7. (Ger). PMID:2238815 OVID-Medline.
Exclude: not stratified by primary/secondary LE or no LE

Stoberl C. [Indirect lymphography--possibilities and limits of roentgen diagnosis of the lymphatic system]. Wien Med Wochenschr 1999;149(2-4):92-4. (Ger). PMID:10378334 OVID-Medline.
Exclude: narrative, primary LE, editorial, conference etc

Suga K, Kume N, Matsunaga N, et al. Assessment of leg oedema by dynamic lymphoscintigraphy with intradermal injection of technetium-99m human serum albumin and load produced by standing. Eur J Nucl Med 2001;28(3):294-303. PMID:11315596 OVID-Medline.
Exclude: Diagnostic Exploratory Study

Szolnoky G, Lakatos B, Keskeny T. Advantage of combined decongestive lymphatic therapy over manual lymph drainage: A pilot study. Lymphology 2002;35(Suppl 1):277-82.
Exclude: Narrative, primary LE, editorial, conference etc.

Szuba A. Literature watch. The addition of manual lymph drainage to compression therapy for breast cancer related lymphedema: a randomized controlled trial. Lymphatic

Assessment of Bilateral Limb Lymphedema by Bioelectrical Impedance Spectroscopy

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Objective: The aim of the present study was to determine if the ratio of extracellular fluid (ECF), including the lymph, to that of intracellular fluid (ICF), as measured by bioimpedance spectroscopy (BIS), could be used to assess bilateral lymphedema (LE).

Background: The presence of LE is commonly determined as an increase in tissue volume due to the presence of excess lymph relative to the volume of a comparable unaffected body region or to comparative normative data. However, in bilateral LE of the limbs, a comparable body region, the contralateral limb, is also affected, precluding normalization. An alternative is to normalize the increase in lymph volume, as ECF, to that of ICF volume.

Methods: Extracellular/intracellular fluid ratios, expressed as the ratio of intracellular impedance (R_i) to extracellular impedance (R_0), for the limbs of 277 female and 224 male controls were determined from an accumulated database of impedance data. Equivalent data were obtained for an opportunistic cross-sectional sample of 37 female and 5 male participants with bilateral LE of the legs. The ratios of R_i/R_0 in the lymphedematous legs of the affected participants were compared with the equivalent ratios in the unaffected arms of the same participants and with those of the controls using box plots and visualized as bivariate data using tolerance ellipses.

Results: Despite R_i/R_0 ratios varying with age, sex, and limb dominance, comparison of the ratio for affected legs (normalized to the ratio in the unaffected arms) with equivalent ratios observed in a control population (as bivariate tolerance plots) was capable of discriminating between 70% and 89% of the participants with LE.

Conclusions: Bioelectrical impedance spectroscopy and determination of R_i/R_0 ratios as indices of ECF/ICF ratios holds promise for the semiquantitative assessment of bilateral LE.

Key Words: Bilateral, Lymphedema, Bioelectrical impedance spectroscopy, BIS

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Lymphedema (LE) will be familiar to many surgeons as a chronic debilitating condition that may occur as a consequence of axillary and inguinal surgery and/or radiotherapy to lymph nodes. The precise incidence and prevalence is uncertain, but it is reported to occur in between 9% and 55% of patients receiving inguinal or ilioinguinal dissection.[1–3] This uncertainty is due largely to the lack of a universally accepted definition for the presence of LE.[2,4] Although a staging classification for LE based primarily on clinical features has been developed to describe the progression of the condition,[5] early detection and severity of LE are commonly based on changes in the volume or circumference of an affected limb relative to that of the contralateral control.[2,5–8] Such measurements are, however, not applicable in cases of bilateral LE, common in postgynecological LE, where there is no unaffected contralateral limb to act as a control.[3,9] Attempts have been made to overcome this problem by estimating the change in volume by comparing, for example, thigh-to-foot ratios of circumferences within the same limb.[10] Clearly, such an approach will again not work where the whole limb is affected.

Bioelectrical impedance spectroscopy (BIS) has been used for the assessment of arm LE since the early 1990s.[11] Bioelectrical impedance spectroscopy is based on the principle that the magnitude of the opposition (impedance) to the flow of a low-frequency alternating electric current through the limb is inversely proportional to the volume of fluid in the tissue. Thus, the impedance at a low frequency is a measure of the ECF volume and, at least in the early stages of LE, where excess lymphatic fluid accumulates, tissue impedance decreases proportionately.[12] (Strictly, the measured impedance is that of the extracellular water, but because the total extracellular fluid is approximately 95% water, the terms fluid and water are often used interchangeably.) Because there is wide biological variation between individuals in impedance values, the impedance per se of a limb cannot discriminate between an affected limb, that is, one with excess lymph, from a normal limb.[11] The impedance of an affected limb is therefore normalized to that of the contralateral unaffected limb and expressed as a ratio; the ratio is then compared with normative values. The method has been shown to detect accurately early-stage upper limb LE and monitor treatment efficacy (see Ward et al[12] for review) and to be suitable for early detection of lower limb LE.[13]

In bilateral LE, as with volumetric measurements, normalization to the contralateral limb is not possible. In 1996, Ward et al[12] showed that the intracellular fluid volume is largely unaffected in the early stages of LE development and subsequently demonstrated that the ratio of extracellular (ECF) to intracellular fluid (ICF) volumes (as the inverse ratio of the respective resistances, R_i/R_0, where R_i and R_0 are the resistances of ICF and ECF, respectively) was a sensitive diagnostic criterion for unilateral LE posttreatment for breast cancer.[14] These authors postulated that this method could be applied to the assessment of bilateral LE, although substantiation of the technique in such cases was necessary.

The purpose of the present study was to determine the normal variation of ECF/ICF ratios (as R_i/R_0) in the limbs of a cross section of a healthy population and to determine whether this ratio could be used to discriminate individuals with clinically ascribed bilateral LE.

METHODS

Participants

The study was an opportunistic cross-sectional design with patients with clinically diagnosed bilateral LE identified from patients attending an LE treatment clinic and invited to participate in the study. Criteria to be met for inclusion were broad, with participants being excluded only if they were younger than 18 years, pregnant, fitted with a pacemaker or other electrical medical device (contraindicated for BIS measurements), or had medical conditions other than LE or were using medications that would disturb their fluid status, for example, diuretics.

Data were obtained for control subjects with no history of LE from a database of impedance data maintained at The University of Queensland. The participants had been recruited for a variety of other studies being undertaken by the authors for whom comparable impedance measurements had been obtained. These data were pooled to provide the data set used for analysis in the present study. Pooling of data was considered acceptable inasmuch as inclusion and exclusion criteria for all of the studies were similar and the impedance measurement protocols were identical in all studies.

All participants were weighed using digital scales, to the nearest 0.1 kg, and their height measured to the nearest 0.1 cm using a wall or stand-mounted stadiometer. All participants were asked to state their arm and leg dominance. The characteristics of the participants are presented in Table 1. The mean ages of the male and female controls were similar at approximately 43 years of age. This was considerably younger than the mean ages of the LE participants at 70 and 63 years for the men and women, respectively. Correspondingly, the age distribution of the LE participants was such that all the men were aged between 60 and 80 years, whereas the women's ages ranged from 25.5 to 84.1 years. The male LE participants were shorter on average by 16 cm but 4.9 kg heavier than the male controls; consequently, their body mass indices (BMIs) were higher: 33.7 kg/m^2 compared to 26.3 kg/m^2. Although the female LE participants were the same height as the female controls, they were considerably heavier: average of 94.2 kg compared to 69.0 kg, with correspondingly higher BMI: 34.7 compared to 25.4 kg/m^2. The number of cases of primary LE in men was 4-fold greater than secondary LE. The ratio was 3:1 for the female participants. There were also 7 female participants with clinically ascribed mixed lipedema-lymphedema. Ethical approvals were obtained from the relevant medical research ethics committee of the participating institutions, and all participants provided written informed consent.

Measurement of Limb Impedance

The impedance of the legs and arms of all participants was measured according to methods described in detail elsewhere.[12] Briefly, whole limb impedance (wrist to axilla and ankle to groin) was determined according to the principle of equipotentials, as this protocol has been shown to have

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TABLE 1. Participants' characteristics

	Controls		Lymphedema	
	Males	**Females**	**Males**	**Females**
Number	224	277	5	37
Age, yrs	42.6 ± 17.5 (18.4–91.0)	43.6 ± 15.3 (18.3–86.0)	70.4 ± 4.2 (64.0–74.3)	63.3 ± 14.6 (25.5–84.1)
Numbers per Decade of Age, yrs				
<20	6 (2.7%)	3 (1.1%)	—	—
20–29.9	51 (22.8%)	56 (20.2%)	—	2 (5.4%)
30–39.9	63 (28.1%)	63 (22.7%)	—	—
40–49.9	40 (17.9%)	70 (25.3%)	—	5 (13.5%)
50–59.9	30 (13.4%)	49 (17.7%)	—	6 (16.2%)
60–69.9	12 (5.4%)	15 (5.4%)	2 (40%)	8 (21.6%)
70–79.9	6 (2.7%)	12 (4.3%)	3 (60%)	11 (29.8%)
>80	16 (7.1%)	9 (3.2%)	—	5 (13.5%)
Height, cm	178.2 ± 7.2 (155.0–197.6)	164.6 ± 6.7 (147.0–187.4)	161.6 ± 2.8 (157.0–164.7)	164.8 ± 8.2 (151.0–182.1)
Weight, kg	83.5 ± 13.4 (54.0–126.9)	69.0 ± 14.7 (40.4–119.0)	88.4 ± 16.4 (68.0–111.1)	94.2 ± 20.7 (62.0–141.0)
Body mass index (BMI), kg/m^2	26.3 ± 3.9 (18.3–40.5)	25.4 ± 4.8 (16.1–45.9)	33.7 ± 5.4 (27.6–41.8)	34.7 ± 7.5 (24.2–50.8)
Numbers per BMI range, kg/m^2				
Underweight (<20)	8 (3.5%)	30 (10.8%)	—	—
Healthy (20–24.9)	81 (36.2%)	116 (41.9%)	—	3 (8.1%)
Overweight (25–29.9)	94 (42.0%)	88 (31.8%)	1 (20%)	8 (24.3)
Obese (>30)	41 (18.3%)	43 (15.5%)	4 (80.0%)	25 (67.6%)
Lymphedema stage (n)			0:4:1:0	3:25:7:0*
Primary: secondary lymphedema (n)			4:1	28:9
Lipedema present			0	7

*Number of subjects per LE grades 1 to 4; 2 subjects were ungraded.

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greater precision than a "paired electrode" approach.[12] Participants assumed a supine position on a nonconductive examination couch, arms by their side and slightly abducted from the body. All jewelry, except rings on fingers, was removed and the skin at the electrode sites cleaned with an alcohol wipe before the application of silver–silver chloride gel electrodes. The electrode locations were those recommended by the manufacturer, that is, current drive electrodes on the dorsal surface of the third metacarpal of the hand and the dorsal surface of the third metatarsal of the foot and the voltage sensing electrodes on the dorsum of the wrists midway between the styloid processes and the dorsal surface of the ankle midway between the malleoli. All measurements were performed on each limb with the exception of a cohort of 73 men and 103 women in the control group for whom only measurements on the dominant side were available.

Impedance data were obtained with 2 different impedance instruments. Data for 224 male controls and 277 female controls drawn from the database had been obtained using an SFB3 frequency-scanning bioimpedance spectrometer (range, 5-1024 kHz; SEAC-ImpediMed Ltd, Brisbane, Australia). All other data were obtained using an SFB7 bioimpedance spectrometer (ImpediMed Ltd), which scans a similar range of frequencies (3-1000 kHz). Equivalence of these instruments has been demonstrated previously (below and in Ward[15]).

Data Processing and Analysis

Data from both SFB3 and SFB7 were processed according to Cole theory[12] using the manufacturer's software (Bioimp v 4.8.0.0, ImpediMed Ltd) to provide values for R_0 and R_∞. R_0 is the resistance at zero frequency and is equal to that of ECF, whereas R_∞ is the resistance at infinite frequency equal to that of total limb water. At zero and infinite frequencies, impedance (Z) and resistance (R) are equal; therefore, for the purposes of this manuscript, these terms are used interchangeably. Generally, the default program settings were used for analyses; however, for some data sets, the analysis was optimized by adjustment of the frequency "window" used for fitting the data to the Cole model guided by minimizing the standard error of the estimate (SEE) for the curve fitting procedure.[16] The SEE for all analyses was less than 1%.

Small, less than 2.5%, but significant differences in R_0 and R_∞ values between the SFB3 and SFB7 instruments have been determined when both are tested on the participants or electronic test circuits, an observation confirmed in a small subset of the present subject group (data not presented). Therefore, to facilitate pooling of data, all SFB3 data were converted to equivalent SFB7 data using the conversion factors provided by Ward.[15]

The resistance of intracellular water (ICF), R_i, was calculated from the relationship:

$$R_i = \frac{(R_0 \times R_\infty)}{(R_0 - R_\infty)}$$

Inasmuch as resistance is inversely related to volume, the ratio of ECF/ICF is given by

$$\frac{ECW}{ICW} = \frac{R_i}{R_o},$$

assuming that the resistivities of ICF and ECF are relatively constant (see "Discussion" section). The ECF/ICF ratios were calculated for each limb.

Statistical Analysis

Participant data were summarized with parametric descriptive statistics. Individuals were classified according to group (LE/control), sex (male/female), age (<30, 30–39.9, 40–49.9, 50-59.9 and >60 years) and BMI (20, 20–24.9, 25–29.9, >30 kg/m^2). The distributions of ECF/ICF ratios, as R_i/R_0, for all limbs were then determined for subgroups.

To determine whether there were significant differences across and between subgroups, 2- and 3-way analyses of variance (ANOVA) were performed using the general linear model procedures in the statistics module of Sigmaplot Version 11.0 (Systat Software Inc, Chicago, Ill). Two-way ANOVAs were used to determine whether there were any overall differences between age strata and between dominant and nondominant limbs within each sex and limbs (arm and

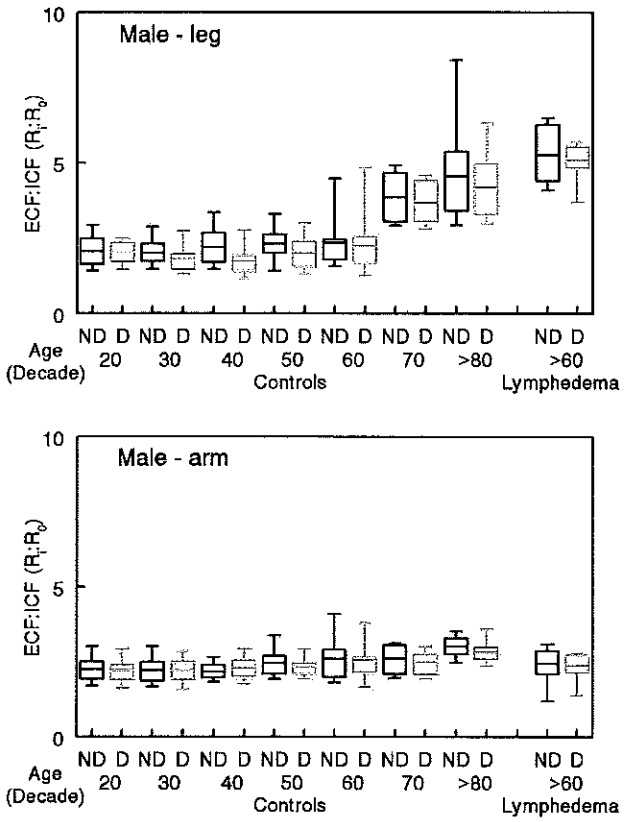

FIGURE 1. Ratio of extracellular to intracellular fluid (as R_i/R_0) in the arms and legs of male controls and of male participants with bilateral lower limb LE stratified by age. Data presented as mean (line), 25th and 75th percentiles (box), and 5th and 95th percentiles (whiskers). Mean values shown for each data set. Upper panel: nondominant arm to dominant arm; lower panel: nondominant leg to dominant leg.

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leg). Three-way ANOVAs were used to determine whether there were any overall differences between age, sex, and between dominant and nondominant limbs for arms and leg separately. Post hoc pairwise comparisons were performed using the Holm-Sidak test. The effect of BMI was similarly explored in a 2-way ANOVA.

To determine whether the ratios for the affected limbs could be discriminated from those of the control group, the distribution of the ECF/ICF ratios of the legs and the arms were analyzed according to the generalized method of Hotteling.[17,18] When the ECF/ICF ratios for the arms are plotted on polar axes against the same ratios for the legs of the control population, they follow a bivariate Gaussian distribution and a confidence interval (CI) for the correlation between the 2 variables determines an ellipsoid shape. Confidence intervals of 50%, 70%, and 95% were calculated for the control data and presented as tolerance ellipses[18] against which the ECF/ICF ratios for the LE participants were then compared. Tolerance ellipses were prepared using both the data for all control subjects and those aged older than 40 years because these more closely matched the age spectrum of the LE participants (Table 1).

RESULTS

Control Subjects

The ratio of ECF to ICF in all 4 limbs, stratified for limb dominance and by age decade, for the male participants is presented in Figure 1 and for the female participants in Figure 2. The mean ECF/ICF ratios of the arms in men varied significantly ($P < 0.001$) among the different age strata after allowing for dominance. The ratios were generally similar up to the fifth decade and then increased progressively into the ninth decade in men. Post hoc analysis among the groups indicated that this was significant for the 80-year-olds versus all other groups and for the 70-year-olds versus the 30- and 40-year-olds only. Despite there being also an overall significant, but weaker, effect ($P < 0.030$) of age in the arms of the female controls, there were no individual pairwise significant differences. As for the men, there was no effect of arm dominance or a significant age-dominance interaction.

The effect of age on ECF/ICF ratios in the legs was the same and highly significant ($P < 0.001$) in both men and women. Post hoc analysis between age strata for each sex indicated that significant differences ($P < 0.001$) were present between 80-year-old subjects and all other age strata. The ECF/ICF ratios of the nondominant leg were significantly larger in both sexes ($P < 0.014$ for men and $P < 0.005$ for women). There was no significant interaction between age and dominance.

Three-way ANOVA revealed that the ECF/ICF ratios were significantly higher ($P < 0.001$) in both the arms and the legs of men compared to those of the women. However, there was no significant interaction among dominance, age, and sex.

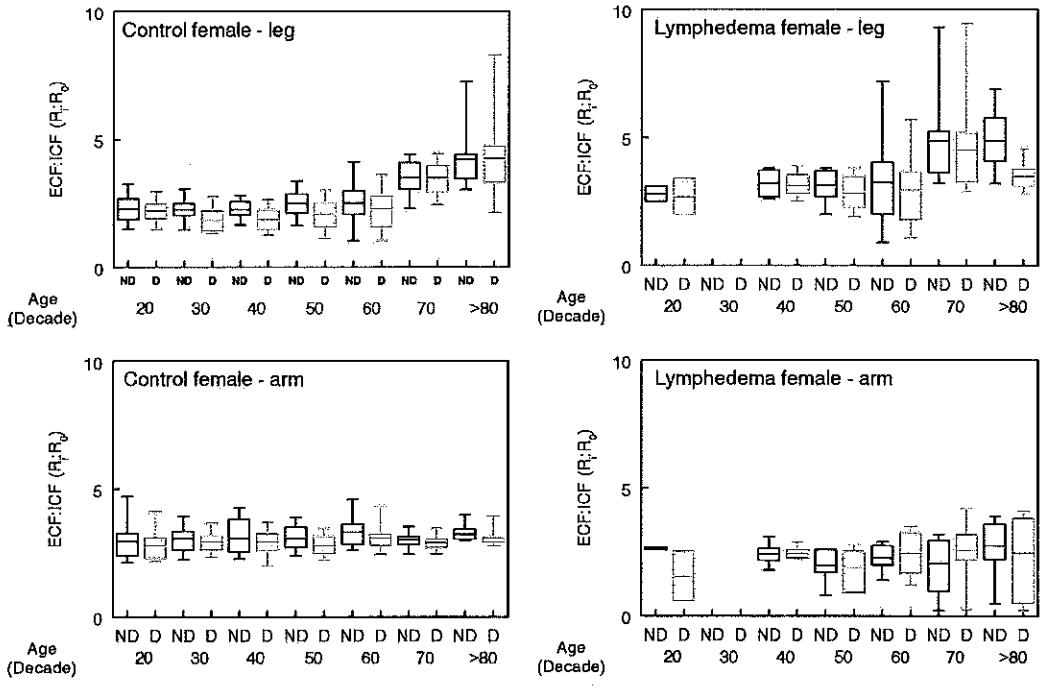

FIGURE 2. Ratio of ECF to ICF (as R_i/R_0) in the arms and legs of female controls and of female participants with bilateral lower limb LE stratified by age. Data presented as mean (line), 25th and 75th percentiles (box), and 5th and 95th percentiles (whiskers). Mean values shown for each data set. Upper left panel: affected leg to nondominant to dominant; upper right panel: control leg to nondominant to dominant; lower left panel: affected arm to nondominant to dominant; lower right panel: control arm to nondominant to dominant.

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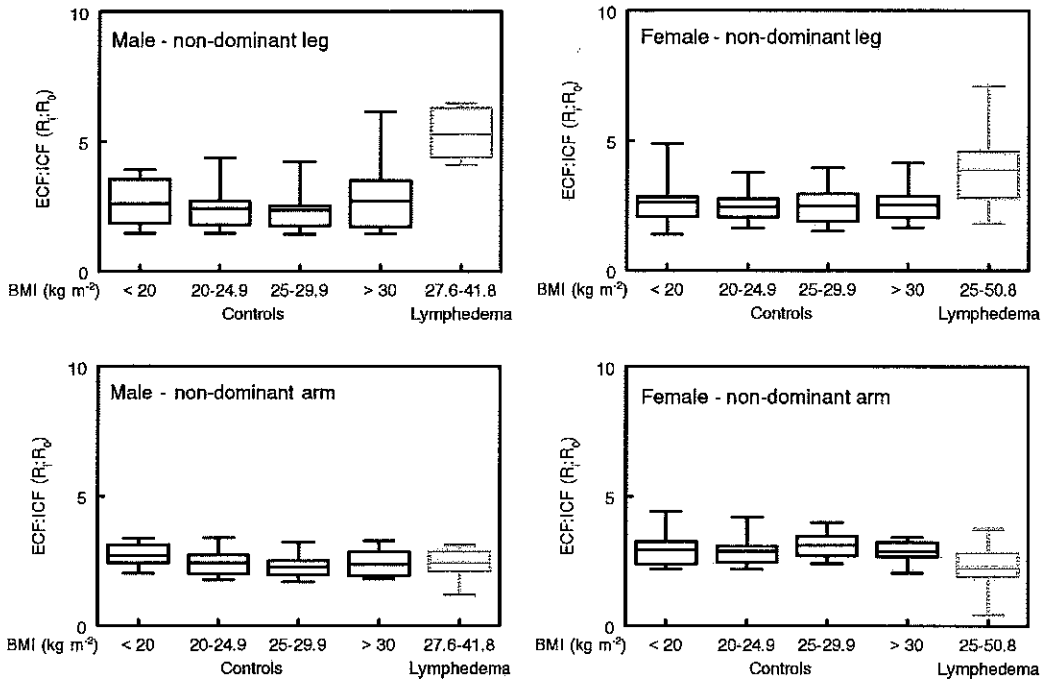

FIGURE 3. Ratio of ECF to ICF (as R_i/R_0) in the arms and legs of control subjects and of participants with bilateral lower limb LE stratified by body mass index (BMI). Data presented as mean (line), 25th and 75th percentiles (box), and 5th and 95th percentiles (whiskers). Upper left panel: male nondominant leg; upper right panel: female nondominant leg; lower left panel: male nondominant arm; lower right panel: female nondominant arm.

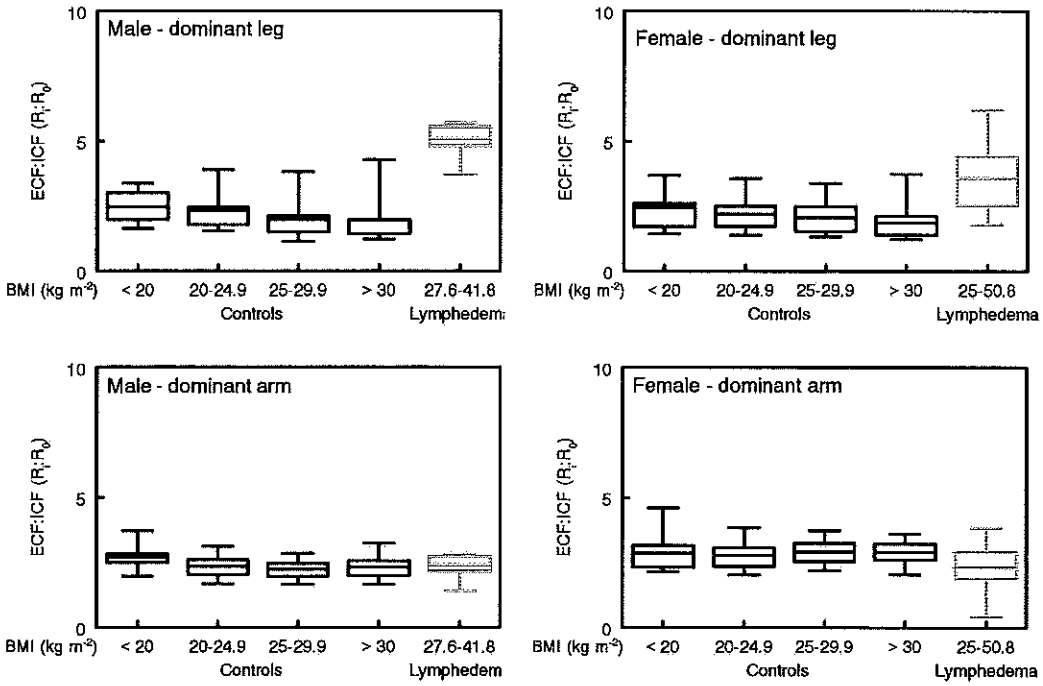

FIGURE 4. Ratio of ECF to ICF (as R_i/R_0) in the arms and legs of control subjects and of participants with bilateral lower limb LE stratified by BMI. Data presented as mean (line), 25th and 75th percentiles (box), and 5th and 95th percentiles (whiskers). Upper left panel: male dominant leg; upper right panel: female dominant leg; lower left panel: male dominant arm; lower right panel: female dominant arm.

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The ratios of ECF to ICF in the nondominant limbs, stratified by BMI, are presented in Figure 3, and the ratios of ECF to ICF in the dominant limbs are shown in Figure 4. Two-way ANOVA showed a significant effect ($P < 0.001$) of BMI, allowing for the effects of dominance, in the arms of men only. There was no significant effect of BMI in the legs irrespective of sex. However, as noted earlier, the ratios were significantly larger in the nondominant leg compared with the dominant leg for both men and women ($P < 0.016$ and $P < 0.001$, respectively).

Participants With Lymphedema

The small sample size of the male LE participants (n = 5 only) precluded stratification in this group by age, and the combined group data are presented in Figure 1. The ratios in the arms of the LE participants were slightly but not significantly less (6%) than those observed in the comparably aged control subjects. The ratios in the legs of the LE participants were, however, significantly greater ($P < 0.007$) than those of the comparably aged controls: 5.1 and 5.3 compared with 2.9 and 3.1 for nondominant and dominant limbs, respectively (Fig. 1).

The mean ratio in the legs of LE females was significantly greater by 29% ($P < 0.001$) than that of comparable control women at all ages and irrespective of limb dominance. Post hoc analysis indicated that the effect of the presence of LE was significant at ($P < 0.001$) for the age strata 40 to 49.9, 60 to 69.9, and older than 70 years of age; ($P < 0.010$) for the 50 to 59.9 age group but not significant in those aged younger than 30 years. This last observation is not surprising given the small number of participants in this age stratum.

The ECF/ICF ratio in the arms of the LE females was significantly lower ($P < 0.001$) by an average of 17%, compared with similarly aged controls (Fig. 2), a finding similar to that observed in the men with leg LE. However, post hoc analysis indicated no difference among age strata. There were no other significant differences or interactions among the variables studied.

Bivariate Analyses

Bivariate tolerance ellipses for control males and females with superimposed data points for the LE participants

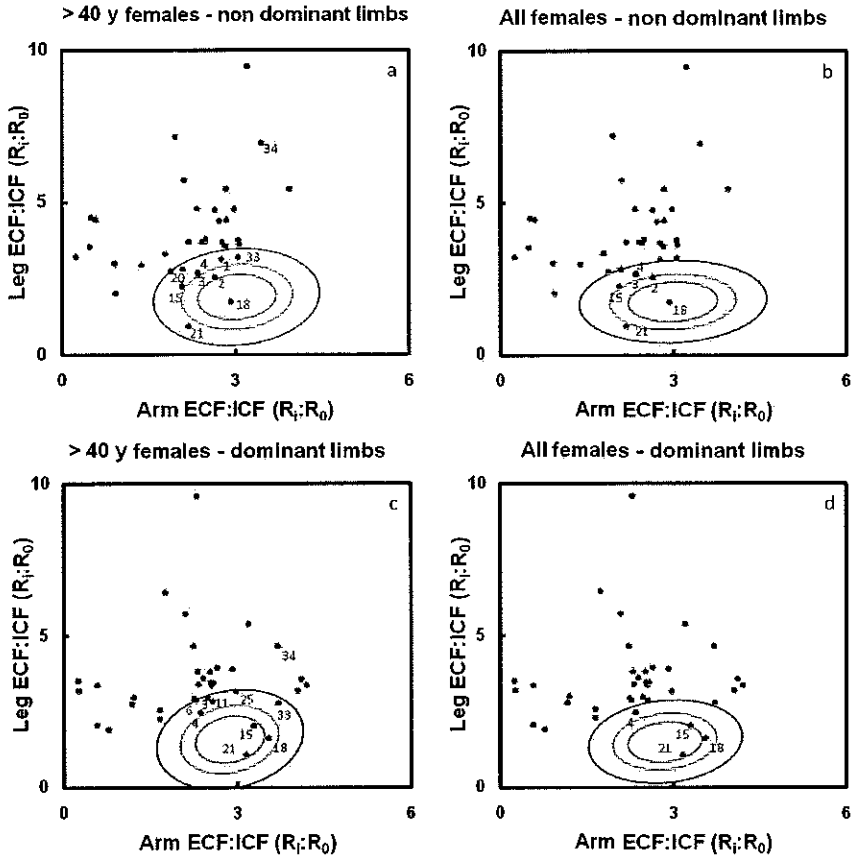

FIGURE 5. Bivariate distributions of ratio of ECF to ICF (as R_i/R_0) in the arms and legs of female controls and of participants with bilateral lower limb LE. Control data presented as tolerance ellipses: 50th (—), 75th (—), and 95th (—) percentiles. Upper left panel: nondominant arm, female participants aged older than 40 years; upper right panel: nondominant arm, female participants aged 18 to 86 years; lower left panel: dominant arm, female participants aged older than 40 years; lower right panel: dominant arm, female participants aged 18 to 86 years. Numbers refer to participants' ID as discussed in text.

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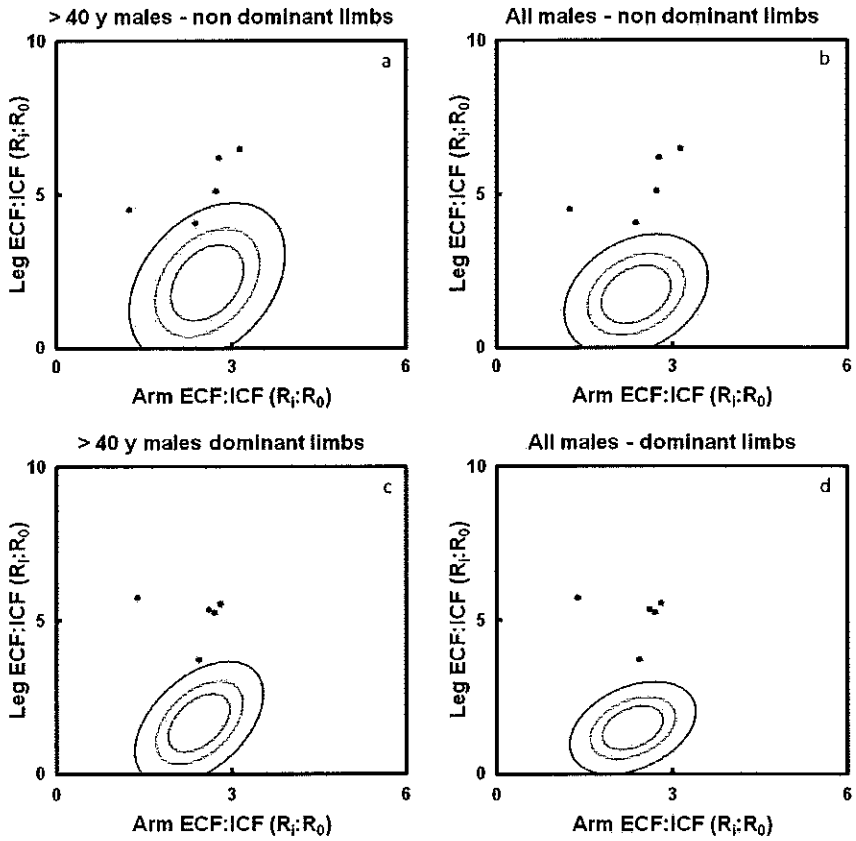

FIGURE 6. Bivariate distributions of ratio of extracellular to intracellular water (as R_i/R_0) in the arms and legs of male controls and of participants with bilateral lower limb LE. Control data presented as tolerance ellipses: 50th (—), 75th (—), and 95th (—) percentiles. Upper left panel: nondominant arm, male participants aged older than 40 years; upper right panel: nondominant arm, male participants aged 18 to 86 years; lower left panel: dominant arm, male participants aged older than 40 years; lower right panel: dominant arm, male participants aged 18 to 86 years.

are presented in Figures 5 and 6, respectively. Tolerance ellipses are presented for participants older than 40 years only (left-hand panels) and for all participants (right-hand panels) in each figure. Depending on limb dominance, all but 4 (dominant) or 6 (nondominant) female LE participants fell outside the 95th percentile for all control subjects (Figs. 5B, D). If the more conservative tolerance ellipses for similarly aged control subjects are used, then 9 of the total 37 women fell within the 95th percentile. These data suggest that, depending on limb dominance and the chosen tolerance limits, between 89% and 70% of women were identified as having ECF/ICF ratios outside the reference range. For the male LE participants, except for the ECF/ICF ratio in the nondominant leg of one subject when compared to age-matched controls (Fig. 6A), all participants exhibited ratios that fell outside the 95th percentile ellipse.

DISCUSSION

The assessment of bilateral limb LE presents particular challenges to the clinician. The commonly used approach of measuring affected limb volume excess compared to the assumed normal contralateral limb is not possible. In this study, a novel approach of referencing the volume excess in a lymphedematous limb to another body region was explored. Using bivariate analysis, up to 89% of the participants with a clinically ascribed bilateral LE were correctly classified.

The approach taken was to quantify the accumulated lymph in LE as a change in the ratio of ECF to ICF in the limb. The present study is, to the authors' knowledge, the first to apply this method to the assessment of bilateral LE in the legs. We found that the ECF/ICF ratio was increased in the legs of those with leg LE compared with that of similarly aged controls but that the range of ratio values found in affected limbs overlapped with that observed in the healthy population, limiting diagnostic discrimination.

Consequently, individual variation was decreased by indexing the ECF/ICF ratio of an affected leg to the equivalent ratio of the arm. The relationship between these variables, ECF/ICF ratios in the arm and leg, of healthy control subjects is depicted as tolerance ellipses. A tolerance ellipse is a reference for a given percentile, which gives the probability that a given datum is located from the mean vector of the ellipse drawn for the reference population, that is, the probability for data from participants with LE that the loci for

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these data fall outside the tolerance ellipse. This procedure proved effective in classifying correctly all the male participants and up to 89% of the female participants at the 95% probability level of having LE.

Interestingly, despite the LE participants having clinically ascribed bilateral leg LE, predominantly of grades 2 and 3, comparison of ECF/ICF ratios with the reference ranges did not consistently detect this. Six LE women had both legs correctly identified as abnormal when compared with age-matched control data (Figs. 5A, C). However, participants 6, 11, and 25 presented with only the dominant leg as abnormal, whereas participants 1, 2, and 20 presented with only the nondominant leg as having an abnormal ratio despite all being clinically described as having bilateral LE. The possibility of an original misdiagnosis of bilateral LE cannot be excluded.

Another interesting observation was that for those participants whose ECF/ICF ratios fell outside the reference ranges, the loci for the ratio for each leg were not identical. For example, subject 34 exhibited a ratio of 6.9 for the nondominant leg compared to 4.6 for the dominant leg despite having similar ratios in both arms: 3.5 and 3.6, respectively (Figs. 5A, C). These results suggest that bilateral LE may not affect both limbs equally.

A number of caveats to the conclusions drawn from this study should be acknowledged. The relationship between the impedance of a given volume conductor is given by

$$V = \rho \frac{L^2}{Z},$$

where V is the volume, L is the conductor length, and ρ is the specific resistivity of the conductor.[12] Hence, the measured impedance of a conductor—in this study, either the ECF or ICF—is determined by both the resistivities of the tissue fluids and their volumes. Thus, comparison of the R_i/R_0 ratios between limbs in both the LE and control conditions to reflect accurately the volume differences assumes that the resistivities of the ICF and ECF compartments remain constant. The correctness of this assumption is unknown. Indeed, accurate knowledge of the resistivities of these fluids is lacking.[19] Resistivity is primarily determined by the concentration of electrolytes in the tissue fluids and is exquisitely sensitive to change in their concentration. Under normal physiological conditions, electrolyte balance is tightly homeostatically regulated such that resistivities remain essentially constant. Whether this is the case in LE is unknown. As LE progresses and protein or fatty infiltration of the lymph occurs, resistivity will undoubtedly alter. It is, however, noteworthy that 7 of the women with LE presented with mixed lipedema/lymphedema yet still had ratios falling outside the ellipses, suggesting that the presence of lipid had not affected adversely the diagnostic power of the method. Further research is required to determine the magnitude of such changes.

There is an assumption that effects of LE are generally confined to a specific body region. For example, secondary LE after inguinal surgery is usually considered to be confined to the legs only. The procedure for indexing the ECF/ICF ratio of an affected limb—in this case, the leg—to that of another body region is predicated on this assumption. The findings from this study challenge this assumption because the ratio was found, on average, to be significantly lower in the arms of those with LE compared with the control subjects.

Superimposition of participant's data on reference tolerance ellipses provides a simple and effective visual representation for LE assessment. It does not, however, provide an exact quantitative measure of the magnitude of the ECF volume increase. The distance of a datum locus from the ellipse is an indication of this magnitude. It may be possible, with more data, to provide better quantification by calculation of Kendall's τ statistic as has been used in other contexts.[20]

Inasmuch as it could not be assumed that the ECF/ICF ratio is constant across all healthy individuals, we attempted to determine the impact of factors such as age, dominance, and BMI, as a surrogate for adiposity, on the reference range of values. An unexpected observation was that the ratio of R_i to R_0 in the legs of older persons was increased, indicative of expansion of the ECF in these individuals. This is suggestive of a degree of edema being present and may reflect the increasing prevalence of vascular disorders in the elderly. It is also noteworthy that many of these older participants were essentially sedentary institutional residents in which pooling of ECF in the legs may occur through lowered calf muscle pump activity due to physical inactivity. With respect to LE assessment, these observations indicate that age-related reference ranges are required. Although less pronounced, a BMI effect was also noted. A further unexpected observation was the lack of effect of dominance on the ECF/ICF ratio in the arms compared with a significant effect in the legs of the control subjects. It is generally assumed that the effects of dominance, and consequently on physiology, are more pronounced in the upper limbs than in the legs. Presumably, although arm dominance is typically associated with a volume difference between the arms of up to 3%,[11] differences in composition are proportionate such that ECF/ICF ratios are unchanged. Why this seems not to be the case in the legs is unknown.

This proof of concept study has demonstrated that bioimpedance spectroscopy shows great promise for the assessment of bilateral LE. The data analytic method of tolerance ellipses provides an effective way to display these data. Further work is, however, required. Definitive normative ranges across a broader spectrum of ages, to allow age-matching of patients with controls, and body habitus in which bilateral LE commonly occurs, need to be established. A prospective study to determine the minimum change in ratio capable of detecting LE, that is, the detection threshold, should also be undertaken. The successful translation of BIS for early detection of unilateral breast cancer–related LE from a research tool to routine clinical practice,[21] however, gives hope that it may eventually be equally successful for the assessment of bilateral LE.

ACKNOWLEDGMENTS

The willing participation of the volunteers is gratefully acknowledged. The assistance of Kendall Sharpe, Jarrod Meerkin, Simon Van Rosendal, and Julia Dyer in collecting control data is also gratefully acknowledged.

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REFERENCES

1. Moffatt CJ, Franks PJ, Doherty DC, et al. Lymphoedema: an underestimated health problem. *QJM*. 2003;96:731–738.
2. Rockson SG, Rivera KK. Estimating the population burden of lymphedema. *Ann NY Acad Sci*. 2008;1131:147–154.
3. Spillane AJ, Saw RP, Tucker M, et al. Defining lower limb lymphedema after inguinal or ilio-inguinal dissection in patients with melanoma using classification and regression tree analysis. *Ann Surg*. 2008;248:286–293.
4. Williams AF, Franks PJ, Moffatt CJ. Lymphoedema: estimating the size of the problem. *Palliat Med*. 2005;19:300–313.
5. Lawenda BD, Mondry TE, Johnstone PAS. Lymphedema: a primer on the identification and management of a chronic condition in oncologic treatment. *CA Cancer J Clin*. 2009;59: 8–24.
6. Purushotham AD, Bennett Britton TM, Klevesath MB, et al. Lymph node status and breast cancer–related lymphedema. *Ann Surg*. 2007;246:42–45.
7. Sander AP, Hajer NM, Hemenway K, et al. Upper-extremity volume measurements in women with lymphedema: a comparison of measurements obtained via water displacement with geometrically determined volume. *Phys Ther*. 2002;82: 1201–1212.
8. Stanton AW, Northfield JW, Holroyd B, et al. Validation of an optoelectronic limb volumeter (Perometer). *Lymphology*. 1997;30:77–97.
9. Brown J. A clinically useful method for evaluating lymphoedema. *Clin J Oncol Nurs*. 2004;8:35–38.
10. Hassall A, Graveline C, Hilliard P. A retrospective study of the effects of the Lymphapress pump on lymphedema in a pediatric population. *Lymphology*. 2001;34:156–165.
11. Ward LC, Bunce I, Cornish BH, et al. Multifrequency bioelectrical impedance augments the diagnosis and management of lymphedema in post-mastectomy patients. *Eur J Clin Invest*. 1992;22:751–754.
12. Ward LC, Kilbreath SL, Cornish BH. Bioelectrical impedance analysis for early detection of lymphoedema. In: Weissleder H, Schuchhardt C, eds. *Lymphedema Diagnosis and Therapy*. 4th ed. Essen, Germany: Viavital Verlag Gmbh Publ.; 2008:502–517.
13. Halska MJ, Novackova M, Mala I, et al. A prospective study of postoperative lymphedema after surgery for cervical cancer. *Int J Gynecol Cancer*. 2010;20:900–904.
14. Cornish BH, Thomas BJ, Ward LC, et al. A new technique for the quantification of peripheral edema with application in both unilateral and bilateral cases. *Angiology*. 2002;53:41–47.
15. Ward LC. Inter-instrument comparison of bioimpedance spectroscopic analysers. *The Open Medical Device Journal*. 2009;1:3–10.
16. Ward LC, Byrne N, Rutter K, et al. Reliability of multiple frequency bioelectrical impedance analysis: an inter-machine comparison. *Am J Hum Biol*. 1997;9:63–72.
17. Batschelet E. Circular *statistics in biology*. London, UK: Academic Press; 1981.
18. Chew V. Confidence, prediction, and tolerance regions for the multivariate normal distribution. *J Am Stat Assoc*. 1966;61:605–617.
19. Ward LC, Czerneic S, Kilbreath SL. Quantitative bioimpedance spectroscopy for the assessment of lymphedema. *Breast Cancer Res Treat*. 2009;117:541–547.
20. Smithies O, Engels WR, Devereux JR, et al. Base substitutions, length differences and DNA strand asymmetries in the human Gg and Ag fetal globin gene region. *Cell*. 1981;26:345–353.
21. Schonholz SM. Preoperative assessment enables the early detection and successful treatment of lymphedema. *Cancer*. 2009;115:909.

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Medical Policy

Subject:	Bioimpedance Spectroscopy Devices for the Detection and Management of Lymphedema		
Policy #:	MED.00105	**Current Effective Date:**	07/15/2014
Status:	Reviewed	**Last Review Date:**	05/15/2014

Description/Scope

This document addresses the use of bioimpedance spectroscopy, a device which uses impedance ratios to measure extracellular fluid volume differences between limbs to aid in the clinical assessment of lymphedema.

Note: Please see the following related document for additional information:

- CG-DME-06 Pneumatic Compression Devices for Lymphedema

Position Statement

Investigational and Not Medically Necessary:

The use of bioimpedance spectroscopy testing in the assessment, diagnosis, or management of individuals with known or suspected lymphedema is considered **investigational and not medically necessary.**

Rationale

In spite of advances in breast conserving surgical procedures and axillary dissection techniques, breast cancer survivors remain at risk for the development of secondary lymphedema, that is, excessive lymph fluid and protein accumulation in the extracellular space of the affected limb. Studies suggest that 6-40% of breast cancer survivors will develop lymphedema at some point during their lifetime (Ridner, 2009). While conventional clinical methods to diagnose and monitor lymphedema include serial measurements of limb circumference and limb volume (water displacement volumetry), multiple methods of evaluating lymphedema are under study including infrared perometry scanning (infrared light beams image the outline of the limb, which can then be used to calculate limb volume), single and multi-frequency bioelectrical impedance, ultrasound, and lymphoscintigraphy. Some of these studies have included comparisons with conventional measures of lymphedema, such as limb circumference and volume.

Bioelectrical impedance analysis has been studied in research settings for a number of years. In 2008 the U.S. Food and Drug Administration (FDA) granted 510(k) market clearance for one such device, the ImpediMED L-Dex® U400 BIS Extra Cellular Fluid Analyzer (ImpediMed Limited, San Diego CA). This device is classified as an impedance plethysomograph and was approved for the measurement of extracellular fluid volume differences between the arms to aid in the clinical assessment of unilateral lymphedema of the arm in women. FDA market clearance did not include approval of the device to either diagnose or predict the development of lymphedema of an extremity. In 2011 the FDA granted 510(k) clearance to another Extra Cellular Fluid Analyzer which is manufactured by the same company and lists the L-Dex U400 as the predicate device. The purpose of this submission was to expand the previously cleared indication to include use for legs in women and men and a clarification limiting the device to be used with humans.

Czerniec and colleagues (2010) reported on individuals with lymphedema (n=33) and individuals without lymphedema (n=18) to examine the relationship between physical methods of measuring lymphedema and self-reported swelling. The purpose of the study was to compare the reliability of the most commonly used measures of lymphedema. Measurement techniques included self-report, bioimpedance spectroscopy, truncated cone (circumference) method and infrared perometry. The study concluded that the physical measurement tools studied were reliable with high concordance (0.89 to 0.99) while self-report correlated only moderately with physical measurements (0.65-0.71) and was therefore only moderately reliable. The authors concluded that while lymphedema assessment methods showed good concordance and reliability, they were not interchangeable.

Ridner (2009) reported a feasibility study of single-frequency bioelectrical impedance to detect upper limb lymphedema in non-laboratory settings. Impedance ratios among healthy normal women and breast cancer survivors, both with and without lymphedema were compared. Ratios of healthy normal controls and breast cancer survivor without clinical evidence of lymphedema were similar with nearly complete overlap in confidence intervals. However, those values were markedly different from the values measured in the breast cancer survivor group with lymphedema ($p< 0.001$).

In a 2011 study by Czerniec, the authors sought to determine if bioimpedance spectroscopy could detect lymphedema of the arm and compare the measurements of bioimpedance spectroscopy to that of perometry. With a sample size of 29 women with known lymphedema and 11 women with no history of lymphedema, arm volume was measured by both perometry and bioimpedance spectroscopy. Both techniques were sensitive enough to detect localized lymphedema of the arm and there was a high degree of concordance between the bioimpedance spectroscopy ratio and that determined by perometry. However, sample size was small and larger studies are necessary.

Fu and colleagues (2013) reported on a study which sought to examine the reliability, sensitivity and specificity of bioimpedance spectroscopy in the detection of lymphedema. Circumferential tape measurement was used to validate the presence of lymphedema in 250 women. Bioimpedance was used to measure lymph fluid changes. The 250 women in the study included healthy females, breast cancer survivors with lymphedema, and those who were at risk for developing lymphedema. Bioelectrical impedance analysis, as indicated by L-Dex ratio, was highly reliable among healthy women (ICC=0.99; 95% CI = 0.99 - 0.99), survivors at-risk for lymphedema (ICC=0.99; 95% CI = 0.99 - 0.99), and all women (ICC=0.85; 95% CI = 0.81 - 0.87); reliability was acceptable for survivors with lymphedema (ICC=0.69; 95% CI = 0.54 to 0.80). The bioimpedance ratio correlated with limb volume by sequential circumferential tape measurement. The L-Dex ratio had a diagnostic cutoff of >+7 which missed 20% of true lymphedema cases. The authors noted that "it is important for clinicians to integrate other assessment methods (such as self-report, clinical observation, or perometry) to ensure the accurate detection of lymphedema."

A technology assessment by the Agency for Healthcare Research and Quality (AHRQ, 2010) concluded that limb volume and circumference remain the de facto 'gold standard' tests to assess the presence of secondary lymphedema. In addition, this technology assessment concluded that there is insufficient evidence to assess the reliability of other tests to measure lymphedema which are under study such as perometry, ultrasound, lymphoscintigraphy, and bioimpedance.

Currently, there is insufficient information to judge the technical and diagnostic performance of bioimpedance spectroscopy testing or its clinical utility as studies are limited to small group sizes (Jain, 2010). There is no evidence from comparative clinical trials which demonstrate an impact on clinical outcome. Further study of this technology as a tool for the diagnosis and treatment of early, subclinical lymphedema continues, but based on current evidence, the use of bioimpedance spectroscopy testing in the diagnosis or management of individuals with known or suspected lymphedema is considered investigational.

Background/Overview

Lymphedema is swelling from fluid build-up caused by improper functioning of the lymphatic system. Symptoms of lymphedema include arm swelling, heaviness, chest/breast swelling, tightness, firmness, pain, numbness, stiffness or impaired limb mobility. There are two types of lymphedema: primary lymphedema which occurs in those who have a congenital abnormality or dysfunction of their lymphatic system and secondary lymphedema which is an acquired condition caused by a disruption or obstruction of the normal lymphatic system. Individuals with breast cancer are at risk for the development of arm lymphedema. Axillary lymph node sampling compromises lymphatic drainage and lymphatic function can be further compromised by radiation delivered to the lymph node beds. Secondary lymphedema can develop days or years after treatment. Most lymphedema occurs within three years of surgery and is associated with discomfort, heaviness, functional limitations and risk of infection. Early lymphedema can be difficult to diagnose. Various methods can be used for evaluating limb volume including water displacement, circumferential measurement, infrared perometry and bioelectrical impedance.

Measurement of limb volume with water displacement requires the individual to submerge the affected limb into a cylinder filled with a known volume of water. The amount of water that is displaced can be weighed or the volume measured. Water displacement is a reliable method to measure limb volume, but it can be cumbersome and difficult to perform in the physician's office. It cannot be used for those individuals with open wounds or sores.

Circumferential measurement of limb volume involves the use of a measuring tape to measure limb circumference at various anatomical landmarks or marked distances from the fingertips or toes. A hindrance to circumferential measurement is that individuals must remove the clothing over the affected limb. There is also concern about the reliability of the measurements. Different individuals measuring the limbs may hold the tape measure tightly or loosely around the limb causing a variation in the measurements.

Infrared perometry uses infrared light to measure the limb volume. The affected limb is placed in a frame and the perometer scans the limb taking measurements at different segments. The limb volume is then calculated by a special computer program. Perometry is a quick method and it does not touch the skin so it can be used for individuals with sensitive or broken skin. Clinical application may be limited by the fact that the equipment is not portable and requires individuals to come into a clinic or facility for limb measurement.

Bioelectrical impedance involves the response of a living organism to a mild electrical current. Skin electrodes are used to pass a small alternating current through a limb and measure the opposition (impedance) to the flow of the current. The measure of the body's resistance to the current can be converted into measurements such as fluid levels. Bioimpedance spectroscopy is a direct measurement of extracellular fluid volume. The device is used to detect extracellular fluid in an individual's limb and is being studied as a tool to detect early signs of subclinical lymphedema.

Definitions

Lymphedema: A condition in which extra lymph fluid builds up in tissues and causes swelling. It may occur in an arm or leg if lymph vessels are blocked, damaged, or removed by surgery.

Coding

The following codes for treatments and procedures applicable to this document are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement policy. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-

coverage of these services as it applies to an individual member.

When services are Investigational and Not Medically Necessary:
When the code describes a procedure indicated in the Position Statement section as investigational and not medically necessary.

CPT

0239T	Bioimpedance spectroscopy (BIS), measuring 100 frequencies or greater, direct measurement of extracellular fluid differences between the limbs

ICD-9 Diagnosis ***[For dates of service prior to 10/01/2015]***
All diagnoses

ICD-10 Diagnosis ***[For dates of service on or after 10/01/2015]***
All diagnoses

References

Peer Reviewed Publications:

1. Bar Ad V, Cheville A, Solin LJ, et al. Time course of mild arm lymphedema after breast conservation treatment for early-stage breast cancer. Int J Radiat Oncol Biol Phys. 2010; 76(1):85-90.
2. Czerniec SA, Ward LC, Lee MJ, et al. Segmental measurement of breast cancer-related arm lymphoedema using perometry and bioimpedance spectroscopy. Support Care Cancer. 2011; 19(5):703-710.
3. Czerniec SA, Ward LC, Refshauge KM, et al. Assessment of breast cancer-related arm lymphedema--comparison of physical measurement methods and self-report. Cancer Invest. 2010; 28(1):54-62.
4. Fu MR, Cleland CM, Guth AA, et al. L-dex ratio in detecting breast cancer-related lymphedema: reliability, sensitivity, and specificity. Lymphology. 2013; 46(2):85-96.
5. Hayes S, Janda M, Cornish B, et al. Lymphedema secondary to breast cancer: how choice of measure influences diagnosis, prevalence, and identifiable risk factors. Lymphology. 2008; 41(1):18-28.
6. Jain MS, Danoff JV, Paul SM. Correlation between bioelectrical spectroscopy and perometry in assessment of upper extremity swelling. Lymphology. 2010; 43(2):85-94.
7. Ridner SH, Dietrich MS, Deng J, et al. Bioelectrical impedance for detecting upper limb lymphedema in nonlaboratory settings. Lymphat Res Biol. 2009; 7(1):11-15.
8. Stout Gergich NL, Pfalzer LA, McGarvey C, et al. Preoperative assessment enables the early diagnosis and successful treatment of lymphedema. Cancer. 2008; 112(12):2809-2819.

Government Agency, Medical Society, and Other Authoritative Publications:

1. Agency for Healthcare Research and Quality. Diagnosis and treatment of secondary lymphedema. Technology Assessment Report. 2010 May. Project ID LYMT0908. Available at: http://www.ahrq.gov/clinic/techix.htm. Accessed on March 17, 2014.
2. U.S. Food and Drug Administration 510(k) Premarket Notification Database. ImpediMed L-Dex U400 BIS Extra Cellular Fluid Analysis. No. K080825. Rockville, MD: FDA. October 3, 2008. Available at: http://www.accessdata.fda.gov/cdrh_docs/pdf8/K080825.pdf. Accessed on March 17, 2014.

Websites for Additional Information

1. National Cancer Institute (NCI). Available at: http://www.cancer.gov/. Accessed on March

17, 2014.

Index

L-DexU400
Lymphedema

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete listing of all products available.

Document History

Status	Date	Action
Reviewed	05/15/2014	Medical Policy & Technology Assessment Committee (MPTAC) review.
Reviewed	05/14/2014	Hematology/Oncology Subcommittee Review. Updated Rationale and References.
Reviewed	05/09/2013	MPTAC review.
Reviewed	05/08/2013	Hematology/Oncology Subcommittee Review. No change to Position Statement.
Revised	05/10/2012	MPTAC review.
Revised	05/09/2012	Hematology/Oncology Subcommittee Review. Clarification to Position Statement. Updated Rationale, Background/Overview, and References.
Reviewed	08/18/2011	MPTAC review. Updated Rationale and References.
New	08/19/2010	MPTAC review. Initial document development. Coding section includes CPT changes effective 01/01/2011.

Using Nontraditional Risk Factors in Coronary Heart Disease Risk Assessment: U.S. Preventive Services Task Force Recommendation Statement

U.S. Preventive Services Task Force

Description: New recommendation from the U.S. Preventive Services Task Force (USPSTF) on the use of nontraditional, or novel, risk factors in assessing the coronary heart disease (CHD) risk of asymptomatic persons.

Methods: Systematic reviews were conducted of literature since 1996 on 9 proposed nontraditional markers of CHD risk: high-sensitivity C-reactive protein, ankle–brachial index, leukocyte count, fasting blood glucose, periodontal disease, carotid intima–media thickness, coronary artery calcification score on electron-beam computed tomography, homocysteine, and lipoprotein(a). The reviews followed a hierarchical approach aimed at determining which factors could practically and definitively reassign persons assessed as intermediate-risk according to their Framingham score to either a high-risk or low-risk strata, and thereby improve outcomes by means of aggressive risk-factor modification in those newly assigned to the high-risk stratum.

Recommendation: The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of using the nontraditional risk factors studied to screen asymptomatic men and women with no history of CHD to prevent CHD events. (I statement).

Ann Intern Med. 2009;151:474-482. www.annals.org

For author affiliation, see end of text.

* For a list of the members of the USPSTF, see the **Appendix** (available at www.annals.org).

The U.S. Preventive Services Task Force (USPSTF) makes recommendations about preventive care services for patients without recognized signs or symptoms of the target condition.

It bases its recommendations on a systematic review of the evidence of the benefits and harms and an assessment of the net benefit of the service.

The USPSTF recognizes that clinical or policy decisions involve more considerations than this body of evidence alone. Clinicians and policymakers should understand the evidence but individualize decision making to the specific patient or situation.

Summary of Recommendation and Evidence

The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of using the nontraditional risk factors discussed in this statement to screen asymptomatic men and women with no history of coronary heart disease (CHD) to prevent CHD events. This is an I statement.

The nontraditional risk factors included in this recommendation are high-sensitivity C-reactive protein (hs-CRP), ankle–brachial index (ABI), leukocyte count, fasting blood glucose level, periodontal disease, carotid intima–media thickness (carotid IMT), coronary artery calcification (CAC) score on electron-beam computed tomography (EBCT), homocysteine level, and lipoprotein(a) level.

See the Clinical Considerations section for suggestions for practice concerning the Insufficient Evidence statement.

See the **Figure** for a summary of the recommendation and suggestions for clinical practice.

See **Table 1** for a description of the USPSTF grades and **Table 2** for a description of the USPSTF classification of levels of certainty about net benefit.

See also:

Print
Related articles 483, 496
Summary for Patients. I-38

Web-Only
Appendix
CME quiz
Conversion of graphics into slides
Downloadable recommendation summary

Rationale

Importance

Coronary heart disease is the most common cause of mortality in adults in the United States. Treatment to prevent CHD events by modifying risk factors is currently based on the Framingham risk model, which sorts individuals into low-, intermediate-, or high-risk groups. If the risk model could be improved, treatment might be bet-

OPEN

2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk

A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines

Endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation, American Society for Preventive Cardiology, American Society of Hypertension, Association of Black Cardiologists, National Lipid Association, Preventive Cardiovascular Nurses Association, and WomenHeart: The National Coalition for Women With Heart Disease

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Preamble and Transition to ACC/AHA Guidelines to Reduce Cardiovascular Risk

The goals of the American College of Cardiology (ACC) and the American Heart Association (AHA) are to prevent cardiovascular diseases (CVD); improve the management of people who have these diseases through professional education and research; and develop guidelines, standards, and policies that promote optimal patient care and cardiovascular health. Toward these objectives, the ACC and AHA have collaborated with the National Heart, Lung, and Blood Institute (NHLBI) and stakeholder and professional organizations to develop clinical practice guidelines for assessment of cardiovascular risk, lifestyle modifications to reduce cardiovascular risk, management of blood cholesterol in adults, and management of overweight and obesity in adults.

In 2008, the NHLBI initiated these guidelines by sponsoring rigorous systematic evidence reviews for each topic by expert panels convened to develop critical questions (CQs), interpret the evidence, and craft recommendations. In response to the 2011 report from the Institute of Medicine on the development of trustworthy clinical guidelines,[1] the NHLBI Advisory Council recommended that the NHLBI focus specifically on reviewing the highest-quality evidence and partner with other organizations to develop recommendations.[2,3] Accordingly, in June 2013 the NHLBI initiated collaboration with the ACC and AHA to work with other organizations to complete and publish the 4 guidelines noted above and make them available to the widest possible constituency. Recognizing that the Expert Work Group/Work Groups did not consider evidence beyond 2011 (except as specified in the methodology), the ACC, AHA, and collaborating societies plan to begin updating these guidelines starting in 2014.

The joint ACC/AHA Task Force on Practice Guidelines (Task Force) appointed a subcommittee to shepherd this transition, communicate the rationale and expectations to the writing panels and partnering organizations, and expeditiously publish the documents. The ACC/AHA and partner organizations recruited a limited number of expert reviewers for fiduciary examination of content, recognizing that each document had undergone extensive peer review by representatives of the NHLBI Advisory Council, key federal agencies, and scientific experts. Each writing panel responded to comments from these reviewers. Clarifications were incorporated where appropriate, but there were no substantive changes because the bulk of the content was undisputed.

Although the Task Force led the final development of these prevention guidelines, they differ from other ACC/AHA guidelines. First, as opposed to an extensive compendium of clinical information, these documents are significantly more limited in scope and focus on selected CQs on each topic, based on the highest-quality evidence available. Recommendations were derived from randomized trials, meta-analyses, and observational studies evaluated for quality and were not formulated when sufficient evidence was not available. Second, the text accompanying each recommendation is succinct, summarizing the evidence for each question. The Full Panel/Work Group Reports include more detailed information about the evidence statements that serve as the basis for recommendations. Third, the format of the recommendations differs from other ACC/AHA guidelines. Each recommendation has been mapped from the NHLBI grading format to the ACC/AHA Classification of Recommendation/Level of Evidence (COR/LOE) construct (Table 1) and is expressed in both formats. Because of the inherent differences in grading systems and the clinical questions driving the recommendations, alignment between the NHLBI and ACC/AHA formats is in some cases imperfect. Explanations of these variations are noted in the recommendation tables, where applicable.

In consultation with NHLBI, the policies adopted by the writing panels to manage relationships of authors with industry and other entities (RWI) are outlined in the methods section of each panel report. These policies were in effect when this

Table 1. Applying Classification of Recommendation and Level of Evidence

ESTIMATE OF CERTAINTY (PRECISION) OF TREATMENT EFFECT	SIZE OF TREATMENT EFFECT: CLASS I *Benefit >>> Risk* Procedure/Treatment **SHOULD** be performed/ administered	CLASS IIa *Benefit >> Risk* *Additional studies with focused objectives needed* **IT IS REASONABLE** to perform procedure/administer treatment	CLASS IIb *Benefit ≥ Risk* *Additional studies with broad objectives needed; additional registry data would be helpful* Procedure/Treatment **MAY BE CONSIDERED**	CLASS III *No Benefit* or CLASS III *Harm* COR III: No benefit — Procedure/Test: Not Helpful — Treatment: No Proven Benefit COR III: Harm — Procedure/Test: Excess Cost w/o Benefit or Harmful — Treatment: Harmful to Patients
LEVEL A Multiple populations evaluated* Data derived from multiple randomized clinical trials or meta-analyses	■ Recommendation that procedure or treatment is useful/effective ■ Sufficient evidence from multiple randomized trials or meta-analyses	■ Recommendation in favor of treatment or procedure being useful/effective ■ Some conflicting evidence from multiple randomized trials or meta-analyses	■ Recommendation's usefulness/efficacy less well established ■ Greater conflicting evidence from multiple randomized trials or meta-analyses	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Sufficient evidence from multiple randomized trials or meta-analyses
LEVEL B Limited populations evaluated* Data derived from a single randomized trial or nonrandomized studies	■ Recommendation that procedure or treatment is useful/effective ■ Evidence from single randomized trial or nonrandomized studies	■ Recommendation in favor of treatment or procedure being useful/effective ■ Some conflicting evidence from single randomized trial or nonrandomized studies	■ Recommendation's usefulness/efficacy less well established ■ Greater conflicting evidence from single randomized trial or nonrandomized studies	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Evidence from single randomized trial or nonrandomized studies
LEVEL C Very limited populations evaluated* Only consensus opinion of experts, case studies, or standard of care	■ Recommendation that procedure or treatment is useful/effective ■ Only expert opinion, case studies, or standard of care	■ Recommendation in favor of treatment or procedure being useful/effective ■ Only diverging expert opinion, case studies, or standard of care	■ Recommendation's usefulness/efficacy less well established ■ Only diverging expert opinion, case studies, or standard of care	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Only expert opinion, case studies, or standard of care
Suggested phrases for writing recommendations	should is recommended is indicated is useful/effective/beneficial	is reasonable can be useful/effective/beneficial is probably recommended or indicated	may/might be considered may/might be reasonable usefulness/effectiveness is unknown/unclear/uncertain or not well established	COR III: No Benefit: is not recommended; is not indicated; should not be performed/ administered/ other; is not useful/ beneficial/ effective COR III: Harm: potentially harmful; causes harm; associated with excess morbidity/mortality; should not be performed/ administered/ other
Comparative effectiveness phrases†	treatment/strategy A is recommended/indicated in preference to treatment B treatment A should be chosen over treatment B	treatment/strategy A is probably recommended/indicated in preference to treatment B it is reasonable to choose treatment A over treatment B		

A recommendation with Level of Evidence B or C does not imply that the recommendation is weak. Many important clinical questions addressed in the guidelines do not lend themselves to clinical trials. Even when randomized trials are unavailable, there may be a very clear clinical consensus that a particular test or therapy is useful or effective.

*Data available from clinical trials or registries about the usefulness/efficacy in different subpopulations, such as sex, age, history of diabetes, history of prior myocardial infarction, history of heart failure, and prior aspirin use.

†For comparative-effectiveness recommendations (Class I and IIa; Level of Evidence A and B only), studies that support the use of comparator verbs should involve direct comparisons of the treatments or strategies being evaluated.

effort began in 2008 and throughout the writing process and voting on recommendations, until the process was transferred to ACC/AHA in 2013. In the interest of transparency, the ACC/AHA requested that panel authors resubmit RWI disclosures as of July 2013. Relationships relevant to this guideline are disclosed in Appendix 1. None of the ACC/AHA expert reviewers had relevant RWI (Appendix 2). See Appendix 3 for a list of abbreviations used in the guideline.

Systematic evidence reports and accompanying summary tables were developed by the expert panels and NHLBI. The guideline was reviewed by the ACC/AHA Task Force and approved by the ACC Board of Trustees, and the AHA Science Advisory and Coordinating Committee. In addition, ACC/AHA sought endorsement from other stakeholders, including professional organizations. It is the hope of the writing panels, stakeholders, professional organizations, NHLBI, and Task Force that the guidelines will garner the widest possible readership for the benefit of patients, providers, and the public health.

These guidelines are meant to define practices that meet the needs of patients in most circumstances and are not a replacement for clinical judgment. The ultimate decision about care of a particular patient must be made by the healthcare provider and patient in light of the circumstances presented by that patient. As a result, situations might arise in which deviations from these guidelines may be appropriate. These considerations notwithstanding, in caring for most patients, clinicians can employ the recommendations confidently to reduce the risks of atherosclerotic cardiovascular disease (ASCVD) events.

See Tables 2 and 3 for an explanation of the NHLBI recommendation grading methodology.

Table 2. NHLBI Grading of the Strength of Recommendations

Grade	Strength of Recommendation*
A	Strong recommendation There is high certainty based on evidence that the net benefit† is substantial.
B	Moderate recommendation There is moderate certainty based on evidence that the net benefit is moderate to substantial, or there is high certainty that the net benefit is moderate.
C	Weak recommendation There is at least moderate certainty based on evidence that there is a small net benefit.
D	Recommendation against There is at least moderate certainty based on evidence that there is no net benefit or that risks/harms outweigh benefits.
E	Expert opinion ("There is insufficient evidence or evidence is unclear or conflicting, but this is what the Work Group recommends.") Net benefit is unclear. Balance of benefits and harms cannot be determined because of no evidence, insufficient evidence, unclear evidence, or conflicting evidence, but the Work Group thought it was important to provide clinical guidance and make a recommendation. Further research is recommended in this area.
N	No recommendation for or against ("There is insufficient evidence or evidence is unclear or conflicting.") Net benefit is unclear. Balance of benefits and harms cannot be determined because of no evidence, insufficient evidence, unclear evidence, or conflicting evidence, and the Work Group thought no recommendation should be made. Further research is recommended in this area.

*In most cases, the strength of the recommendation should be closely aligned with the quality of the evidence; however, under some circumstances, there may be valid reasons for making recommendations that are not closely aligned with the quality of the evidence (eg, strong recommendation when the evidence quality is moderate, such as smoking cessation to reduce cardiovascular disease risk or ordering an ECG as part of the initial diagnostic work-up for a patient presenting with possible MI). Those situations should be limited and the rationale explained clearly by the Work Group.

†Net benefit is defined as benefits minus risks/harms of the service/intervention.

ECG indicates electrocardiogram; MI, myocardial infarction; and NHLBI, National Heart, Lung, and Blood Institute.

Table 3. NHLBI Quality Rating of the Strength of Evidence

Type of Evidence	Quality Rating*
• Well-designed, well-executed† RCT that adequately represent populations to which the results are applied and directly assess effects on health outcomes. • Meta-analyses of such studies. Highly certain about the estimate of effect. Further research is unlikely to change our confidence in the estimate of effect.	High
• RCT with minor limitations‡ affecting confidence in, or applicability of, the results. • Well-designed, well-executed nonrandomized controlled studies§ and well-designed, well-executed observational studies‖. • Meta-analyses of such studies. Moderately certain about the estimate of effect. Further research may have an impact on our confidence in the estimate of effect and may change the estimate.	Moderate
• RCT with major limitations. • Nonrandomized controlled studies and observational studies with major limitations affecting confidence in, or applicability of, the results. • Uncontrolled clinical observations without an appropriate comparison group (eg, case series, case reports). • Physiological studies in humans. • Meta-analyses of such studies. Low certainty about the estimate of effect. Further research is likely to have an impact on our confidence in the estimate of effect and is likely to change the estimate.	Low

*In some cases, other evidence, such as large all-or-none case series (eg, jumping from airplanes or tall structures), can represent high- or moderate-quality evidence. In such cases, the rationale for the evidence rating exception should be explained by the Work Group and clearly justified.

†"Well-designed, well-executed" refers to studies that directly address the question; use adequate randomization, blinding, and allocation concealment; are adequately powered; use intention-to-treat analyses; and have high follow-up rates.

‡Limitations include concerns with the design and execution of a study that result in decreased confidence in the true estimate of the effect. Examples of such limitations include but are not limited to: inadequate randomization, lack of blinding of study participants or outcome assessors, inadequate power, outcomes of interest that are not prespecified for the primary outcomes, low follow-up rates, and findings based on subgroup analyses. Whether the limitations are considered minor or major is based on the number and severity of flaws in design or execution. Rules for determining whether the limitations are considered minor or major and how they will affect rating of the individual studies will be developed collaboratively with the methodology team.

§Nonrandomized controlled studies refer to intervention studies where assignment to intervention and comparison groups is not random (eg, quasi-experimental study design).

‖Observational studies include prospective and retrospective cohort, case-control, and cross-sectional studies.

NHLBI indicates National Heart, Lung, and Blood Institute; and RCT, randomized controlled trials.

1. Introduction

1.1. Organization of the Work Group

The Risk Assessment Work Group (Work Group) was composed of 11 members and 5 ex-officio members, including internists, cardiologists, endocrinologists, and experts in cardiovascular epidemiology, biostatistics, healthcare management and economics, and guideline development.

1.2. Document Review and Approval

A formal peer review process, which included 12 expert reviewers and representatives of federal agencies, was initially completed under the auspices of the NHLBI. This document was also reviewed by 3 expert reviewers nominated by the ACC and the AHA when the management of the guideline transitioned to the ACC/AHA. The ACC and AHA Reviewers' RWI information is published in this document (Appendix 2).

This document was approved for publication by the governing bodies of the ACC and AHA and endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation, American Society for Preventive Cardiology, American Society of Hypertension, Association of Black Cardiologists, National Lipid Association, Preventive Cardiovascular Nurses Association, and WomenHeart: The National Coalition for Women With Heart Disease.

1.3. Charge to the Work Group

The Work Group was 1 of 3 work groups appointed by the NHLBI to develop its own recommendations and provide cross-cutting input to 3 Panels for updating guidelines on blood cholesterol, blood pressure (BP), and overweight/obesity. The Work Group was asked to examine the scientific evidence on risk assessment for initial ASCVD events and to develop an approach for quantitative risk assessment that could be used in practice and used or adapted by the risk factor panels (blood cholesterol, hypertension, and obesity) in their guidelines and algorithms. Specifically, the Work Group was charged with 2 tasks:

1. To develop or recommend an approach to quantitative risk assessment that could be used to guide care; and
2. To use systematic review methodology to pose and address a small number of questions judged to be critical to refining and adopting risk assessment in clinical practice.

1.4. Methodology and Evidence Review

This guideline is based on the Full Work Group Report supplement, which is provided as a supplement to the guideline. The Full Work Group Report supplement contains background and additional material related to content, methodology, evidence synthesis, rationale, and references and is supported by the NHLBI Systematic Evidence Review, which can be found at (http://www.nhlbi.nih.gov/guidelines/cvd_adult/risk_assessment/). These documents also describe the process for the development of novel, comprehensive multivariable risk equations for the prediction of 10-year risk of development of ASCVD in non-Hispanic African-American and non-Hispanic white men and women from 40 to 79 years of age. These equations were developed from several long-standing population-based cohort studies funded by the NHLBI. Ten-year risk was defined as the risk of developing a first ASCVD event, defined as nonfatal myocardial infarction or coronary heart disease (CHD) death or fatal or nonfatal stroke, over a 10-year period among people free from ASCVD at the beginning of the period.

In addition, through evaluation of evidence developed by systematic reviews of the literature, the Work Group addressed the following 2 CQs:

CQ1. "What is the evidence with regard to reclassification or contribution to risk assessment when high-sensitivity C-reactive protein (hs-CRP), apolipoprotein B (ApoB), glomerular filtration rate, microalbuminuria, family history, cardiorespiratory fitness, ankle-brachial index (ABI), carotid intima-media thickness (CIMT), or coronary artery calcium (CAC) score is considered in addition to the variables that are in the traditional risk scores?"

CQ2. "Are models constructed to assess the long-term (≥15 years or lifetime) risk of a first cardiovascular disease (CVD) event in adults effective in assessing variation in long-term risk among adults at low and/or intermediate short-term risk, whether analyzed separately or in combination?"

The evidence and recommendations in the guideline focus on the large proportion of the adult population without clinical signs or symptoms of ASCVD who merit evaluation for the primary prevention of ASCVD. They do not apply to those with clinically manifest ASCVD, who require secondary prevention approaches, or to highly-selected patient subgroups, such as those with symptoms suggestive of CVD, who require diagnostic strategies rather than risk assessment. Furthermore, these recommendations were not developed for use in specific subgroups of asymptomatic individuals at unusually high risk, such as those with genetically determined extreme values of traditional risk factors (eg, patients with familial hypercholesterolemia).

2. Risk Assessment: Recommendations

See Table 4 for a summary of the recommendations for risk assessment.

3. Approach to Risk Assessment

In addressing its charge, the Work Group recognized the need for a risk assessment approach that was based on the types of data that primary care providers could easily collect and that could be implemented in routine clinical practice. After deliberation, the Work Group endorsed the existing and widely used paradigm of matching the intensity of preventive efforts with the individual's absolute risk.[23,24] The Work Group acknowledges that none of the risk assessment tools or novel risk markers examined in the present document have been formally evaluated in randomized controlled trials of screening strategies with clinical events as outcomes. Nevertheless, this approach balances an understanding of an individual's absolute risk of CVD and potential treatment benefits against the potential absolute risks for harm from therapy. With the use of this framework, treatment can be targeted to those most likely to benefit without undue risk of harm, in the context of a "risk discussion." A risk discussion could include the assessment of the patient's risk of ASCVD, as well as potential benefits, negative aspects, risks, and patient preferences with regard to initiation of relevant preventive therapies.

By its nature, such an approach requires a platform for reliable quantitative estimation of absolute risk based on data from representative population samples. It is important to note that risk estimation is based on group averages, which are then applied to individual patients in practice. This process is admittedly imperfect; no one has 10% or 20% of a heart attack during a 10-year period. Individuals with the same estimated risk will either have or not have the event of interest, and only those patients who are destined to have an event can have their event prevented by therapy. The criticism of the risk-estimation approach to treatment decision making also applies to the alternative, and much less efficient approach, of checking the patient's characteristics against numerous and complex inclusion and exclusion criteria for a potentially large number of pertinent trials. Only a small fraction of trial participants have events, and only a fraction of these events are prevented by therapy. Using either approach, the clinician must apply the average results obtained from groups of patients to the individual patient in practice.

Given the modification and adoption of the Framingham 10-year risk score for CHD risk assessment by the Third Report of the National Cholesterol Education Program Expert Work Group on Diagnosis, Evaluation, and Treatment of High

Table 4. Summary of Recommendations for Risk Assessment

Recommendations	NHLBI Grade	NHLBI Evidence Statements	ACC/AHA COR	ACC/AHA LOE
Assessment of 10-Year Risk of a First Hard ASCVD Event				
1. The race- and sex-specific Pooled Cohort Equations* to predict 10-year risk of a first hard ASCVD event should be used in non-Hispanic African Americans and non-Hispanic whites, 40–79 years of age.	B (Moderate)	N/A	I	B[4–8]
2. Use of the sex-specific Pooled Cohort Equations for non-Hispanic whites may be considered for estimation of risk in patients from populations other than African Americans and non-Hispanic whites.	E (Expert Opinion)	N/A	IIb	C
CQ1: Use of Newer Risk Markers After Quantitative Risk Assessment				
1. If, after quantitative risk assessment, a risk-based treatment decision is uncertain, assessment of ≥1 of the following—family history, hs-CRP, CAC score, or ABI—may be considered to inform treatment decision making.	E (Expert Opinion)	Appendix 4	IIb†	B[9–17]
2. Routine measurement of CIMT is not recommended in clinical practice for risk assessment for a first ASCVD event.	N (No recommendation for or against)	Appendix 4	III: No Benefit†	B[12,16,18]
3. The contribution of ApoB, CKD, albuminuria, and cardiorespiratory fitness to risk assessment for a first ASCVD event is uncertain at present.	N (No recommendation for or against)	Appendix 4	—	—
CQ2: Long-Term Risk Assessment				
1. It is reasonable to assess traditional ASCVD risk factors‡ every 4–6 years in adults 20–79 years of age who are free from ASCVD and to estimate 10-year ASCVD risk every 4–6 years in adults 40–79 years of age who are free from ASCVD.	B (Moderate)	Appendix 5 CQ2/ES7	IIa	B[19,20]
2. Assessment of 30-year or lifetime ASCVD risk on the basis of traditional risk factors‡ may be considered in adults 20–59 years of age who are free from ASCVD and are not at high short-term risk.	C (Weak)	Appendix 5 CQ2/ES2, CQ2/ES3, CQ2/ES4, CQ2/ES5, CQ2/ES6	IIb	C[20–22]

A downloadable spreadsheet enabling estimation of 10-year and lifetime risk of ASCVD and a Web-based calculator is available at http://my.americanheart.org/cvriskcalculator and http://www.cardiosource.org/en/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/2013-Prevention-Guideline-Tools.aspx.

*Derived from the ARIC (Atherosclerosis Risk in Communities) study,[8] Cardiovascular Health Study,[5] CARDIA (Coronary Artery Risk Development in Young Adults) study,[7] and Framingham original and offspring cohorts.[4,6]

†Based on new evidence reviewed during ACC/AHA update of evidence.

‡Age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic BP, use of antihypertensive therapy, diabetes, and current smoking.

ABI indicates ankle-brachial index; ACC, American College of Cardiology; AHA, American Heart Association; ApoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; CAC, coronary artery calcium; CIMT, carotid intima-media thickness; CKD, chronic kidney disease; COR, Class of Recommendation; CQ, critical question, ES, evidence statement; hs-CRP, high-sensitivity C-reactive protein; LOE, Level of Evidence; NHLBI, National Heart, Lung, and Blood Institute; and —, not applicable.

Blood Cholesterol in Adults (Adult Treatment Panel III)[24] and the uptake of this algorithm by practice sites across the United States, the Work Group began by discussing the value of retaining this algorithm. In collaboration with other NHLBI panels, the Work Group decided not to use this algorithm in its 2013 recommendations because of the algorithm's derivation in an exclusively white sample population and the limited scope of the outcome (in determining CHD alone). Rather, the Work Group derived risk equations from community-based cohorts that are broadly representative of the US population of whites and African Americans, and the Work Group focused on estimation of first hard ASCVD events (defined as first occurrence of nonfatal myocardial infarction, CHD death, or fatal or nonfatal stroke) as the outcome of interest because they were deemed to be of greater relevance to both patients and providers. The focus on hard ASCVD, rather than CHD alone, is also consistent with evidence reviewed in a statement from the AHA and American Stroke Association calling for the inclusion of ischemic stroke in the outcome of interest for CVD risk assessment.[25]

Numerous multivariable risk scores and equations have been derived and published (Appendix 6; for more details, see the Full Work Group Report supplement). As part of its deliberations, the Work Group considered previously published risk scores with validation in NHLBI cohort data as one possible approach. However, several persistent concerns with existing risk equations were identified, including nonrepresentative or historically dated populations, limited ethnic diversity, narrowly defined endpoints, endpoints influenced by provider preferences (eg, elective revascularizations), and endpoints with poor reliability (eg, angina and heart failure). Given the inherent limitations of existing scores, the Work Group judged that a new risk score was needed to address some of the deficiencies of existing scores—for example, the need for a population sample that approaches, to the degree possible, the ideal sample for algorithm development and closely represents the US population.

Data are sparse on the use and impact of absolute risk scores in clinical practice in primary-prevention settings.[26]

Two systematic reviews, based on few studies, support the conclusion that risk assessment, combined with counseling, is associated with favorable but modest changes in patient knowledge and intention to change and in provider prescribing behavior and risk factor control.[27,28] No data are available on hard event outcomes. The Work Group specifically calls for research in this area (Section 8).

The Work Group notes that the "2009 ACCF/AHA Performance Measures for the Primary Prevention of Cardiovascular Disease in Adults" specifically recommended use of global CVD risk estimation in clinical practice.[29] Likewise, the US Preventive Services Task Force recommendations for aspirin,[30] the NHLBI Adult Treatment Panel III recommendations,[24] and European[31] and Canadian[32,33] guidelines for primary prevention of CVD, among others, have all recommended the use of absolute risk assessment for decision making about the intensity of lifestyle and pharmacological preventive interventions. Risk scores have been implemented in practice through paper scoring sheets and, increasingly, through Web sites and downloadable applications. The electronic medical record can be adapted to estimate absolute risks automatically by using patient data and published equations, and it is anticipated that risk estimation with this technology will become a mainstream application of the current and future risk algorithms.

4. Development of New Pooled Cohort ASCVD Risk Equations

Having made the decision to develop new equations to estimate the 10-year risk of developing a first ASCVD event, the Work Group used the best available data from community-based cohorts of adults, with adjudicated endpoints for CHD death, nonfatal myocardial infarction, and fatal or nonfatal stroke. Cohorts that included African-American or white participants with at least 12 years of follow-up were included. Data from other racial/ethnic groups were insufficient, precluding their inclusion in the final analyses. The final pooled cohorts included participants from several large, racially and geographically diverse, modern NHLBI-sponsored cohort studies, including the ARIC (Atherosclerosis Risk in Communities) study,[8] the Cardiovascular Health Study,[5] and the CARDIA (Coronary Artery Risk Development in Young Adults) study,[7] combined with applicable data from the Framingham Original and Offspring Study cohorts.[4,6]

The Work Group used state-of-the-art statistical methods to derive and internally validate the Pooled Cohort Equations, which provide sex- and race-specific estimates of the 10-year risk of ASCVD for African-American and white men and women 40 to 79 years of age. The variables that statistically merit inclusion in the risk assessment equations are age, total cholesterol, high-density lipoprotein cholesterol, systolic BP (including treated or untreated status), diabetes mellitus (diabetes), and current smoking status.

An expanded description of the derivation and validation of the Pooled Cohort Equations, as well as the means for implementing them in clinical practice, is provided in Appendix 7. Additional details are provided in the Full Work Group Report supplement. A specific clinical vignette is also provided as an example in Appendix 7. In the clinical vignette, the 10-year risk is calculated for a patient 55 years of age who is a nonsmoker without diabetes, and with total cholesterol level of 213 mg/dL, high-density lipoprotein cholesterol level of 50 mg/dL, and untreated systolic BP of 120 mm Hg. With these values used in the Pooled Cohort Equations, the predicted 10-year ASCVD risks are 2.1% for white women, 3.0% for African-American women, 5.3% for white men, and 6.1% for African-American men.

Numerous other potential risk markers were considered for inclusion in the Pooled Cohort Equations: for many, no additional utility was demonstrated when they were included; for others, data are insufficient at the present time to determine their additional value. The equations were also assessed in external validation studies with data from other available cohorts. Other than the Framingham CHD risk score (and its derivative ATP III risk assessment profile) and the European SCORE (System for Cardiac Operative Risk Evaluation) algorithm for CVD death, these equations have been subjected to more rigorous validation than other currently available equations, and they are the only risk assessment equations that include significant numbers of African Americans and that focus on estimation of 10-year risk of the clinically relevant endpoint of ASCVD. The Work Group specifically calls for further research to develop similar equations applicable to other ethnic groups, to validate the utility of the Pooled Cohort Equations in diverse primary-prevention settings, and to assess the potential benefit of novel risk markers when added to these equations, so that the equations maybe modified or expanded over time as new data become available.

4.1. Recommendations for Assessment of 10-Year Risk of a First Hard ASCVD Event

Recommendation 1. The race- and sex-specific Pooled Cohort Equations* to predict 10-year risk of a first hard ASCVD event should be used in non-Hispanic African Americans and non-Hispanic whites, 40 to 79 years of age.

NHLBI Grade: B (Moderate); ACC/AHA COR: I; LOE: B

Recommendation 2. Use of the sex-specific Pooled Cohort Equations for non-Hispanic whites may be considered for estimation of risk in patients from populations other than African Americans and non-Hispanic whites.

NHLBI Grade: E (Expert Opinion); ACC/AHA COR: IIb; LOE: C

A Web-based application enabling estimation of 10-year and lifetime risk of ASCVD is available at http://my.americanheart.org/cvriskcalculator and http://www.cardiosource.org/en/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/2013-Prevention-Guideline-Tools.aspx.

5. Implications for Risk Assessment

A range of estimated 10-year risk of a first hard ASCVD event is illustrated in the Full Work Group Report supplement (Tables 8 through 11), across a broad range of risk factor burdens for

*Ten-year risk was defined as the risk of developing a first ASCVD event, defined as nonfatal myocardial infarction, CHD death, or fatal or nonfatal stroke, over a 10-year period among people free from ASCVD at the beginning of the period.

selected combinations of the risk factors in sex–race groups (African-American and white women and men). The estimated risks are specific to defined combinations of the risk factors and demonstrate how they vary over a broad spectrum of potential profiles. Risk factor levels that are more adverse than those shown in these tables should always be associated with a higher estimated risk. For example, if a given risk factor combination indicates an estimated 10-year risk of hard ASCVD of 8%, but a patient has a higher level of systolic BP or total cholesterol, or a lower level of high-density lipoprotein cholesterol, than shown for that table cell, then the estimated risk would be ≥8%. Because the estimated probabilities can become unstable when approaching the limits of the sample data, the risk probabilities are truncated at 1% and 30%. The proportions of the US adult population, 40 to 79 years of age, in selected strata of estimated 10-year risk of hard ASCVD events, are shown overall and by sex and race/ethnicity in Table 5. When compared with non-Hispanic whites, estimated 10-year risk of ASCVD is generally lower in Hispanic-American and Asian-American populations and higher in American-Indian populations;[34,35] hence, the lack of race/ethnicity-specific risk algorithms is an important gap in our efforts to understand and prevent ASCVD in these populations. Although the development of algorithms specific to these racial/ethnic groups is encouraged, in the interim, providers may consider using the equations for non-Hispanic whites for these patients. When doing so, the estimated risks may be overestimates, especially for Hispanic and Asian Americans.

6. CQs and Systematic Evidence Review

6.1. Critical Question 1

"What is the evidence with regard to reclassification or contribution to risk assessment when hs-CRP, ApoB, glomerular filtration rate, microalbuminuria, family history, cardiorespiratory fitness, ABI, CAC, or CIMT is considered in addition to the variables that are in the traditional risk scores?"

The concept of matching the intensity of risk factor management to the estimated risk of CVD has been well established since the 27th Bethesda Conference in 1996.[23] As a consequence, widespread attention has focused on the accuracy and reliability of risk assessment. Claims that a minority of the risk of CVD can be explained by the major traditional risk factors or that most patients presenting with CHD have no elevated traditional risk factors have been disproved.[36,37] Nonetheless, the desire to improve existing quantitative risk-estimation tools has helped to stimulate and maintain interest in the search for new risk markers for CVD that might further enhance risk assessment.

CQ1 was developed to address whether newer risk markers have been identified that actually improve risk assessment enough to warrant routine measurement in clinical practice. This question applies to risk assessment in the general population—that is, the typical asymptomatic adult in routine clinical practice. This question does not address other highly selected patient subgroups, such as those with symptoms suggestive of CVD.

CQ1 was addressed through 2 independent approaches. First, in the process of developing the Pooled Cohort Equations, the additional risk markers listed in CQ1 were tested for inclusion in the model if they were available in the databases and could be evaluated on the basis of at least 10 years of follow-up. A review of meta-analyses and systematic reviews published before September 19, 2013, was conducted in 2 stages. In the first stage, meta-analyses and systematic reviews published before April 2011 were identified and reviewed. In a second stage, conducted to update the evidence base before publication, additional meta-analyses and systematic reviews published before September 19, 2013, were identified and reviewed against the same criteria applied in the first stage. The reliance on published meta-analyses to evaluate novel biomarkers is a conservative approach that helps avoid the influence of positive publication bias that can occur early in the evaluation of a novel association and assures that we relied on a mature body of evidence.[38]

Members of the Work Group proposed an initial list of novel risk markers for inclusion in CQ1, which was then prioritized during several rounds of discussion. In selecting the final list, the Work Group gave priority to factors that have engendered substantial discussion in the scientific community and that could be reasonably considered as potentially feasible for widespread population use by primary care providers in routine clinical settings in the United States. In these deliberations, the Work Group considered availability, cost, assay reliability, and risks of the test or downstream testing. The final list of new risk markers to be evaluated included several blood and urine biomarkers (hs-CRP, ApoB, creatinine [or estimated glomerular filtration rate], and microalbuminuria), several measures of subclinical cardiovascular disease (CAC, CIMT, and ABI), family history, and cardiorespiratory fitness. Other novel potential screening tools maybe the subject of future guideline updates. Guidance published by Hlatky et al[39] was considered during discussion of the utility of incorporating these new risk factors into routine risk assessment. Special attention was given to the additional value these markers contributed to risk assessment in terms of discrimination, calibration, reclassification, and cost-effectiveness, in the context of any potential harm.

6.1.1. Summary of Systematic Reviews and Meta-Analyses for CQ1

Thirteen systematic review articles or meta-analyses met the inclusion/exclusion criteria.[9–18,40–42] Publication dates ranged from 2008 to 2013. The Work Group reviewed the 13 systematic reviews and meta-analyses and created a table to list their key findings (Appendix 4). None of these markers has been evaluated as a screening test in randomized controlled trials with clinical events as outcomes. On the basis of current (limited) evidence, it is the opinion of the Work Group that among the novel risk markers, assessments of family history of premature CVD, as well as measurement of hs-CRP, CAC, and ABI, show some promise for clinical utility. Table 6 provides expert opinion on thresholds of these measures that may be considered for clinical decision making.

The Work Group notes that the review by Peters et al[16] provides evidence to support the contention that measuring CAC is

Table 5. Distribution of Estimated 10-Year Risk of a First Hard ASCVD Event in the CVD-Free, Nonpregnant US Population, 40 to 79 Years of Age, by Sex and Race/Ethnicity*

	Predicted 10-Year Risk of Hard ASCVD Event						
	<2.5%	2.5%–4.9%	5.0%–7.4%	7.5%–9.9%	10.0%–14.9%	15.0%–19.9%	≥20.0%
Total							
% (95% CI)	33.4 (31.2–35.5)	21.0 (19.4–22.7)	12.7 (11.4–14.0)	7.4 (6.5–8.3)	8.9 (8.1–9.6)	6.3 (5.6–7.1)	10.2 (9.5–11.0)
n	33 534 000	21 151 000	12 766 000	7 470 000	8 940 000	6 380 000	10 300 000
Sex							
Men							
% (95% CI)	17.4 (15.2–19.7)	22.7 (20.3–25.1)	15.6 (13.8–17.4)	10.1 (8.5–11.6)	12.1 (10.7–13.5)	8.8 (7.4–10.2)	13.3 (12.1–14.4)
n	8 386 000	10 950 000	7 511 000	4 847 000	5 849 000	4 248 000	6 388 000
Women							
% (95% CI)	48.0 (44.8–51.3)	19.5 (17.3–21.6)	10.0 (8.3–11.8)	5.0 (3.8–6.2)	5.9 (5.1–6.7)	4.1 (3.4–4.7)	7.5 (6.5–8.4)
n	25 148 000	10 200 000	5 256 000	2 622 000	3 091 000	2 131 000	3 912 000
Race/Ethnicity							
White							
Men							
% (95% CI)	18.0 (15.0–21.1)	22.4 (19.4–25.3)	15.7 (13.3–18.1)	10.0 (8.2–11.8)	11.7 (9.9–13.5)	8.7 (7.0–10.4)	13.6 (12.3–14.9)
n	6 467 000	8 016 000	5 616 000	3 584 000	4 189 000	3 112 000	4 870 000
Women							
% (95% CI)	47.1 (43.0–51.1)	20.4 (17.7–23.0)	10.7 (8.6–12.8)	5.1 (3.6–6.7)	5.5 (4.6–6.5)	4.1 (3.4–4.9)	7.1 (5.9–8.2)
n	18 175 000	7 863 000	4 136 000	1 984 000	2 132 000	1 596 000	2 725 000
African American							
Men							
% (95% CI)	1.4 (0.3–2.6)	23.9 (19.9–28.0)	20.6 (17.0–24.2)	11.8 (8.8–14.8)	17.4 (14.3–20.5)	11.1 (8.2–13.9)	13.8 (11.0–16.7)
n	60 000	1 008 000	866 000	495 000	731 000	466 000	583 000
Women							
% (95% CI)	36.5 (32.4–40.6)	18.7 (15.6–21.8)	10.9 (8.6–13.2)	6.5 (5.0–7.9)	9.4 (7.2–11.7)	5.7 (4.2–7.2)	12.3 (9.5–15.0)
n	1 921 000	985 000	572 000	339 000	496 000	300 000	645 000
Hispanic							
Men							
% (95% CI)	24.0 (19.8–28.1)	22.1 (17.9–26.2)	13.2 (10.8–15.6)	10.6 (8.1–13.0)	11.4 (9.9–12.9)	6.2 (4.6–7.9)	12.6 (9.4–15.7)
n	1 303 000	1 200 000	718 000	574 000	619 000	339 000	683 000
Women							
% (95% CI)	59.4 (54.3–64.4)	14.5 (11.5–17.5)	7.5 (5.4–9.6)	4.5 (2.6–6.4)	4.9 (3.4–6.5)	3.0 (2.0–3.9)	6.3 (4.7–7.9)
n	3 293 000	803 000	418 000	248 000	273 000	164 000	347 000
Others							
Men							
% (95% CI)	20.8 (10.8–30.7)	27.1 (18.0–36.3)	11.6 (4.9–18.2)	7.2 (0.6–13.8)	11.5 (4.5–18.6)	12.3 (5.9–18.8)	9.4 (3.0–15.8)
n	555 000	726 000	310 000	193 000	309 000	330 000	251 000
Women							
% (95% CI)	59.8 (50.2–69.3)	18.6 (10.8–26.5)	4.4 (0–8.7)	1.7 (0–3.5)	6.4 (2.1–10.7)	2.4 (0.4–4.5)	6.7 (2.3–11.0)
n	1 757 000	548 000	128 000	49 000	188 000	71 000	195 000

*Data derived by applying the Pooled Cohort Equations to the National Health and Nutrition Examinations Surveys, 2007–2010 (*N*=5367, weighted to 100 542 000 US population).

ASCVD indicates atherosclerotic cardiovascular disease; and CVD, cardiovascular disease.

likely to be the most useful of the current approaches to improving risk assessment among individuals found to be at intermediate risk after formal risk assessment. Furthermore, the Work Group recognizes that the "2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults" made recommendations for CAC testing.[43] However, the Work Group notes that the outcomes in the studies reviewed by Peters et al[16] and by Greenland et al[43] were CHD outcomes, not hard ASCVD events that included stroke; hence, uncertainty remains about the contribution of CAC assessment to estimation of 10-year risk of first hard ASCVD events after formal risk assessment with the new Pooled Cohort Equations. Furthermore,

Table 6. Expert Opinion Thresholds for Use of Optional Screening Tests When Risk-Based Decisions About Initiation of Pharmacological Therapy Are Uncertain After Quantitative Risk Assessment

Measure	Support Revising Risk Assessment Upward	Do Not Support Revising Risk Assessment
Family history of premature CVD	Male <55 years of age Female <65 years of age (first-degree relative)	Occurrences at older ages only (if any)
hs-CRP	≥2 mg/L	<2 mg/L
CAC score	≥300 Agatston units or ≥75th percentile for age, sex, and ethnicity*	<300 Agatston units and <75th percentile for age, sex, and ethnicity*
ABI	<0.9	≥0.9

*For additional information, see http://www.mesa-nhlbi.org/CACReference.aspx.

ABI indicates ankle-brachial index; CAC, coronary artery calcium; CVD, cardiovascular disease; and hs-CRP, high-sensitivity C-reactive protein.

issues of cost and radiation exposure related to measuring CAC were discussed, resulting in some uncertainty about potential risks of more widespread screening, which resulted in a decision in the present guideline to make assessment of CAC an ACC/AHA COR IIb recommendation among individuals for whom a risk-based treatment decision is uncertain after formal risk estimation. The Work Group notes that this ACC/AHA COR IIb recommendation is consistent with the recommendations in the 2010 ACCF/AHA guideline[43] for patients with a 10-year CHD risk of <10%, as well as for many other patients, because of the lower risk threshold (7.5% 10-year risk of a first hard ASCVD event) adopted by the "2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults"[44] for recommending initiation of statin therapy for ASCVD risk reduction.

Furthermore, it was noted that measurement of ApoB, albuminuria, glomerular filtration rate, or cardiorespiratory fitness is of uncertain value. Finally, the Work Group judged that the evidence provided by Den Ruijter et al,[18] reviewed during the ACC/AHA update period, in combination with the concerns about measurement quality, provided sufficient rationale to recommend against measuring CIMT in routine clinical practice for risk assessment for a first ASCVD event. If any of the 9 markers considered in the present report is assessed in selected patients, the use of the information to guide treatment decisions will require sound clinician judgment and should be based on shared decision making.

6.1.2. Recommendations for CQ1: Use of Newer Risk Markers After Quantitative Risk Assessment

Recommendation 1. If, after quantitative risk assessment, a risk-based treatment decision is uncertain, assessment of 1 or more of the following—family history, hs-CRP, CAC score, or ABI—may be considered to inform treatment decision making.

NHLBI Grade: E (Expert Opinion); ACC/AHA COR: IIb†, LOE: B†

†Based on new evidence reviewed during ACC/AHA update of the evidence.

Recommendation 2. Routine measurement of CIMT is not recommended in clinical practice for risk assessment for a first ASCVD event.

NHLBI Grade: N (No recommendation for or against); ACC/AHA COR III: No Benefit†, LOE: B

Recommendation 3. The contribution of ApoB, chronic kidney disease, albuminuria, and cardiorespiratory fitness to risk assessment for a first ASCVD event is uncertain at present.

NHLBI Grade: N (No recommendation for or against)

6.2. Critical Question 2

"Are models constructed to assess the long-term (≥15 years or lifetime) risk of a first CVD event in adults effective in assessing variation in long-term risk among adults at low or intermediate short-term risk, whether analyzed separately or in combination?"

Younger men (typically <50 years of age) and most women have low (eg, <5% or <10%) predicted 10-year risks of CHD and more broad CVD outcomes, even in the presence of significant risk factor burden.[45,46] However, extensive epidemiological, pathological, and basic science data indicate that the development of atherosclerosis, the precursor of ASCVD, occurs over decades and is related to long-term and cumulative exposure to causal, modifiable risk factors. Thus, a life-course perspective on risk assessment and prevention must be taken, especially among younger individuals. The primary value of risk factor measurement and quantitative long-term risk estimation in younger adults is 2-fold: first, to identify risk in individuals with extreme values of risk factors (eg, familial hypercholesterolemia); and second, to provide risk information and context for the potential benefits of lifestyle modification. When posing CQ2, the Work Group did not anticipate that long-term or lifetime risk would replace 10-year risk assessment as the foundation for absolute risk assessment and clinical decision making. Rather, longer-term risk estimates, if found to be useful, could provide adjunctive information for risk communication.

CQ2 was developed to assess the utility of long-term and lifetime risk assessment as an adjunct to short-term (10-year) risk assessment. It was recognized that there is little "disconnect" with regard to approaches to prevention when the 10-year risk estimate is high (eg, >10% predicted 10-year risk); such patients merit intensive prevention efforts and should be considered for drug therapy to reduce or modify adverse levels of causal risk factors. CQ2 was selected for evaluation to determine whether quantitative or semiquantitative long-term risk assessment would provide differential information that could be useful in risk communication, specifically to patients estimated to be at lower short-term risk. However, it is unclear what the long-term predicted and observed risks for CHD and CVD are among individuals who are at low predicted 10-year risk. CQ2 was designed to identify studies that assessed both short- and long-term risk, focusing in particular on those studies that provide long-term outcomes data for groups predicted to be at low 10-year risk. If a sufficiently large proportion of the population is at high long-term risk despite being at low short-term risk, then incorporating long-term risk assessment

into routine clinical practice might have value for informing risk discussions with patients and guiding therapeutic lifestyle counseling and other aspects of care.

6.2.1. Summary of Evidence for CQ2

Ten studies that met inclusion/exclusion criteria were identified by the systematic review performed in April 2011 and were examined.[19–22,47–52] Publication dates ranged from 1999 to 2009. All of the studies were observational. On the basis of these studies, 7 evidence statements were adopted (Appendix 5).

Multiple sources provided consistent evidence for the associations of traditional risk factors with events occurring during both short-term and long-term follow-up. The important associations are best represented and understood in the context of multivariable risk equations that reliably predict absolute risk of ASCVD events. In addition, most of these risk factors are both causal and modifiable, which indicates their central clinical importance for ASCVD prevention efforts. Given the additional evidence suggesting improved risk prediction with updated clinical covariates, the Work Group makes the following recommendations.

6.2.2. Recommendations for CQ2: Long-Term Risk Assessment

Recommendation 1. It is reasonable to assess traditional ASCVD risk factors‡ every 4 to 6 years in adults 20 to 79 years of age who are free from ASCVD and to estimate 10-year ASCVD risk every 4 to 6 years in adults 40 to 79 years of age who are free from ASCVD.

NHLBI Grade: B (Moderate); ACC/AHA COR: IIa, LOE: B

Recommendation 2. Assessment of 30-year or lifetime ASCVD risk on the basis of traditional risk factors‡ may be considered in adults 20 to 59 years of age who are free from ASCVD and are not at high short-term risk.

NHLBI Grade: C (Weak); ACC/AHA COR: IIb, LOE: C

A Web-based application enabling estimation of 10-year and lifetime risk of ASCVD is available at http://my.americanheart.org/cvriskcalculator and http://www.cardiosource.org/en/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/2013-Prevention-Guideline-Tools.aspx.

Evidence was not found on the utility of lifetime risk assessment for guiding pharmacological therapy decisions, and the Work Group judged that long-term and lifetime risk information may be used more appropriately at this time to motivate therapeutic lifestyle change in younger individuals. This perspective influenced the choice of age 20 years as the starting point for long-term risk assessment, despite a threshold of age 40 years for short-term 10-year ASCVD risk assessment.

Long-term and lifetime risk estimation may be less valuable for individuals who are found to be at high short-term (10-year) risk according to multivariable equations, for whom decisions about prevention efforts may be clear. However, an understanding of long-term risk may provide a means of encouraging adherence to lifestyle or pharmacological therapies, especially for patients who might have difficulty understanding the importance of their short-term risk. Likewise, for older individuals or those with limited life expectancy, clinical considerations should dictate the intensity of risk assessment and prevention efforts.

‡Age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic BP, use of antihypertensive therapy, diabetes, and current smoking.

7. Implementation Considerations for Risk Assessment

A suggested approach for incorporating these recommendations into clinical practice is shown in Figure 1. For patients 20 to 79 years of age who are free from clinical ASCVD, the first step is to assess ASCVD risk factors. Although it is reasonable to assess ASCVD risk factors in individuals younger or older than this age range, limitations of available data prevented the development of robust risk assessment algorithms in those populations. Hence, for patients outside this age range, providers should refer to applicable clinical practice guidelines (ie, pediatric[53] and adult primary prevention guidelines.[44,54,56] Risk assessment should be repeated every 4 to 6 years in persons who are found to be at low 10-year risk (<7.5%). Beginning at age 40 years, formal estimation of the absolute 10-year risk of ASCVD is recommended.[20,21] Long-term or lifetime risk estimation is recommended for all persons who are between 20 and 39 years of age and for those between 40 and 59 years of age who are determined to be at low 10-year risk (<7.5%). As shown in Figure 1, all patients should receive applicable risk information and appropriate lifestyle counseling. The 10-year risk estimates provided by the new Pooled Cohort Equations differ from those generated by the Adult Treatment Panel III algorithm in several respects,[24] as discussed in detail in the Full Work Group Report supplement. To summarize, on the basis of the risk estimation algorithm recommended by Adult Treatment Panel III, approximately 31.9% of the ASCVD-free, nonpregnant US population between 40 and 79 years of age have a 10-year risk of a first hard CHD event of at least 10% or have diabetes. On the basis of the new Pooled Cohort Equations described here, approximately 32.9% have a 10-year risk of a first hard ASCVD of at least 7.5%. The outcomes and thresholds of these 2 approaches are different, but the overlap of these 2 means of defining high-risk groups is substantial, at roughly 75%. Nonetheless, these important differences make simple linear conversions imprecise. We recommend that healthcare organizations convert to these new Pooled Cohort Equations as soon as practical (Appendix 7). A Web-based application enabling estimation of 10-year and lifetime risk of ASCVD is available at http://my.americanheart.org/cvriskcalculator and http://www.cardiosource.org/en/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/2013-Prevention-Guideline-Tools.aspx.

8. Evidence Gaps and Future Research Needs

The Work Group strongly recommends continued research to fill gaps in knowledge about short- and long-term ASCVD risk assessment and outcomes in all racial/ethnic groups, across the age spectrum, and in women and men. Future research should include analyses of short- and long-term risk in diverse groups, optimal communication of ASCVD risk information, utility of short-and long-term risk assessment for motivating behavioral change and adherence to therapy, utility of short- and long-term risk assessment for influencing risk factor levels and clinical outcomes, utility of differential information

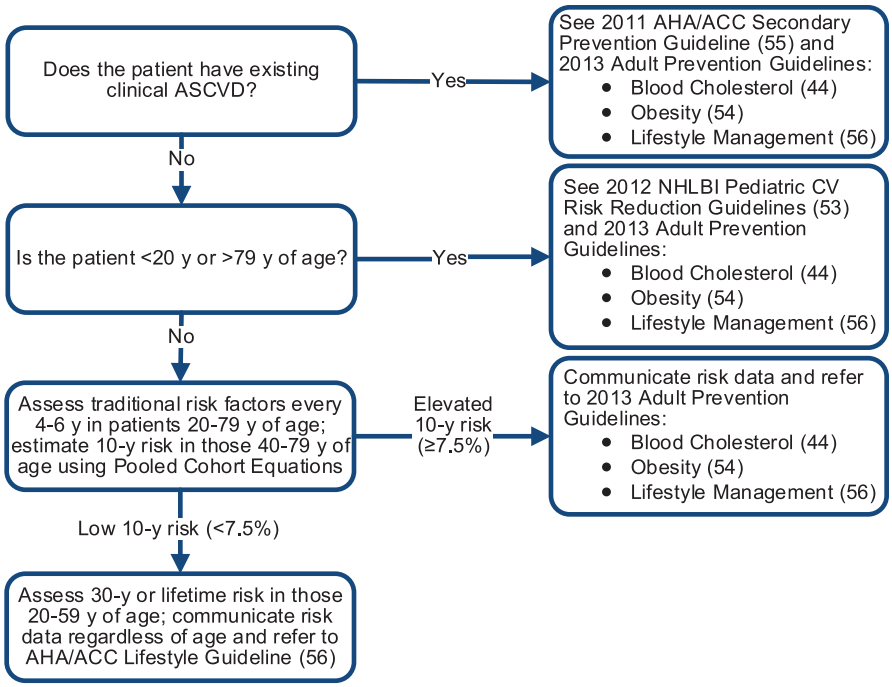

Figure 1. Implementation of Risk Assessment Work Group Recommendations. ACC indicates American College of Cardiology; AHA, American Heart Association; ASCVD, atherosclerotic cardiovascular disease; CV, cardiovascular; and NHLBI, National Heart, Lung, and Blood Institute.

conveyed by short- and long-term risk assessment, and utility of novel risk markers in short- and long-term risk assessment.

9. Conclusions

The Work Group's approach to risk assessment represents a step forward in ASCVD prevention that is large enough to justify the challenges inherent in implementing a new approach, rather than staying with the CHD risk assessment approach recommended previously. The final recommendations are summarized in Table 4 and Figure 1. Two major advantages of this approach are the ability to estimate risk for a broader-based ASCVD outcome that is more relevant to additional segments of the population, including women and African Americans, and the ability to provide risk estimates specific to African Americans. Promotion of lifetime risk estimation may represent an additional step forward in supporting lifestyle behavior change counseling efforts. Periodic updates of the guidelines should address numerous issues related to risk assessment.

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References

1. Institute of Medicine (US) Committee on Standards for Developing Trustworthy Clinical Practice Guidelines. Clinical Practice Guidelines We Can Trust. Washington, DC: National Academies Press; 2011.
2. Gibbons GH, Harold JG, Jessup M, et al. The next steps in developing clinical practice guidelines for prevention. J Am Coll Cardiol. 2013;62:1399–400.
3. Gibbons GH, Shurin SB, Mensah GA, et al. Refocusing the agenda on cardiovascular guidelines: an announcement from the National Heart, Lung, and Blood Institute. J Am Coll Cardiol. 2013;62:1396–8.
4. Dawber TR, Kannel WB, Lyell LP. An approach to longitudinal studies in a community: the Framingham Study. Ann N Y Acad Sci. 1963;107:539–56.
5. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol. 1991;1:263–76.
6. Kannel WB, Feinleib M, McNamara PM, et al. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol. 1979;110:281–90.
7. Friedman GD, Cutter GR, Donahue RP, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. J Clin Epidemiol. 1988;41:1105–16.

8. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989;129:687–702.
9. Buckley DI, Fu R, Freeman M, et al. C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the US Preventive Services Task Force. Ann Intern Med. 2009;151:483–95.
10. Empana JP, Tafflet M, Escolano S, et al. Predicting CHD risk in France: a pooled analysis of the D.E.S.I.R., Three City, PRIME, and SU.VI.MAX studies. Eur J Cardiovasc Prev Rehabil. 2011;18:175–85.
11. Ankle Brachial Index Collaboration; Fowkes FGR, Murray GD, et al. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. JAMA. 2008;300:197–208.
12. Helfand M, Buckley DI, Freeman M, et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the US Preventive Services Task Force. Ann Intern Med. 2009;151:496–507.
13. Emerging Risk Factors Collaboration; Kaptoge S, Di Angelantonio E, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 2010;375:132–40.
14. Kashani M, Eliasson A, Vernalis M, et al. Improving assessment of cardiovascular disease risk by using family history: an integrative literature review. J Cardiovasc Nurs. 2013;28:E18–27.
15. Moyer VA; US Preventive Services Task Force. Screening for peripheral artery disease and cardiovascular disease risk assessment with the ankle-brachial index in adults: US Preventive Services Task Force recommendation statement. Ann Intern Med. 2013;159:342–8.
16. Peters SAE, den Ruijter HM, Bots ML, et al. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart. 2012;98:177–84.
17. Schnell-Inderst P, Schwarzer R, Göhler A, et al. Prognostic value, clinical effectiveness, and cost-effectiveness of high-sensitivity C-reactive protein as a marker for major cardiac events in asymptomatic individuals: a health technology assessment report. Int J Technol Assess Health Care. 2010;26:30–9.
18. Den Ruijter HM, Peters SAE, Anderson TJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA. 2012;308:796–803.
19. Karp I, Abrahamowicz M, Bartlett G, et al. Updated risk factor values and the ability of the multivariable risk score to predict coronary heart disease. Am J Epidemiol. 2004;160:707–16.
20. Pencina MJ, D'Agostino RB Sr., Larson MG, et al. Predicting the 30-year risk of cardiovascular disease: the Framingham Heart Study. Circulation. 2009;119:3078–84.
21. Lloyd-Jones DM, Leip EP, Larson MG, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. Circulation. 2006;113:791–98.
22. Lloyd-Jones DM, Wilson PWF, Larson MG, et al. Framingham Risk Score and prediction of lifetime risk for coronary heart disease. Am J Cardiol. 2004;94:20–4.
23. 27th Bethesda Conference. Matching the Intensity of Risk Factor Management with the Hazard for Coronary Disease Events. September 14-15, 1995. J Am Coll Cardiol. 1996;27:957–1047.
24. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143–421.
25. Lackland DT, Elkind MSV, D'Agostino R Sr., et al. Inclusion of stroke in cardiovascular risk prediction instruments: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2012;43:1998–2027.
26. Shillinglaw B, Viera AJ, Edwards T, et al. Use of global coronary heart disease risk assessment in practice: a cross-sectional survey of a sample of US physicians. BMC Health Serv Res. 2012;12:20.
27. Sheridan SL, Crespo E. Does the routine use of global coronary heart disease risk scores translate into clinical benefits or harms? A systematic review of the literature. BMC Health Serv Res. 2008;8:60.
28. Sheridan SL, Viera AJ, Krantz MJ, et al. The effect of giving global coronary risk information to adults: a systematic review. Arch Intern Med. 2010;170:230–9.
29. Redberg RF, Benjamin EJ, Bittner V, et al. AHA/ACCF [corrected] 2009 performance measures for primary prevention of cardiovascular disease in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Performance Measures (Writing Committee to Develop Performance Measures for Primary Prevention of Cardiovascular Disease). Circulation. 2009;120:1296–336.
30. US Preventive Services Task Force. Aspirin for the prevention of cardiovascular disease: US Preventive Services Task Force recommendation statement. Ann Intern Med. 2009;150:396–404.
31. Perk J, De Backer G, Gohlke H, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. 2012;33:1635–701.
32. Anderson TJ, Grégoire J, Hegele RA, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. Can J Cardiol. 2013;29:151–67.
33. Genest J, McPherson R, Frohlich J, et al. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult–2009 recommendations. Can J Cardiol. 2009;25:567–79.
34. National Heart, Lung, and Blood Institute. Morbidity & Mortality: 2012 Chartbook on Cardiovascular, Lung, and Blood Diseases. Bethesda, MD: National Heart, Lung, and Blood Institute; 2012.
35. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. Circulation. 2013;127:e6–245.
36. Greenland P, Knoll MD, Stamler J, et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA. 2003;290:891–7.
37. Magnus P, Beaglehole R. The real contribution of the major risk factors to the coronary epidemics: time to end the "only-50%" myth. Arch Intern Med. 2001;161:2657–60.
38. Ioannidis JPA, Panagiotou OA. Comparison of effect sizes associated with biomarkers reported in highly cited individual articles and in subsequent meta-analyses. JAMA. 2011;305:2200–10.
39. Hlatky MA, Greenland P, Arnett DK, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation. 2009;119:2408–16.
40. Emerging Risk Factors Collaboration; Di Angelantonio E, Sarwar N, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009;302:1993–2000.
41. Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA. 2009;301:2024–35.
42. Sniderman AD, Williams K, Contois JH, et al. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. Circ Cardiovasc Qual Outcomes. 2011;4:337–45.
43. Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2010;122:e584–636.
44. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC Jr, Watson K, Wilson PWF. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(suppl 2):S1–S45.
45. Cavanaugh-Hussey MW, Berry JD, Lloyd-Jones DM. Who exceeds ATP-III risk thresholds? Systematic examination of the effect of varying age and risk factor levels in the ATP-III risk assessment tool. Prev Med. 2008;47:619–23.
46. Marma AK, Lloyd-Jones DM. Systematic examination of the updated Framingham Heart Study general cardiovascular risk profile. Circulation. 2009;120:384–90.
47. Berry JD, Dyer A, Carnethon M, et al. Association of traditional risk factors with cardiovascular death across 0 to 10, 10 to 20, and >20 years follow-up in men and women. Am J Cardiol. 2008;101:89–94.
48. Håheim LL, Tonstad S, Hjermann I, et al. Predictiveness of body mass index for fatal coronary heart disease in men according to length of follow-up: a 21-year prospective cohort study. Scand J Public Health. 2007;35:4–10.
49. Jousilahti P, Tuomilehto J, Rastenyte D, et al. Headache and the risk of stroke: a prospective observational cohort study among 35 056 Finnish men and women. Arch Intern Med. 2003;163:1058–62.
50. Liao Y, McGee DL, Cooper RS, et al. How generalizable are coronary risk prediction models? Comparison of Framingham and two national cohorts. Am Heart J. 1999;137:837–45.

51. Menotti A, Lanti M. Coronary risk factors predicting early and late coronary deaths. Heart. 2003;89:19–24.
52. Wannamethee SG, Shaper AG, Lennon L, et al. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. Arch Intern Med. 2005;165:2644–50.
53. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics. 2011;128(Suppl 5):S213–56.
54. Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. Circulation. 2014;129(suppl 2):S102–S138.
55. Smith SC Jr., Benjamin EJ, Bonow RO, et al. AHA/ACCF secondary prevention and risk reduction therapy for patients with coronary and other atherosclerotic vascular disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation. Circulation. 2011;124:2458–73.
56. Eckel RH, Jakicic JM, Ard JD, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(suppl 2):S76–S99.
57. Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837–47.
58. D'Agostino RB Sr, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117:743–53.
59. Assmann G, Cullen P, Schulte H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Münster (PROCAM) study. Circulation. 2002;105:310–15.
60. Hippisley-Cox J, Coupland C, Vinogradova Y, et al. Derivation and validation of QRISK, a new cardiovascular disease risk score for the United Kingdom: prospective open cohort study. BMJ. 2007;335:136.
61. Ridker PM, Paynter NP, Rifai N, et al. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. Circulation. 2008;118:2243–51; 4p following 2251.
62. Ridker PM, Buring JE, Rifai N, et al. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007;297:611–9.
63. Conroy RM, Pyörälä K, Fitzgerald AP, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003;24:987–1003.
64. Patel MR, Dehmer GJ, Hirshfeld JW, et al. ACCF/SCAI/STS/AATS/AHA/ASNC 2009 appropriateness criteria for coronary revascularization: a report by the American College of Cardiology Foundation Appropriateness Criteria Task Force, Society for Cardiovascular Angiography and Interventions, Society of Thoracic Surgeons, American Association for Thoracic Surgery, American Heart Association, and the American Society of Nuclear Cardiology. Circulation. 2009;119:1330–52.
65. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150:604–12.
66. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr., et al. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27:157–72; discussion 207–212.

KEY WORDS: AHA Scientific Statements ■ cardiovascular disease ■ cholesterol ■ primary prevention ■ biomarkers ■ risk assessment ■ risk reduction behavior

Appendix 1. Author Relationships With Industry and Other Entities (Relevant)—2013 ACC/AHA Guideline on Assessment of Cardiovascular Risk

Work Group Member	Employment	Consultant	Speaker's Bureau	Ownership/ Partnership/ Principal	Personal Research	Expert Witness
David C. Goff, Jr, Co-Chair	Colorado School of Public Health—Dean	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: • Merck 2013: None	2008–2012: None 2013: None
Donald M. Lloyd-Jones, Co-Chair	Northwestern University Feinberg School of Medicine—Senior Associate Dean; Chair and Professor of Preventive Medicine; Professor of Medicine (Cardiology)	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Glen Bennett, Ex-Officio	NHLBI—Coordinator	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Sean Coady, Ex-Officio	NHLBI—Statistician	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Ralph B. D'Agostino, Sr	Boston University—Professor of Mathematics and Statistics; Mathematics and Statistics Department—Chair	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None

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Appendix 1. Continued

Work Group Member	Employment	Consultant	Speaker's Bureau	Ownership/ Partnership/ Principal	Personal Research	Expert Witness
Raymond Gibbons	Nuclear Cardiology Laboratory Mayo Clinic—Professor of Medicine and Co-Director	2008–2012: None 2013: • AstraZeneca • Lantheus Medical Imaging	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Philip Greenland	Northwestern University Feinberg School of Medicine—Senior Associate Dean for Clinical and Translational Research; Harry W. Dingman Professor of Medicine	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Daniel T. Lackland	Medical University of South Carolina—Professor of Epidemiology and Medicine	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Daniel Levy, Ex-Officio	NHLBI—Framingham Heart Study, Director	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: • BG Medicine 2013: None	2008–2012: None 2013: None
Christopher J. O'Donnell, Ex-Officio	NHLBI—Associate Director and Senior Investigator	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Jennifer G. Robinson	University of Iowa—Professor of Epidemiology and Medicine; Director, Prevention Intervention Center	2008–2012: None	2008–2012: None	2008–2012: None	2008–2012: • Aegerion • Amarin* • Amgen* • AstraZeneca* • Daiichi-Sankyo* • Esperion • Genentech/Hoffman LaRoche* • GlaxoSmithKline* • Merck* • Sanofi-aventis/ Regeneron*	2008–2012: None
		2013: None	2013: None	2013: None	2013: • Amarin* • Amgen* • AstraZeneca* • Daiichi-Sankyo* • Genentech/ Hoffman LaRoche* • GlaxoSmithKline* • Merck* • Sanofi-aventis/ Regeneron*	2013: None

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Appendix 1. Continued

Work Group Member	Employment	Consultant	Speaker's Bureau	Ownership/ Partnership/ Principal	Personal Research	Expert Witness
J. Sanford Schwartz	University of Pennsylvania—Leon Hess Professor of Internal Medicine, Health Management and Economics	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Susan T. Shero, Ex-Officio	NHLBI—Public Health Advisor	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Sidney C. Smith, Jr	University of North Carolina—Professor of Medicine; Center for Cardiovascular Science and Medicine—Director	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Paul Sorlie, Ex-Officio	NHLBI—Chief of Division of Epidemiology and Clinical Applications	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Neil J. Stone	Northwestern Memorial Hospital—Bonow Professor of Medicine, Feinberg School of Medicine, Northwestern University	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None
Peter W. F. Wilson	Atlanta VA Medical Center; Emory Clinical Cardiovascular Research Institute—Professor of Medicine	2008–2012: • Merck • XZK 2013: None	2008–2012: None 2013: None	2008–2012: None 2013: None	2008–2012: • Merck • LipoScience 2013: None	2008–2012: None 2013: None

This table reflects the relevant healthcare-related relationships of authors with industry and other entities provided by the panels during the document development process (2008–2012). Both compensated and uncompensated relationships are reported. These relationships were reviewed and updated in conjunction with all meetings and conference calls of the Expert Work Group during the document development process. Authors with relevant relationships during the document development process recused themselves from voting on recommendations relevant to their relationships. In the spirit of full transparency, the ACC and AHA asked Expert Work Group members to provide updates and approve the final version of this table, which includes current relevant relationships (2013). To review the NHLBI and ACC/AHA's current comprehensive policies for managing RWI, please refer to http://www.nhlbi.nih.gov/guidelines/cvd_adult/coi-rwi_policy.htm and http://www.cardiosource.org/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/Relationships-With-Industry-Policy.aspx.

Per ACC/AHA policy: A person is deemed to have a significant interest in a business if the interest represents ownership of ≥5% of the voting stock or share of the business entity, or ownership of ≥$10 000 of the fair market value of the business entity; or if funds received by the person from the business entity exceed 5% of the person's gross income for the previous year. Relationships that exist with no financial benefit are also included for the purpose of transparency. Relationships in this table are modest unless otherwise noted.

*Significant relationship.

ACC indicates American College of Cardiology; AHA, American Heart Association; and NHLBI, National Heart, Lung, and Blood Institute.

Appendix 2. Expert Reviewer Relationships With Industry and Other Entities—2013 ACC/AHA Guideline on Assessment of Cardiovascular Risk

Reviewer	Employment	Representing	Consultant	Speaker's Bureau	Ownership/ Partnership/ Principal	Personal Research	Expert Witness
Ezra A. Amsterdam	University of California (Davis) Medical Center, Division of Cardiology—Professor	ACC/AHA	None	None	None	None	None
Ralph G. Brindis	University of California, San Francisco—Department of Medicine & the Phillip R. Lee Institute for Health Policy Studies—Clinical Professor of Medicine	ACC/AHA Task Force on Practice Guidelines	None	None	None	None	None
Frederick A. Masoudi	University of Colorado, Anschutz Medical Campus—Professor of Medicine (Cardiology)	ACC/AHA	None	None	None	None	None

This table represents the relationships of reviewers with industry and other entities that were self-disclosed at the time of peer review. It does not necessarily reflect relationships with industry at the time of publication. To review the NHLBI and ACC/AHA's current comprehensive policies for managing relationships with industry and other entities, please refer to http://www.nhlbi.nih.gov/guidelines/cvd_adult/coi-rwi_policy.htm and http://www.cardiosource.org/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/Relationships-With-Industry-Policy.aspx.

ACC indicates American College of Cardiology; and AHA, American Heart Association.

Appendix 3. Abbreviations

ABI = ankle-brachial index
ApoB = apolipoprotein B
ASCVD = atherosclerotic cardiovascular disease
BP = blood pressure
CAC = coronary artery calcium
CHD = coronary heart disease
CIMT = carotid intima-media thickness
COR = Class of Recommendation
CQ = critical question
CVD = cardiovascular disease
hs-CRP = high-sensitivity C-reactive protein
LOE = Level of Evidence
NHLBI = National Heart, Lung, and Blood Institute
RWI = relationships of authors with industry and other entities

Appendix 4. Evidence Statements for CQ1

Evidence Statement Number	Author/Group	Factor	Evidence Statement/Conclusion
1	USPSTF[9]	hs-CRP	"Strong evidence indicates that CRP is associated with CHD events. Moderate, consistent evidence suggests that adding CRP to risk prediction models among initially intermediate-risk persons improves risk stratification." "Few studies directly assessed the effect of CRP on risk reclassification in intermediate-risk persons." hs-CRP was associated with risk, and its use resulted in some reclassification in intermediate-risk persons, but it was not clear whether this reclassification led to a net improvement in prediction. Values of receiver operating curve *C*-statistics (measures of discrimination) are mentioned but not reported; hence, no evidence on discrimination, calibration, net reclassification index, or cost-effectiveness was provided. Reports some impact on reclassification, probably modest (pp. 488–491).
2	Helfand et al, 2009[12]	hs-CRP, CAC, CIMT, ABI	With regard to risk assessment for major CHD, the authors concluded that, "The current evidence does not support the routine use of any of the 9 risk factors for further risk stratification of intermediate-risk persons." The 9 risk factors examined were: hs-CRP, CAC score as measured by electron-beam computed tomography, lipoprotein (a) level, homocysteine level, leukocyte count, fasting blood glucose, periodontal disease, ABI, and CIMT. hs-CRP was associated with CHD and led to some reclassification. The authors cite the JUPITER results to support the conclusion that hs-CRP testing may be useful in intermediate-risk patients to drive statin therapy. The Work Group recognizes that more recent individual study results have been published. Updated systematic reviews addressing discrimination, calibration, reclassification, and cost issues in the context of the newer ASCVD risk assessment model proposed in the present document are needed. CAC was associated with CHD and with some reclassification, but the size and value of this reclassification are uncertain. The document provides little evidence with regard to discrimination, calibration, and cost-effectiveness. The Work Group also is concerned about radiation and incidental findings. The Work Group recognizes that more recent individual study results have been published. Updated systematic reviews addressing discrimination, calibration, reclassification, cost, and safety issues in the context of the newer ASCVD risk assessment model proposed in the present document are needed. CIMT was associated with CHD, but the document provides little evidence for reclassification, discrimination, calibration, and cost-effectiveness. The Work Group also has concerns about measurement issues. Standardization of CIMT measurement is a major challenge. The Work Group recognizes that more recent individual study results have been published. Updated systematic reviews addressing discrimination, calibration, reclassification, cost, and measurement (standardization) issues in the context of the newer ASCVD risk assessment model proposed in this document are needed. ABI was associated with CHD and some reclassification, but the size and value of this reclassification are uncertain. Evidence suggests some improvement in discrimination, but the document provides little evidence with regard to calibration and cost-effectiveness. The Work Group members are uncertain whether more recent individual study results have been published relevant to ABI. Updated systematic reviews addressing discrimination, calibration, reclassification, and cost issues in the context of the newer ASCVD risk assessment model proposed in this document are needed.
3	Emerging Risk Factors Collaboration[13]	hs-CRP	"CRP concentration has continuous associations with the risk for coronary heart disease, ischaemic stroke, vascular mortality, and death from several cancers and lung disease that are each of broadly similar size. The relevance of CRP to such a range of disorders is unclear. Associations with ischaemic vascular disease depend considerably on conventional risk factors and other markers of inflammation." hs-CRP is associated with risk of CVD. This analysis did not directly assess value in risk prediction. No additional evidence was provided for discrimination, calibration, reclassification, or cost-effectiveness.
4	Schnell-Inderst et al, 2010[17]	hs-CRP	For MI and cardiovascular mortality, "Adding hs-CRP to traditional risk factors improves risk prediction, but the clinical relevance and cost-effectiveness of this improvement remain unclear." Absolute differences in *C*-statistics between models including and not including hs-CRP ranged from 0.00 to 0.027. Some evidence was provided to support the cost-effectiveness of hs-CRP testing in some modeling scenarios, characterized by intermediate- and higher-risk populations and lower-cost (generic) statins of at least moderate efficacy.
5	Emerging Risk Factors Collaboration[40]	ApoB	This article provided evidence of rough equivalence of associations of CVD with non–HDL-C and ApoB after multivariable adjustment (including HDL-C). See Figure 1 for CHD and the text for stroke. By inference, this finding means there would be rough equivalence between ApoB and total cholesterol with similar adjustment.

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Appendix 4. Continued

Evidence Statement Number	Author/Group	Factor	Evidence Statement/Conclusion
6	Sniderman et al, 2011[42]	ApoB	ApoB was more strongly related to risk of ASCVD than either non–HDL-C or LDL-C in a substitution model that also included HDL-C. No evidence was presented pertinent to an addition model in which ApoB might be added to a model that included total cholesterol, LDL-C, or non–HDL-C. Additional models are the type of model of interest to this question. By inference, these results maymean that ApoB is more strongly related to risk than is total cholesterol. This article did not address directly the value of adding ApoB to a model with traditional risk factors. No information was presented for discrimination, calibration, reclassification, or cost. The relative risks evaluated in the meta-analysis were adjusted for various sets of covariates in the various primary reports, and the adjustments were judged to be incomplete. Furthermore, studies of varying designs and quality were included, leaving the Work Group members concerned about the validity of the evidence.
7	Kodama et al, 2009[41]	Cardiorespiratory fitness	Better cardiorespiratory fitness was associated with lower risk of all-cause mortality and CHD/CVD. According to the sensitivity analyses in Table 2, evidence of association was weaker for CHD/CVD, but still significant, when based on studies with more complete adjustment for other risk factors. The utility of assessing cardiorespiratory fitness in risk prediction was not assessed (discrimination, calibration, reclassification, and cost).
8	Ankle Brachial Index Collaboration[11]	ABI	ABI is associated with total CHD risk and leads to significant reclassification, and the pattern of reclassification is different by sex. Among men, the effect is to down-classify high-risk men. Among women, the effect is to up-classify low-risk women. Overall, the FRS, as applied by the investigators, showed relatively poor discrimination in this meta-analysis, with *C*-statistics of 0.646 (95% CI: 0.643–0.657) in men and 0.605 (0.590–0.619) in women. There was an improvement in *C*-statistic in both men (0.655 [0.643–0.666]) and women (0.658 [0.644–0.672]) when ABI was added to a model with FRS. The improvement in the *C*-statistic was greater and significant in women but was not significant in men. No evidence on calibration, net reclassification index, or cost-effectiveness was provided.
9	Empana et al, 2011[10]	Family history of CHD	"In separate models adjusted for age, gender, and study cohort, a family history of CHD, BMI, and waist circumference were all predictors of CHD. When traditional risk factors were controlled for, family history of CHD ($P<0.001$) and BMI ($P=0.03$) but not waist circumference ($P=0.42$) remained associated with CHD. However, the addition of family history of CHD or BMI to the traditional risk factors model did not improve the discrimination of the model (not shown)." This article developed a CHD risk prediction algorithm based on 4 French population studies and evaluated, among other factors, the contribution of family history to traditional risk factors. Family history of CHD was defined as the self-report of a MI in first-degree relatives (parents and siblings) in the D.E.S.I.R. and SU.VI.MAX studies, as a history of MI before age 55 years in men and before age 65 years in women in parents, siblings, and grandparents in the PRIME study, and as a death due to MI in first-degree relatives in the Three City study. No evidence on calibration, net reclassification index, or cost-effectiveness was provided.
10	Moyer et al, 2013[15]	ABI	This article is an updated review of the utility of assessing ABI for the USPSTF. "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for PAD and CVD risk assessment with the ABI in adults. (I statement)" "The USPSTF found no evidence that screening for and treatment of PAD in asymptomatic patients leads to clinically important benefits. It also reviewed the potential benefits of adding the ABI to the FRS and found evidence that this results in some patient risk reclassification; however, how often the reclassification is appropriate or whether it results in improved clinical outcomes is not known." The Work Group notes that this review provides some evidence that assessing ABI may improve risk assessment; however, no evidence was found by the USPSTF reviewers pertinent to the question of whether measuring ABI leads to better patient outcomes.

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Appendix 4. Continued

Evidence Statement Number	Author/Group	Factor	Evidence Statement/Conclusion
11.	Peters et al, 2012[16]	CIMT, CAC	This article is a systematic review of the literature on the contribution to risk assessment of imaging for subclinical atherosclerosis. "Published evidence on the added value of atherosclerosis imaging varies across the different markers, with limited evidence for FMD and considerable evidence for CIMT, carotid plaque and CAC. The added predictive value of additional screening may be primarily found in asymptomatic individuals at intermediate cardiovascular risk. Additional research in asymptomatic individuals is needed to quantify the cost-effectiveness and impact of imaging for subclinical atherosclerosis on cardiovascular risk factor management and patient outcomes." With regard to CIMT: "The c-statistic of the prediction models without CIMT increased from 0.00 to 0.03 when CIMT was added. In the Atherosclerosis Risk In Communities (ARIC) study, addition of CIMT to the prediction model resulted in an NRI overall of 7.1% (95% CI 2.2% to 10.6%) and an IDI of 0.007 (95% CI 0.004 to 0.010). The NRI intermediate was 16.7% (95% CI 9.3% to 22.4%). In contrast, 10 year results from the Carotid Atherosclerosis Progression Study showed that addition of CIMT to the prediction model resulted in an IDI of 0.04% and NRI overall of –1.41%. Analysis of 1,574 participants from the Firefighters and Their Endothelium study showed an NRI overall of 11.6% (P=0.044) and an NRI intermediate of 18.0% (P=0.034)." The Work Group notes that this article provides some evidence to consider assessing CIMT; however, this conclusion was not supported by the article by Den Ruijter et al described below.[18] With regard to CAC: "The c-statistic increased from 0.04 to 0.13 when CAC was added to the model. Four recently published studies also reported results on the NRI and/or the IDI. One of these studies comprised a subgroup analysis of an earlier publication in the total population in individuals without indications for statin therapy. Analyses of the MESA study showed that addition of CAC to the conventional prediction model resulted in an NRI overall of 25% (95% CI 16% to 34%) and an NRI intermediate of 55% (95% CI 41% to 69%). The IDI in the MESA study was 0.026. Results were similar in the Rotterdam study. Addition of CAC to the prediction model led to an NRI overall of 14% (P<0.01) which was mainly driven by correctly reclassifying those at intermediate risk according to the traditional prediction model. Results from the Heinz Nixdorf Recall study also showed large NRIs when CAC was added to the Framingham Risk Score. Using different thresholds to define the intermediate risk category (10%–20% or 6%–20%), the NRI overall was 22% and 20%, respectively. The NRI intermediate was 22% for intermediate risk thresholds of 10%–20% and 31% for intermediate risk thresholds of 6%–20%. In addition, the IDI was 0.0152 when the prediction models with and without CAC were compared. The NRI overall was 25.1% and the IDI was 0.0167 in individuals from the Heinz Nixdorf Recall study without indications for statin therapy." The Work Group notes that this article provides evidence to support the conclusion that assessing CAC is likely to be the most useful approach to improving risk assessment among individuals found to be at intermediate risk after formal risk assessment. Furthermore, we note that the outcomes in the studies reviewed above were CHD, not ASCVD. The Work Group discussed concerns about cost, radiation exposure, and the uncertainty of the contribution of assessing CAC to estimation of 10-year risk of hard ASCVD after formal risk assessment.
12.	Kashani et al, 2013[14]	Family history	This article is an integrative literature review on the contribution of assessing family history to risk appraisal. "The evidence demonstrates that family history is an independent contributor to risk appraisal and unequivocally supports its incorporation to improve accuracy in global CVD risk estimation." The Work Group notes that a variety of endpoints, clinical and subclinical, were included in the reviewed articles. No evidence on discrimination, calibration, net reclassification index, or cost-effectiveness was provided.
13.	Den Ruijter et al, 2012[18]	CIMT	This article is an individual-level meta-analysis of "14 population-based cohorts contributing data for 45 828 individuals. During a median follow-up of 11 years, 4007 first-time MIs or strokes occurred." "We first refitted the risk factors of the FRS and then extended the model with common CIMT measurements to estimate the absolute 10-year risks to develop a first-time MI or stroke in both models. The *C*-statistic of both models was similar (0.757; 95% CI, 0.749–0.764; and 0.759; 95% CI, 0.752–0.766). The net reclassification improvement with the addition of common CIMT was small (0.8%; 95% CI, 0.1%–1.6%). In those at intermediate risk, the net reclassification improvement was 3.6% in all individuals (95% CI, 2.7%–4.6%) and no differences between men and women." "The addition of common CIMT measurements to the FRS was associated with small improvement in 10-year risk prediction of first-time MI or stroke, but this improvement is unlikely to be of clinical importance." The Work Group judged this article to provide the strongest evidence available on the potential value of CIMT to risk assessment. The Work Group also has concerns about measurement issues. Standardization of CIMT measurement is a major challenge.

ABI indicates ankle-brachial index; ApoB, apolipoprotein B; BMI, body mass index; ASCVD, atherosclerotic cardiovascular disease; CAC, coronary artery calcium; CHD, coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CRP, C-reactive protein; CVD, cardiovascular disease; FMD, flow-mediated dilation; FRS, Framingham Risk Score; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IDI, integrative discrimination index; JUPITER, Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin; LDL-C, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; NRI, net reclassification index; PAD, peripheral artery disease; MI, myocardial infarction; and USPSTF, US Preventive Services Task Force.

Appendix 5. Evidence Statements for CQ2

Evidence Statement Number	Evidence Statement	References
1.	We found no evidence assessing variations in long-term or lifetime risk of CVD outcomes among persons at low or intermediate short-term risk in racial/ethnic groups other than non-Hispanic whites in the United States and Europe. *Strength of Evidence: None*	—
2.	ASCVD risk factors measured in young and middle-aged adults, considered singly or jointly, generally are associated with short-term (≤10 years), long-term (≥15 years), and lifetime risk of ASCVD. *Strength of Evidence:* Low (for diabetes and metabolic syndrome) to Moderate (for BMI, cholesterol, systolic BP, and smoking).	20,21,47,48,51,52
3.	Multivariable short-term (10-year) CHD risk prediction models underestimate absolute lifetime risk of CHD but maystratify relative lifetime risk of CHD in women and older men.* *Strength of Evidence: Low* *CHD is defined as all manifestations of CHD, or as CHD death/nonfatal MI.	22
4.	Long-term (30-year) risk equations based on traditional ASCVD risk factors* provide more accurate prediction of long-term ASCVD† risk than do extrapolations of short-term (10-year) risk equations among individuals 20–59 years of age who are free from ASCVD. *Strength of Evidence: Low* *Age, sex, total cholesterol, HDL-C, systolic BP, use of antihypertensive therapy, diabetes, and current smoking. †CHD death, nonfatal MI, or fatal/nonfatal stroke; or all ASCVD.	20
5.	The presence and severity of selected traditional ASCVD risk factors* stratify absolute levels of lifetime risk of ASCVD† among non-Hispanic white adults 45–50 years of age who are free of ASCVD and not at high short-term risk. *Strength of Evidence: Low* *Risk factors were considered in 5 mutually exclusive strata encompassing the full spectrum of risk levels, as follows: 1) ≥2 major risk factors (defined as total cholesterol ≥240 mg/dL or treated, systolic BP ≥160 or diastolic BP ≥100 mm Hg or treated, or diabetes, or current smoking) and lifetime risk of ASCVD >50%; 2) only 1 major risk factor and lifetime risk of ASCVD 39%–50%; 3) ≥1 elevated risk factor (defined as untreated total cholesterol 200 to 239 mg/dL, or untreated systolic BP 140–159 mm Hg or diastolic BP 90–99 mm Hg, and no diabetes and no current smoking) and lifetime risk of ASCVD 39%–46%; 4) ≥1 risk factor at nonoptimal levels (untreated total cholesterol 180–199 mg/dL, or untreated systolic BP 120–139 mm Hg or diastolic BP 80–89 mm Hg, and no diabetes and no current smoking) and lifetime risk of ASCVD 27%–36%; and 5) all optimal levels of risk factors (defined as untreated total cholesterol <180 mg/dL, and untreated BP <120/<80 mm Hg, and no diabetes, and no current smoking) and lifetime risk of ASCVD <10%. †CHD death, MI, coronary insufficiency, angina, fatal/nonfatal atherothrombotic stroke, claudication, other CVD death.	21
6.	Long-term (≥15 years) risk prediction models based on selected traditional ASCVD risk factors* predict CHD death with good discrimination and calibration, and better in women than men, in US non-Hispanic white populations. *Strength of Evidence: Low* *Age, sex, total cholesterol, systolic BP, diabetes, and smoking.	50
7.	Measuring and updating ASCVD risk factors every 4–6 years improves short- and long-term risk prediction. *Strength of Evidence: Moderate*	19,20

ASCVD indicates atherosclerotic cardiovascular disease; BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CQ, critical question; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; MI, myocardial infarction; and —, none.

Appendix 6. Characteristics of Previously Published Risk Scores and Current Pooled Cohort Equations (Including Data Sources, Covariates, and Outcomes)

Risk Score				Risk Factors/Covariates Included																CVD Events								
																				Hard CVD, Including Cardiac Failure								
																				Hard ASCVD								
																				Hard CHD								
																				Total CHD								
																				Total CHD, Including Revascularization								
Study Group	Study and Region	Data Source	Publication Year	Age	Sex	Total Chol	LDL Chol	HDL Chol	CRP	Systolic BP	BP Rx	Diabetes	HbA1c*	Smoking	Family Hx CVD†	Body Mass Index	Social	Region	Coronary Revasc	Angina Pectoris	Unstable Angina	Myocardial Infarct	CHD Death	Stroke	Stroke Death	Cardiac Failure	TIA
Framingham CHD[57]	Framingham MA, USA	EAF, EAM	1998	x	x	x	x	x		x		x		x						x	x	x	x				
ATP III[24]	Framingham MA, USA	EAF, EAM	2001	x	x	x		x		x	x			x									x	x				
Framingham Global[58]	Framingham MA, USA	EAF, EAM	2008	x	x	x		x		x	x	x		x									x	x	x	x	x	
PRO-CAM[59]	Muenster, Germany	EM	2002	x			x	x		x		x		x	x								x	x				
QRISK[60]	QRESEARCH, United Kingdom	EF, EM	2007	x	x	x		x		x	x			x	x	x	x‡	x	x	x	x	x	x	x	x	x		x
Reynolds Men[61]	Phys Health Study USA	EAF	2008	x		x		x	x	x				x	x				x			x	x	x	x			
Reynolds Women[62]	Women's Health Study USA	EAM	2007	x		x		x	x	x			x	x	x				x			x	x	x	x			
EURO-SCORE[63]	12 cohorts Europe	EF, EM	2003	x	x	x		x		x				x				x					x		x			
Pooled Cohort (current)	CARDIA, Framingham, ARIC, CHS,USA	EAF, EAM AAF, AAM		x	x	x		x		x	x	x		x								x	x	x	x			

*Only among those with diabetes.

†Definitions of a positive family history vary.

‡Measure of social deprivation.

AAF indicates African-American females; AAM, African-American males; ARIC, Atherosclerosis Risk in Communities study; ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; CARDIA, Coronary Artery Risk Development in Young Adults study; CHD, coronary heart disease; Chol, cholesterol; CHS, Cardiovascular Health Study; CRP, C-reactive protein; CVD, cardiovascular disease; EAF, European-American females; EAM, European-American males; EF, European females; EM, European males; EURO-SCORE, European System for Cardiac Operative Risk Evaluation; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; Hx, history; LDL, low-density lipoprotein; Revasc, revascularization; Rx, medication; and TIA, transient ischemic attack.

Appendix 7. Development and Steps for Implementation of the ASCVD Pooled Cohort Risk Equations

Prior experience with the development of the Framingham Heart Study 10-year *CHD* risk prediction equations[24,57] and the more recent Framingham 10-year *general CVD* risk prediction equations,[58] was used as a basis for developing the new Pooled Cohort Risk Equations. To expand the utility and generalizability of the new equations, extensive data were used from several large, racially and geographically diverse, modern NHLBI-sponsored cohort studies, including the ARIC (Atherosclerosis Risk in Communities) study,[8] Cardiovascular Health Study,[5] and the CARDIA (Coronary Artery Risk Development in Young Adults) study,[7] combined with applicable data from the Framingham Original and Offspring Study cohorts.[4,6]

A total of 11 240 white women (who experienced 902 hard ASCVD events), 9098 white men (1259 hard ASCVD events), 2641 African-American women (290 hard ASCVD events), and 1647 African-American men (238 hard ASCVD events) who met the following criteria were included: 40 to 79 years of age, apparently healthy, and free of a previous history of nonfatal myocardial infarction (recognized or unrecognized), stroke, heart failure, percutaneous coronary intervention, coronary artery bypass surgery, or atrial fibrillation. Data from the included participants were used to develop sex- and race-specific equations to predict 10-year risk of a first hard ASCVD event. Because of the growing health burden of heart failure, the Work Group examined the possibility of including heart failure as an outcome. However, study-by-study ascertainment and adjudication of heart failure varied considerably, and therefore heart failure could not be included as an outcome. Because of known substantial geographic variation in use (Dartmouth Atlas of Healthcare, http://www.dartmouthatlas.org/), self-selection, and physician recommendation biases,[64] coronary revascularization was also not included as an endpoint.

The Pooled Cohort Equations for estimating ASCVD were developed from sex- and race-specific proportional-hazards models that included the covariates of age, treated or untreated systolic BP level, total cholesterol and high-density lipoprotein cholesterol levels, current smoking status (yes/no), and history of diabetes (yes/no). A variable representing lipid treatment was considered but not retained in the final model because lipid therapy was relatively uncommon in the cohorts and statistical significance was lacking. Baseline characteristics of the participants included in the equation derivation model are shown in the Full Work Group Report Data supplement, as are details of the methods used to derive, evaluate, and validate (internally and externally) the resulting risk equations and their potential limitations. In summary, discrimination and calibration of the models were very good. *C*-statistics ranged from a low of 0.713 (African-American men) to a high of 0.818 (African-American women). Calibration chi-square statistics ranged from a low of 4.86 (non-Hispanic white men) to a high of 7.25 (African-American women). The coefficients for the equations for calculating an estimate of an individual's 10-year risk of a first hard ASCVD event are provided in Table A, along with examples based on a specific risk profile for each race–sex group. The step-by-step process for estimating the risk in the specific examples of Table A is provided in Table B. These 2 tables are intended to enable programmers to integrate these equations into electronic health records.

The Work Group also considered the inclusion of additional and novel risk markers in the risk equations. On the basis of the availability of data across cohorts at applicable examination cycles, additional risk markers were evaluated for potential inclusion if they improved model performance within the framework of Hlatky et al.[39] The additional risk markers that were evaluated included diastolic BP, family history of ASCVD, moderate or severe chronic kidney disease (defined as an estimated glomerular filtration rate of <60 mL/min per 1.73 m^2),[65] and body mass index (continuous or categorical). None of these variables significantly improved discrimination for 10-year hard ASCVD risk prediction when added to the final base models. Other risk markers (hs-CRP, ApoB, microalbuminuria, cardiorespiratory fitness, CAC score, CIMT, and ABI) could not be evaluated in creating this new model because of absence of data or lack of inclusion in the appropriate examination cycle of 1 or more of the studies. Therefore, these and the other risk markers were addressed in CQ1 as potential adjuncts to quantitative risk estimation.

Further research using state-of-the art statistical techniques (including net reclassification improvement and integrative discrimination index[66]) is needed to examine the utility of novel biomarkers when added to these new Pooled Cohort Equations in different populations and patient subgroups. Randomized clinical trials demonstrating the utility of screening with novel risk markers would represent the best evidence for their inclusion in future risk assessment algorithms. In the absence of evidence from trials, methodologically rigorous observational studies should be conducted to evaluate utility.

Table A. Equation Parameters of the Pooled Cohort Equations for Estimation of 10-Year Risk of Hard ASCVD* and Specific Examples for Each Race and Sex Group

	White			African American		
	Coefficient	Individual Example Value	Coefficient × Value†	Coefficient	Individual Example Value	Coefficient × Value†
Women (Example: 55 years of age with total cholesterol 213 mg/dL, HDL-C 50 mg/dL, untreated systolic BP 120 mm Hg, nonsmoker, and without diabetes)						
Ln Age (y)	–29.799	4.01	–119.41	17.114	4.01	68.58
Ln Age, Squared	4.884	16.06	78.44	N/A	N/A	N/A
Ln Total Cholesterol (mg/dL)	13.540	5.36	72.59	0.940	5.36	5.04
Ln Age × Ln Total Cholesterol	–3.114	21.48	–66.91	N/A	N/A	N/A
Ln HDL-C (mg/dL)	–13.578	3.91	–53.12	–18.920	3.91	–74.01
Ln Age × Ln HDL-C	3.149	15.68	49.37	4.475	15.68	70.15
Ln Treated Systolic BP (mm Hg)	2.019	—	—	29.291	—	—
Ln Age × Ln Treated Systolic BP	N/A	N/A	N/A	–6.432	—	—
Ln Untreated Systolic BP (mm Hg)	1.957	4.79	9.37	27.820	4.79	133.19
Ln Age × Ln Untreated Systolic BP	N/A	N/A	N/A	–6.087	19.19	–116.79
Current Smoker (1=Yes, 0=No)	7.574	0	0	0.691	0	0
Ln Age × Current Smoker	–1.665	0	0	N/A	N/A	N/A
Diabetes (1=Yes, 0=No)	0.661	0	0	0.874	0	0
Individual Sum			–29.67			86.16
Mean (Coefficient × Value)	N/A	N/A	–29.18	N/A	N/A	86.61
Baseline Survival	N/A	N/A	0.9665	N/A	N/A	0.9533
Estimated 10-y Risk of Hard ASCVD	N/A	N/A	2.1%	N/A	N/A	3.0%
Men (Example: 55 years of age with total cholesterol 213 mg/dL, HDL-C 50 mg/dL, untreated systolic BP 120 mm Hg, nonsmoker, and without diabetes)						
Ln Age (y)	12.344	4.01	49.47	2.469	4.01	9.89
Ln Total Cholesterol (mg/dL)	11.853	5.36	63.55	0.302	5.36	1.62
Ln Age × Ln Total Cholesterol	–2.664	21.48	–57.24	N/A	N/A	N/A
Ln HDL-C (mg/dL)	–7.990	3.91	–31.26	–0.307	3.91	–1.20
Ln Age × Ln HDL-C	1.769	15.68	27.73	N/A	N/A	N/A
Ln Treated Systolic BP (mm Hg)	1.797	—	—	1.916	—	—
Ln Untreated Systolic BP (mm Hg)	1.764	4.79	8.45	1.809	4.79	8.66
Current Smoker (1=Yes, 0=No)	7.837	0	0	0.549	0	0
Ln Age × Current Smoker	–1.795	0	0	N/A	N/A	N/A
Diabetes (1=Yes, 0=No)	0.658	0	0	0.645	0	0
Individual Sum			60.69			18.97
Mean (Coefficient × Value)	N/A	N/A	61.18	N/A	N/A	19.54
Baseline Survival	N/A	N/A	0.9144	N/A	N/A	0.8954
Estimated 10-y Risk of Hard ASCVD	N/A	N/A	5.3%	N/A	N/A	6.1%

*Defined as first occurrence of nonfatal myocardial infarction or CHD death, or fatal or nonfatal stroke.

†Coefficient × Value: For age, lipids, and BP, defined as the natural log of the value multiplied by the parameter estimate. When an age interaction is present with lipids or BP, the natural log of age is multiplied by the natural log of the lipid or BP, and the result is multiplied by the parameter estimate. N/A indicates that that specific covariate was not included in the model for that sex–race group; — indicates that this value was not included in the example (eg, this example used untreated systolic BP, not treated systolic BP).

ASCVD indicates atherosclerotic cardiovascular disease; BP indicates blood pressure; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; Ln, natural logarithm; and N/A, not included.

Table B. Estimating an Individual's 10-Year Risk of Incident Hard ASCVD

The hypothetical profile provided in Table 5 (the "Individual Example Value" column) is identical for each race and sex group and is based on the overall sample mean. The profile assumes an individual 55 years of age (for which the Ln[Age]=4.01), with a total cholesterol of 213 mg/dL, HDL-C of 50 mg/dL, and an untreated systolic BP of 120 mm Hg. This individual is not a current smoker and does not have diabetes. For the equations, the values for age, lipids, and systolic BP are Ln transformed. Interactions between age and lipids or age and systolic BP use the natural log of each variable (eg, Ln[Age]×Ln[Total Cholesterol]).

Calculation of the 10-year risk estimate for hard ASCVD can best be described as a series of steps. The natural log of age, total cholesterol, HDL-C, and systolic BP are first calculated with systolic BP being either a treated or untreated value. Any appropriate interaction terms are then calculated. These values are then multiplied by the coefficients from the equation ("Coefficient" column of Table A) for the specific race-sex group of the individual. The "Coefficient × Value" column in the table provides the results of the multiplication for the risk profile described above.

The sum of the "Coefficient × Value" column is then calculated for the individual. For the profile shown in Table A, this value is shown as "Individual Sum" for each race and sex group.

The estimated 10-year risk of a first hard ASCVD event is formally calculated as 1 minus the survival rate at 10 years ("Baseline Survival" in Table A), raised to the power of the exponent of the "Coefficient × Value" sum minus the race- and sex-specific overall mean "Coefficient × Value" sum; or, in equation form:

$1 - S_{10}^{(\text{IndX'B}-\textit{Mean}X'\text{B})}$

Using white men as an example:

$1 - 0.9144e^{(60.69-61.18)}$

equates to a 5.3% probability of a first hard ASCVD event within 10 years.

ASCVD indicates atherosclerotic cardiovascular disease; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; and Ln, natural logarithm.

Negative Pressure Wound Therapy

Question: where should negative pressure wound therapy be located on the Prioritized List?

Issue: Negative pressure wound therapy was an excluded service for many years. It was reviewed in 2005 and 2009 and kept on the Excluded List. The Health Resources Review Commission reviewed this technology in 2010 and recommended coverage. During the discussion in 2010, the deep open wound line was identified as possibly being the only appropriate code for this type of treatment. The final decision was to place the codes for this service on the Ancillary List with a guideline.

New codes have been released for 2015 regarding negative pressure wound therapy. HERC staff review of claims data for this service found many inappropriate diagnoses on claims, including conditions for which NPWT is contraindicated (osteomyelitis, cancers, etc.). HERC staff identified a limited number of lines which would be appropriate for these codes.

CPT/HCPCS codes for negative pressure wound therapy

Code	Code Description	Current Placement
97605	Negative pressure wound therapy (eg, vacuum assisted drainage collection), utilizing durable medical equipment (DME), including topical application(s), wound assessment, and instruction(s) for ongoing care, per session; total wound(s) surface area less than or equal to 50 square centimeters	Ancillary
97606	total wound(s) surface area greater than 50 square centimeters	Ancillary
97607	Negative pressure wound therapy, (eg, vacuum assisted drainage collection), utilizing disposable, non-durable medical equipment including provision of exudate management collection system, topical application(s), wound assessment, and instructions for ongoing care, per session; total wound(s) surface area less than or equal to 50 square centimeters	New code
97608	total wound(s) surface area greater than 50 square centimeters	New code
A6550	Wound care set, for negative pressure wound therapy electrical pump, includes all supplies and accessories	Ancillary
E2402	Negative pressure wound therapy electrical pump, stationary or portable	Ancillary
G0456	Negative pressure wound therapy, (e.g. vacuum assisted drainage collection) using a mechanically-powered device, not durable medical equipment, including provision of cartridge and dressing(s), topical application(s), wound assessment, and instructions for ongoing care, per session; total wounds(s) surface area less than or equal to 50 square centimeters	Ancillary
G0457	total wounds(s) surface area greater than 50 square centimeters	Ancillary

ANCILLARY GUIDELINE A1, NEGATIVE PRESSURE WOUND THERAPY

Negative pressure wound therapy (97605, 97606) is a covered benefit only for patients who:

- Have wounds that are refractory to or have failed standard therapies;
- Are not suitable candidates for surgical wound closure; or,
- Are at high risk for delayed or non-healing wounds due to factors such as compromised blood flow, diabetic complications, wounds with high risk of fecal contamination, extremely exudative wounds, and similar situations.

Appropriate Lines for Negative Pressure Wound Therapy

Line	**Examples of appropriate diagnoses on line**
8 TYPE 1 DIABETES MELLITUS	250.61 Diabetes with neurological manifestations, type I
30 TYPE 2 DIABETES MELLITUS	250.60 Diabetes with neurological manifestations, type II (includes diabetic ulcers)
51 DEEP ABSCESSES, INCLUDING APPENDICITIS AND PERIORBITAL ABSCESS	675.13 Abscess of breast
84 INJURY TO INTERNAL ORGANS	868.10 Injury to other intra-abdominal organs with open wound into cavity, unspecified intra-abdominal organ
209 SUPERFICIAL ABSCESSES AND CELLULITIS	682.5 Cellulitis and abscess of buttock
211 DEEP OPEN WOUND, WITH OR WITHOUT TENDON OR NERVE INVOLVEMENT	879.3 Open wound of abdominal wall, anterior, complicated
239 LIMB THREATENING VASCULAR DISEASE, INFECTIONS, AND VASCULAR COMPLICATIONS	728.86 Necrotizing fasciitis
290 COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT	998.32 Disruption of external operation (surgical) wound
383 CHRONIC ULCER OF SKIN	707.05 Pressure ulcer, buttock
427 COMPLICATIONS OF A PROCEDURE USUALLY REQUIRING TREATMENT	998.83 Non-healing surgical wound

HERC staff recommendations:

1) Place all negative pressure wound therapy CPT codes (CPT 97605-97608) and appropriate HCPCS codes (HCPCS G0456 and G0457) on the limited number of lines from the table above
    a. Lines 8,30,51,84,209,211,239, 290, 383, 427
    b. Advise DMAP to remove CPT 97605 and 97606 and HCPCS G0456 and G0457 from the Ancillary List
2) Change the current Ancillary Guideline into a guideline note as shown below

~~**ANCILLARY GUIDELINE A1**~~ **GUIDELINE NOTE XXX, NEGATIVE PRESSURE WOUND THERAPY**

Lines 8,30,51,84,209,211,239,290,383,427

Negative pressure wound therapy (CPT 97605-97608, HCPCS G0456, G0457 ~~97605, 97606~~) is a covered benefit only for patients who:

- Have wounds that are refractory to or have failed standard therapies;
- Are not suitable candidates for surgical wound closure; or,
- Are at high risk for delayed or non-healing wounds due to factors such as compromised blood flow, diabetic complications, wounds with high risk of fecal contamination, extremely exudative wounds, and similar situations.

Arthrocentesis

Issue: the current arthrocentesis codes are one some inappropriate lines. There are new arthrocentesis codes in the 2015 CPT code set. These codes should be on consistent lines with the current codes. The current codes are on some lines with no joint diagnoses or on lines with inappropriate diagnoses (i.e. shoulder diagnosis for a small joint procedure). Some of the existing codes are missing from one or more lines with joint diagnoses.

HERC staff recommendation:

1) Placement of codes as shown in the table below (red=suggested for deletion, blue=suggested for addition)

CPT code	Code description	Current Placement	Recommended Placement
20600	Arthrocentesis, aspiration and/or injection, small joint or bursa (eg, fingers, toes); without ultrasound guidance	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **51** DEEP ABSCESSES, INCLUDING APPENDICITIS AND PERIORBITAL ABSCESS **157** PYOGENIC ARTHRITIS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **422** DISORDERS OF SHOULDER, INCLUDING SPRAINS/STRAINS GRADE 3 THROUGH 6 **430** ACUTE PERIPHERAL MOTOR AND DIGITAL NERVE INJURY **468** OSTEOARTHRITIS AND ALLIED DISORDERS **509** OTHER DISORDERS OF SYNOVIUM, TENDON AND BURSA, COSTOCHONDRITIS, AND CHONDRODYSTROPHY **511** PERIPHERAL ENTHESOPATHIES **597** SYNOVITIS AND TENOSYNOVITIS **601** GANGLION **612** DISORDERS OF SOFT TISSUE	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **157** PYOGENIC ARTHRITIS 290 COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **468** OSTEOARTHRITIS AND ALLIED DISORDERS **511** PERIPHERAL ENTHESOPATHIES **533** BENIGN NEOPLASM OF BONE AND ARTICULAR CARTILAGE INCLUDING OSTEOID OSTEOMAS; BENIGN NEOPLASM OF CONNECTIVE AND OTHER SOFT TISSUE **597** SYNOVITIS AND TENOSYNOVITIS

CPT code	Code description	Current Placement	Recommended Placement
20604	with ultrasound guidance	New code	**50, 157, 290, 306, 468, 511, 533, 597**
20605	Arthrocentesis, aspiration and/or injection, intermediate joint or bursa (eg, temporomandibular, acromioclavicular, wrist, elbow or ankle, olecranon bursa); without ultrasound guidance	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **51** DEEP ABSCESSES, INCLUDING APPENDICITIS AND PERIORBITAL ABSCESS **157** PYOGENIC ARTHRITIS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **422** DISORDERS OF SHOULDER, INCLUDING SPRAINS/STRAINS GRADE 3 THROUGH 6 **430** ACUTE PERIPHERAL MOTOR AND DIGITAL NERVE INJURY **468** OSTEOARTHRITIS AND ALLIED DISORDERS **511** PERIPHERAL ENTHESOPATHIES **544** LESION OF PLANTAR NERVE; PLANTAR FASCIAL FIBROMATOSIS **597** SYNOVITIS AND TENOSYNOVITIS **601** GANGLION **612** DISORDERS OF SOFT TISSUE	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **157** PYOGENIC ARTHRITIS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **468** OSTEOARTHRITIS AND ALLIED DISORDERS **511** PERIPHERAL ENTHESOPATHIES **533** BENIGN NEOPLASM OF BONE AND ARTICULAR CARTILAGE INCLUDING OSTEOID OSTEOMAS; BENIGN NEOPLASM OF CONNECTIVE AND OTHER SOFT TISSUE **597** SYNOVITIS AND TENOSYNOVITIS
20606	with ultrasound guidance	New code	**50, 157, 290, 306, 468, 511, 533, 597**

CPT code	Code description	Current Placement	Recommended Placement
20610	Arthrocentesis, aspiration and/or injection, major joint or bursa (eg, shoulder, hip, knee, subacromial bursa); without ultrasound guidance	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **51** DEEP ABSCESSES, INCLUDING APPENDICITIS AND PERIORBITAL ABSCESS **145** SYSTEMIC LUPUS ERYTHEMATOSUS, OTHER DIFFUSE DISEASES OF CONNECTIVE TISSUE **157** PYOGENIC ARTHRITIS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **359** RHEUMATOID ARTHRITIS, OSTEOARTHRITIS, OSTEOCHONDRITIS DISSECANS, AND ASEPTIC NECROSIS OF BONE Treatment: ARTHROPLASTY/RECONSTRUCTION **422** DISORDERS OF SHOULDER, INCLUDING SPRAINS/STRAINS GRADE 3 THROUGH 6 **430** ACUTE PERIPHERAL MOTOR AND DIGITAL NERVE INJURY **435** INTERNAL DERANGEMENT OF KNEE AND LIGAMENTOUS DISRUPTIONS OF THE KNEE, RESULTING IN SIGNIFICANT INJURY/IMPAIRMENT **468** OSTEOARTHRITIS AND ALLIED DISORDERS **509** OTHER DISORDERS OF SYNOVIUM, TENDON AND BURSA, COSTOCHONDRITIS, AND CHONDRODYSTROPHY **511** PERIPHERAL ENTHESOPATHIES **533** BENIGN NEOPLASM OF BONE AND ARTICULAR CARTILAGE INCLUDING OSTEOID OSTEOMAS; BENIGN NEOPLASM OF CONNECTIVE AND OTHER SOFT TISSUE **597** SYNOVITIS AND TENOSYNOVITIS **601** GANGLION **612** DISORDERS OF SOFT TISSUE	**50** RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY POLYARTHROPATHIES **157** PYOGENIC ARTHRITIS **290** COMPLICATIONS OF A PROCEDURE ALWAYS REQUIRING TREATMENT **306** GOUT **422** DISORDERS OF SHOULDER, INCLUDING SPRAINS/STRAINS GRADE 3 THROUGH 6 **435** INTERNAL DERANGEMENT OF KNEE AND LIGAMENTOUS DISRUPTIONS OF THE KNEE, RESULTING IN SIGNIFICANT INJURY/IMPAIRMENT **468** OSTEOARTHRITIS AND ALLIED DISORDERS **511** PERIPHERAL ENTHESOPATHIES **533** BENIGN NEOPLASM OF BONE AND ARTICULAR CARTILAGE INCLUDING OSTEOID OSTEOMAS; BENIGN NEOPLASM OF CONNECTIVE AND OTHER SOFT TISSUE **597** SYNOVITIS AND TENOSYNOVITIS

CPT code	Code description	Current Placement	Recommended Placement
20611	with ultrasound guidance	New code	**50, 157, 290, 306, 422, 435, 468, 511, 533, 597**

2015 CPT Code Review Issues

Cryoablation of bone tumors (CPT 20982)

1) Minimally invasive treatment for metastatic cancer to bone; also used for primary osteosarcoma treatment
2) Similar minimally invasive treatment--radiofrequency ablation of bone tumors (CPT 20982) is Excluded. Last reviewed 2004, found to be experimental
3) Evidence
    i. **Callstrom 2013**
        1. Prospective multi-center trial of 61 patients with 1 or 2 painful bone metastases, treated with percutaneous cryoablation.
        2. Significant reduction in pain scores seen at 4, 8 and 24 weeks
        3. 1 patient developed osteomyelitis at the site as a complication
        4. CONCLUSIONS: Percutaneous cryoablation is a safe, effective, and durable method for palliation of pain due to metastatic disease involving bone.
    ii. **McMenomy 2013** (only abstract available)
        1. Retrospective study of 40 patients who underwent cryoablation of 5 or fewer musculoskeletal metastases with the goal of complete disease remission
        2. RESULTS: Local control was achieved in 45 of 52 tumors (87%; 95% confidence interval [CI], 75%-93%) at a median follow-up of 21 months (range, 4-62 mo). Median overall survival was 47 months (95% CI, 26-62 mo). Median disease-free survival was 7 months (95% CI, 5-10 mo).
4) No guidance or studies found from Cochrane, NICE, SIGN. No reviews or meta-analyses found
5) HERC staff recommendation: **Non-covered List**
    i. Experimental
    ii. Standard treatment with external beam radiation is available to patients

Cryoablation of liver tumors (CPT 47383)

1) Cryoablation of liver tumors is a minimally invasive treatment of either primary hepatocellular carcinoma or metastatic disease to the liver
2) Radiofrequency ablation of liver tumors (CPT 47382) is covered on the liver cancer line
3) Evidence
    a. **NICE 2010,** guidance for treatment of liver metastases
        i. Current evidence on the safety of cryotherapy for the treatment of liver metastases appears adequate in the context of treating patients whose condition has such a poor prognosis, but the evidence on efficacy is inadequate in quality. Therefore cryotherapy for the treatment of liver metastases should only be used with special arrangements for clinical governance, consent and audit or research.
    b. **Bala 2013**, Cochrane review of cryotherapy for liver metastases
        i. 1 RCT, with high risk of bias
            1. 123 patients, randomized to cryotherapy or conventional surgery
            2. The patients were followed for up to 10 years (minimum five months). Mortality at the last follow-up was 81% (51/63) in the cryotherapy group and 92% (55/60) in the conventional surgery group (RR 0.88; 95% CI 0.77 to 1.02); that is, no statistically significant difference was observed.
            3. Recurrence in the liver was observed in 86% (54/63) of the patients in the cryotherapy group and 95% (57/60) of the patients in the conventional surgery group (relative risk (RR) 0.9; 95% CI 0.8 to 1.01); that is, no statistically significant difference was observed.
        ii. **Authors' conclusions** On the basis of one randomised clinical trial with high risk of bias, there is insufficient evidence to conclude if in patients with liver metastases from various primary sites cryotherapy brings any significant benefit in terms of survival or recurrence compared with conventional surgery. In addition, there is no evidence for the effectiveness of cryotherapy when compared with no intervention. At present, cryotherapy cannot be recommended outside randomised clinical trials.
    c. **Awad 2009**, Cochrane review of cryotherapy for hepatocellular carcinoma
        i. No trials identified
        ii. **Authors' conclusions** At present, there is no evidence to recommend or refute cryotherapy for patients with hepatocellular carcinoma. Randomised clinical trials with low-risk of bias may help in defining the role of cryotherapy in the treatment of hepatocellular carcinoma.
4) HERC staff recommendation: **Non-covered List**
    a. Experimental for both hepatocellular carcinoma and metastatic disease

Transversus abdominis plane (TAP) block (CPT 64486-64489)

1) Definition: Transversus abdominis plane (TAP) block is a regional anesthetic technique used to block sensation to the anterior abdominal wall. This can be used for pain control after surgical procedures such as cesarean section, colorectal surgery, hysterectomy, and other abdominal surgeries.
2) Evidence
    a. **De Oliveira 2014**, meta-analysis of TAP for pain control after laparoscopic surgery
        i. N=10 studies (633 patients)
        ii. The weighted mean difference (99% confidence interval) of the combined effects favored TAP block over control for pain at rest (≤4 hours, −2.41 [−3.6 to −1.16]) and (at 24 hours, −1.33 [−2.19 to −0.48]) (0–10 numerical scale).
        iii. Postoperative opioid consumption was decreased in the TAP block group compared with control, weighted mean difference (99% confidence interval) of −5.74 (−8.48 to −2.99) mg morphine IV equivalents.
        iv. CONCLUSIONS: TAP block is an effective strategy to improve early and late pain at rest and to reduce opioid consumption after laparoscopic surgical procedures.
    b. **Champaneria 2013**, meta-analysis of TAP for pain control after hysterectomy
        i. N=5 studies (225 patients)
        ii. Study quality was moderate.
        iii. Compared with no block or saline placebo, transversus abdominis plane block provided significantly less postoperative pain at rest on a 10 cm visual analog scale at 2 h (mean difference -2.14 cm, 95% confidence interval (CI) –3.57 to -0.71) but not at 24 h postoperatively (–0.52 cm, 95% CI –1.49 to 0.45). Pain on movement showed similar results. Transversus abdominis plane block resulted in significantly less postoperative requirement for morphine use at 24 h (-11.76 mg, 95% CI -18.77 to -4.75) but not at 48 h (-16.01 mg, 95% CI -39.40 to 7.39).
        iv. Conclusions: until further robust evidence is available, anesthetists should not rush to adopt this procedure into routine practice.
    c. **Abdallah 2012**, meta-analysis of TAP for pain control after cesarean section
        i. N=5 trials (312 patients)
        ii. TAP block reduced the mean 24 h i.v. morphine consumption by 24 mg [95% confidence interval (CI) 239.65 to 27.78] when spinal morphine was not used. TAP block also reduced visual analogue scale pain scores (10 cm line where 0 cm, no pain, and 10 cm, worst pain) by 0.8 cm (95% CI 21.53 to 20.05, P¼0.01), and decreased the incidence of opioid-related side effects.
        iii. Conclusions: TAP block provides superior analgesia compared with placebo and can reduce the first 24 h morphine consumption in the setting of a multimodal analgesic regimen that excludes spinal morphine. TAP block can provide effective analgesia when spinal morphine is contraindicated or not used.
    d. HERC staff recommendation:

i. **Ancillary Codes File**
    1. Most anesthesia procedures are on the Ancillary List
    2. Appears to be an effective adjuvant pain control strategy for abdominal surgery post-operative pain

Digital breast tomosynthesis (CPT 77061-77063)

1) Definition: a new technology which acquires 3D images of the breast to assist in breast cancer screening and diagnosis. Generally, tomosynthesis is done together with traditional digital mammography.
2) Other guidelines/guidances
    a. **NCCN 2014**, breast cancer screening guideline
        i. Early studies show promise for tomosynthesis mammography. Two large trials showing a combined use of digital mammography and tomosynthesis resulted in improved cancer detection and decreased call back rates; of note, this is double the dose of radiation and is a factor in recommending this modality. Definitive studies are still pending.
        ii. Tomosynthesis is not included in their flow charts for routine breast cancer screening
3) Evidence
    a. **TEC 2014**, technology report
        i. N=6 studies
        ii. Insufficient evidence to permit conclusions on whether digital breast tomosynthesis improves detection of breast cancer or improves any health outcomes compared to conventional mammography
    b. **Houssami 2013**, systematic review
        i. N=13 studies
        ii. Authors conclusions: At present, there is insufficient evidence to justify a change from standard digital mammography to digital breast tomosynthesis however the available data strongly support investment in new large-scale population screening trials.
    c. **Rafferty 2013**, trial of tomosynthesis + digital mammography vs digital mammography alone
        i. N=1192 patients
            1. Included women scheduled for breast biopsy (i.e. not a screening population) as well as women who presented for screening
        ii. Diagnostic accuracy of tomosynthesis plus digital mammography was superior to digital mammography alone.
        iii. Recall rates for noncancerous cases for all readers decreased with the addition of tomosynthesis (range, 6%–67%; P = 0.001 for 25 readers, P = 0.03 for all readers).
        iv. The increase in the rate of sensitivity was greatest for invasive cancers: 15% and 22% in studies 1 and 2 versus 3% for in situ cancers in both studies.
        v. The authors concluded that the addition of tomosynthesis to digital mammography provides the dual benefit of significantly increased diagnostic accuracy and significantly reduced recall rates for noncancerous cases.

vi. Several authors had significant conflicts of interest; one was an employee of the company that manufactures the tomosynthesis machines and the study was funded by the manufacturer

d. **Skaane 2013**, trial of tomosynthesis tomosynthesis + digital mammography vs digital mammography alone
   i. N=12,631 patients
   ii. Detection rates, including those for invasive and in situ cancers, were 6.1 per 1000 examinations for mammography alone and 8.0 per 1000 examinations for mammography plus tomosynthesis (27% increase, adjusted for reader; *P* =.001). False-positive rates before arbitration were 61.1 per 1000 examinations with mammography alone and 53.1 per 1000 examinations with mammography plus tomosynthesis (15% decrease, adjusted for reader; *P* , .001). After arbitration, positive predictive values for recalled patients with cancers verified later were comparable (29.1% and 28.5%, respectively, with mammography alone and mammography plus tomosynthesis; *P* = .72). Twenty-five additional invasive cancers were detected with mammography plus tomosynthesis (40% increase, adjusted for reader; *P*, .001). The mean interpretation time was 45 seconds for mammography alone and 91 seconds for mammography plus tomosynthesis (*P* , .001).
   iii. Conclusion: The use of mammography plus tomosynthesis in a screening environment resulted in a significantly higher cancer detection rate and enabled the detection of more invasive cancers.
   iv. Study funded by the manufacturer and several authors had significant conflicts of interest; 2 were employees of the manufacturer

4) Other coverage policies:
   a. **BCBS 2014**: does not cover, investigational
   b. **Aetna 2014**: does not cover, investigational
5) HERC staff recommendation: **Non-covered List**
   a. Experimental, unclear what added value is for general population screening, has risk of increased radiation exposure
   b. Re-evaluate coverage when the results of a large NHS trial currently underway in the UK (TOMMY trial) and/or other large scale, definitive studies are released in a few years

Laboratory Tests

FTL3 (CPT 81246)

1) Definition: FTL3 internal tandem duplications occur in some acute myeloid leukemia cell lines
2) Evidence
    a. **Etsy 2013**, review of treatment updates for Acute Myeloid Leukemia
        i. patients with a FLT3 ITD have only a 30–40% chance of cure even after stem cell transplant. Accordingly analyses of NPM1, FLT3, and CEBPA should be part of routine evaluation
        ii. Suggests adaptations to chemotherapy regimens determined in part by FLT3 status
    b. **Port 2014**, meta-analysis of FLT3 for prognosis
        i. Meta-analysis for overall survival (OS) and relapse-free survival (RFS) showed FLT3-ITD to predict an unfavorable prognosis, with hazard ratios (HR) of 1.86 and 1.75, respectively.
3) HERC staff recommendation: Lines with acute myeloid leukemia diagnoses
        i. 97 CHILDHOOD LEUKEMIAS
        ii. 401 ACUTE PROMYELOCYTIC LEUKEMIA
        iii. 402 ACUTE MYELOID LEUKEMIA Treatment: BONE MARROW TRANSPLANT
        iv. 241 MYELOID DISORDERS
        v. Appears to affect prognosis and possibly treatment decisions, does not appear to be used in diagnosis of AML

PCA3/KLK3 ratio (CPT 81313)

1) Definition: PCA3 is a urine test which can be used for screening for prostate cancer; it can be used to determine which patient requires prostate biopsy. KLK3 is another term used for PSA (prostate specific antigen), the standard screening test for prostate cancer.
2) Evidence
    a. **Bradley 2013,** review of PCA3
        i. N=34 observations studies
        ii. PCA3 was more discriminatory than total prostate specific antigen increases (eg at an observed 50% specificity, summary sensitivities were 77% and 57%, respectively). Analyses indicated that this finding holds for initial and repeat biopsies, and that the markers were independent predictors. For all other biopsy decision making comparisons and associated health outcomes, strength of evidence was insufficient. For treatment decision making, strength of evidence was insufficient for all outcomes and comparators.
        iii. Conclusions: PCA3 had a higher diagnostic accuracy than total prostate specific antigen increases, but strength of evidence was low (limited confidence in effect estimates). Strength of evidence was insufficient to conclude that PCA3 testing leads to improved health outcomes. For all other outcomes and comparators, strength of evidence was insufficient.

a. **Choudhury 2013**, review genetic markers in prostate cancer
    iv. A PCA3 score >35 in the urine has correlated with an average sensitivity and specificity of 66% and 76%, respectively, for the diagnosis of PCa (compared to a specificity of 47% for serum PSA at the cutoff for 65% sensitivity). Elevated PCA3 scores have also been demonstrated to increase the probability of a positive repeat biopsy in men with one or two prior negative biopsy results.
    v. Conclusions: Large clinical trials involving multi-institutional collaborations will be required to prospectively validate the utility of these markers for clinical decision making.
b. **Auprich 2011**, review of role of PCA3
    i. Despite its costs, prostate cancer antigen 3 (PCA3) is superior to prostate-specific antigen (PSA) and percent free PSA in the early detection of PCa. PCA3 improves the diagnostic accuracy of externally validated nomograms among men with an elevated PSA undergoing biopsy. PCA3 independently predicts low-volume disease and pathologically insignificant PCa but is not associated with locally advanced disease and is limited in the prediction of aggressive cancer. Preliminary data demonstrate that combining PCA3 with other new biomarkers further improves diagnostic and prognostic accuracy.
    ii. Conclusions: PCA3, integrated in novel biopsy nomograms or risk stratification tools, can be used to counsel or confirm biopsy indications. If confirmed in further studies, using PCA3 together with established staging risk factors could assist clinicians in specific pretreatment decision making. So far no evidence for the usefulness of PCA3 in active surveillance programs has been presented.

3) Other policies
    a. **Aetna 2014**: Experimental
4) HERC staff recommendation: **Non-covered List**
        i. Experimental

Breast mRNA gene expression (CPT 81519)

1) Definition: Breast mRNA gene expression is marketed as Oncotype Dx Breast.
2) Evidence
    a. **AHRQ 2014**, review of oncology genetics tests
        i. Oncotype Dx breast found to have multiple studies associating the test result and prognosis
        ii. Oncotype Dx breast found to have ~30% of treatment decisions changed by the test (moderate evidence)
        iii. Conclusion: Oncotype DX assay in breast cancer [has] a sizeable body of evidence to suggest an effect on treatment decisions (resulting in fewer recommendations for chemotherapy), though not yet a clear effect on downstream outcomes.
    b. **NICE 2013**, review of gene expression profiling in oncology
        i. Oncotype DX is recommended as an option for guiding adjuvant chemotherapy decisions for people with oestrogen receptor positive (ER+), lymph node negative (LN−) and human epidermal growth factor receptor 2 negative (HER2−) early breast cancer if:
            1. the person is assessed as being at intermediate risk **and**
            2. information on the biological features of the cancer provided by Oncotype DX is likely to help in predicting the course of the disease and would therefore help when making the decision about prescribing chemotherapy **and**
            3. the manufacturer provides Oncotype DX to NHS organisations according to the confidential arrangement agreed with NICE.
3) Other policies
    a. Aetna 2014: Oncotype Dx to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:
        i. Breast cancer is nonmetastatic (node negative); *and*
        ii. Breast tumor is estrogen receptor positive; *and*
        iii. Breast tumor is HER2 receptor negative or breast tumor is HER2 receptor positive and less than 1 cm in diameter. (Rationale: adjuvant chemotherapy with trastuzumab (Herceptin) is considered to be medically necessary regardless of an Oncotype Dx score for HER2 receptor positive lesions 1 cm or more in diameter); *and*
        iv. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); *and*
        v. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy (i.e., member will forgo adjuvant chemotherapy if Oncotype Dx score is low).
4) HERC staff recommendation: line 195 CANCER OF BREAST; AT HIGH RISK OF BREAST CANCER
    a. Note: this is scheduled for review and possible coverage guidance with HTAS at their November 24th meeting. If the HTAS coverage recommendation is different than the HERC staff recommendation above, this topic will need to be re-addressed (for example, addition of a guideline for coverage specifics).

ST2 (CPT 83006)

1) Definition: Growth stimulation expressed gene 2 (ST2, Interleukin 1 receptor like-1) is a novel biomarker for heart failure (CHF)
2) Evidence
    a. **Gaggin 2013**, review of novel biomarkers for CHF
        i. Novel biomarkers, such as mid-regional pro atrial natriuretic peptide (MR-proANP),mid-regional pro adrenomedullin (MR-proADM), highly sensitive troponins, soluble ST2 (sST2), growth differentiation factor (GDF)-15 and Galectin-3, show potential in determining prognosis beyond the established natriuretic peptides, but their role in the clinical care of the patient is still partially defined and more studies are needed.
    b. **Ciccone 2013**, review of ST2 as a biomarker
        i. Conclusions: sST2 measurements in blood samples could be a clinical prognostic biomarker useful in risk stratification of patients suffering from myocardial infarction, HF and dyspnea. Further studies are needed in order to better point out the evidence for a routine use of sST2 evaluation in the clinical evaluation of a patient suffering from myocardial infarction and HF.
3) HERC staff recommendation: **Non-covered List**
    a. Experimental

Liver elastography (CPT 91200)

1) Definition: a non-invasive test to assess liver stiffness measurement (LSM) for the diagnosis of liver fibrosis. Promoted as a way to avoid liver biopsy in some patients; also may be more accurate than biopsy which can have sampling error. A device to conduct this test (Fibroscan) was FDA approved in 2012.
2) Guidances
    a. **NICE 2013**, guidance on the diagnosis and management of hepatitis C
        i. Offer transient elastography as the initial test for liver disease in adults newly referred for assessment.
        ii. Offer antiviral treatment without a liver biopsy to adults with a transient elastography score greater than or equal to 11 kPa
        iii. Consider liver biopsy to confirm the level of fibrosis in adults with a transient elastography score between 6 and 10 kPa
        iv. Offer liver biopsy to adults with a transient elastography score less than 6 kPa if they are younger than 30 years and have HBV DNA greater than 2000 IU/ml and abnormal ALT (greater than or equal to 30 IU/L for males and greater than or equal to 19 IU/L for females) on 2 consecutive tests conducted 3 months apart
        v. Do not offer liver biopsy to adults with a transient elastography score less than 6 kPa who have normal ALT (less than 30 IU/L in males and less than 19 IU/L in females) and HBV DNA less than 2000 IU/ml as they are unlikely to have advanced liver disease or need antiviral treatment
        vi. Offer an annual reassessment of liver disease using transient elastography to adults who are not taking antiviral treatment.
    b. SIGN 2013, guidance for diagnosis and management of hepatitis C
        i. Measurement of liver stiffness may be useful as a non-invasive assessment of liver fibrosis.
    c. **AASLD 2014**, management of hepatitis C
        i. Liver elastography can only reliably distinguish cirrhosis from non-cirrhosis.
        ii. Vibration-controlled transient liver elastography is a noninvasive way to measure liver stiffness and correlates well with measurement of substantial fibrosis or cirrhosis in patients with chronic HCV infection. A cutoff value of 8.7 kPa correlates with Metavir F2 or higher fibrosis stage; greater than 9.5 kPa with F3; and 14.5 or higher kPa with F4 or cirrhosis. The measurement range does overlap between stages. The most efficient approach to fibrosis assessment is to combine direct biomarkers and vibration-controlled transient liver elastography. A biopsy should be considered for any patient who has discordant results between the 2 modalities that would affect clinical decision making.
3) Evidence
    a. Cochrane has a protocol for evaluation of liver elastography
    b. **NHS 2008**, technology assessment of liver elastography
        i. FibroScan can be used as an initial screening tool to reduce the number of patients requiring liver biopsy and also to measure ongoing response to treatment. In some patients the initial screening results may be sufficiently

well-defined to provide a confident assessment of the patient's stage of liver fibrosis.

c. **Canadian Agency for Drugs and Technology in Health (CADTH) 2008**, technology assessment of liver elastography
    i. The diagnostic performance of FibroScan is good for identifying severe fibrosis or cirrhosis, but it is less accurate for milder presentations.
    ii. FibroScan is a promising technology, but large multi-centre trials comparing a range of emerging non-invasive fibrosis staging technologies are required.
d. **Tshochatzis 2013**, meta-analysis of liver elastrography accuracy
    i. N=40 studies
    ii. Summary sensitivity and specificity was 0.79 (95% CI 0.74–0.82) and 0.78 (95% CI 0.72–0.83) for F2 stage and 0.83 (95% CI 0.79–0.86) and 0.89 (95% CI 0.87–0.91) for cirrhosis. After an elastography result at/over the threshold value for F2 or cirrhosis (''positive'' result), the corresponding post-test probability for their presence (if pre-test probability was 50%) was 78%, and 88% respectively, while, if values were below these thresholds (''negative'' result), the post-test probability was 21% and 16%, respectively.
    iii. Conclusions: Elastography theoretically has good sensitivity and specificity for cirrhosis (and less for lesser degrees of fibrosis); however, it should be cautiously applied to everyday clinical practice because there is no validation of the stiffness cut-offs for the various stages. Such validation is required before elastography is considered sufficiently accurate for non-invasive staging of fibrosis.
e. **Stebbing 2012**, Meta-analysis of Transient Elastography for the Detection of Hepatic Fibrosis
    i. N=22 studies (4430 patients)
    ii. The pooled estimates for significant fibrosis (ZF2) measured 7.71 kPa (LSM cutoff value) with a sensitivity of 71.9% [95% confidence interval (CI): 71.4%-72.4%] and specificity of 82.4% (95% CI: 81.9-82.9%), whereas for cirrhosis (F4) the results showed a cutoff of 15.08 kPa with a sensitivity of 84.45% (95% CI: 84.2-84.7%) and specificity of 94.69% (95% CI: 94.3%-95%).
    iii. Conclusions: Further evaluation of transient elastography to assess LSM is required in prospective studies to potentially increase the sensitivity and establish its clinical utility.
f. **Singh 2013**, meta-analysis to determine predictive value of liver elastography
    i. N=17 studies (7058 patients) with chronic liver disease
    ii. Baseline LSM was associated significantly with risk of hepatic decompensation (6 studies; RR, 1.07; 95% CI, 1.03–1.11), HCC (9 studies; RR, 1.11; 95% CI, 1.05–1.18), death (5 studies; RR, 1.22; 95% CI, 1.05–1.43), or a composite of these outcomes (7 studies; RR, 1.32; 95% CI, 1.16–1.51).
    iii. CONCLUSIONS: Based on a meta-analysis of cohort studies, the degree of liver stiffness is associated with risk of decompensated cirrhosis, HCC,

and death in patients with CLDs. LSM therefore might be used in risk stratification

4) Other policies
    a. Aetna 2014
        i. Aetna considers transient elastography (e.g., FibroScan) medically necessary for distinguishing hepatic cirrhosis from noncirrhosis in persons with hepatitis C or other chronic liver diseases. Performance of transient elastography more than twice per year is considered not medically necessary. Performance of transient elastography within six months following a liver biopsy is considered not medically necessary. Transient elastography is considered experimental and investigational for all other indications.
5) Expert input:
    a. Dr. Atif Zaman, hepatologist at OHSU
        i. "The cost and complications of liver biopsy in HCV patients will be avoided. It (Fibroscan) has excellent performance for stage 3 and 4…it can be a part of practice and reduce liver biopsy use by at least 65%. Of note, liver biopsies cost $3-5K, which FibroScan is only at $300-500."
6) Summary: Significant controversy exists in the literature and in various guidelines regarding liver elastography. It has the potential to affect management of hepatitis B and C, and to have a significant number of patients avoid liver biopsy, which would reduce complications and costs. However, most meta-analyses conclude that further work needs to be done to increase its sensitivity and determine its clinical utility.
7) HERC staff recommendation: **Non-covered List**
    a. Unclear clinical utility. Gold standard test of liver biopsy is available.
    b. MED report is expected in the next few months. Will readdress once the MED report is available. Additionally, a Cochrane review is expected in the near future.

Corneal hysteresis determination (CPT 92145)

1) Definition: Measuring intraocular pressure (IOP) is important for the diagnosis and monitoring of glaucoma. IOP measurements can be affected by corneal thickness and other characteristics of the cornea. Corneal hysteresis (CH) was developed to measure the viscous damping in the corneal tissue in order to provide a correction to IOP measurements. Corneal hysteresis assesses corneal resistance to deformation in the cornea. The Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY) is a new instrument that measures corneal hysteresis, the corneal biomechanical response to rapid indentation by an air jet.
2) Evidence:
    a. **McMonnies 2012**, review of corneal hysteresis (CH)
        i. Corneal thickness, the level of edema, intraocular pressure, and corneal temperature as well as the area, location, rate, duration, and sequence of corneal unloading and loading may need to be considered as significant variables when assessing CH.
        ii. CH values may be specific to measurement method and conditions rather than representing an unequivocal corneal property.
        iii. The potential for the ORA to contribute to improved clinical management appears to be considerable but so does the need for better understanding and further development of its functions and applications.
    b. **Touboul 2008**, study of correlation between corneal hysteresis (CH) and intraocular pressure (IOP)
        i. N=498 eyes of 258 patients
        ii. Corneal hysteresis was not strongly correlated with IOP
        iii. CONCLUSIONS: Corneal hysteresis, a new corneal parameter, had a moderate dependence on IOP
    c. **Kotecha 2007**, review of the clinical relevance of corneal measurement
        i. Further population and longitudinal studies are needed to clarify whether current in vivo measures of corneal biomechanical properties, including corneal hysteresis, prove to be independent predictors of glaucoma susceptibility.
3) Other policies
    a. **Aetna 2014**: Aetna considers measurement of corneal hysteresis experimental and investigational for the screening, diagnosis and monitoring of glaucoma and for all other indications because of insufficient evidence of its effectiveness
4) HERC staff recommendation: **Non-covered List**
    a. Experimental

Bioimpedance spectroscopy for lymphedema assessment (CPT 93702)

1) Definition: Bioimpedance has been proposed as a method of diagnosing Stage 0 lymphedema, which is difficult to detect with current diagnostic methods. Bioelectrical impedance analysis measures the body's response to an electrical current. A low level current is applied through the body and the impedance (or resistance) to flow is measured. Current flows along the path of least resistance through the body and thus follows tissues with the highest water content, thereby allowing for edema to be measured. The L-Dex U400 BIS (Impedimed LTD) has been cleared by the FDA specifically for lymphedema use.
2) Alternatives: other methods of diagnosing lymphedema include arm circumference measurements, water displacement (volumetry), perometry (infrared optoelectric volumetry), tonometry, and the Lymphedema and Breast Cancer Questionnaire (LBCQ).
3) Evidence
    a. **AHRQ 2011**, technology assessment of diagnostic tests for lymphedema
        i. There is too little evidence to draw conclusions about the reliability of tests such as…bioimpedence.
        ii. No evidence was found that tests such as bioimpedence affected treatment decisions or patient outcomes
        iii. The heterogeneity of the available evidence was too substantial to enable one to draw conclusions about the type of diagnostic test that would be most appropriate for diagnosing secondary lymphedema
        iv. It is recommended that limb volume or circumference be the de facto 'gold standard' tests from which to assess the presence of secondary lymphedema.
    b. **Ward 2011**, assessment of bioimpedence spectroscopy for diagnosis of lymphedema
        i. N=37 female patients and N=5 male patients with bilateral lower extremity lymphedema
            1. Controls: N=277 female and N=224 male controls
        ii. Results varied with age, sex, and limb dominance
        iii. comparison of the ratio for affected legs (normalized to the ratio in the unaffected arms) with equivalent ratios observed in a control population (as bivariate tolerance plots) was capable of discriminating between 70% and 89% of the participants with lymphedema
        iv. Conclusions: Bioelectrical impedance spectroscopy and determination of Ri/R0 ratios as indices of ECF/ICF ratios holds promise for the semiquantitative assessment of bilateral lymphedema.
4) Other policies:
    a. **BCBS 2013**: The use of bioimpedance spectroscopy testing in the assessment, diagnosis, or management of individuals with known or suspected lymphedema is considered investigational and not medically necessary.
5) HERC staff recommendation: **Non-covered List**
    a. Experimental

Carotid intima media thickness (CPT 93895)

1) Definition: Carotid intima-media thickness testing (CIMT or IMT) is an ultrasound-based test to determine the thickness of the intima and media, part of the carotid artery wall. This thickness can be related to prediction of risk for cardiovascular disease. This test requires a high-resolution B-mode ultrasound, which currently is only available in specialized centers
2) Evidence
    a. **USPSTF 2009**, review of non-traditional risk factors for screening for cardiovascular disease
        i. Fair-quality evidence indicates, on the basis of 1 fair and 2 good-quality population-based longitudinal studies in the United States and the Netherlands, that carotid IMT predicts CHD independent of Framingham risk factors in asymptomatic persons (1300 to 16 000 men and women who showed a relative risk of 1.19 to 3.80). Adding carotid IMT scores to a risk prediction equation based on traditional risk factors modestly improved the prediction of subsequent CHD among healthy adults, particularly for me. However, the studies that show an association of carotid IMT with CHD outcome have all been done in research settings, and the ability to conduct carotid IMT with precision in nonresearch settings has not been established. No information is available about the prevalence or applicability of carotid IMT to populations at intermediate risk for CHD events.
        ii. Lack of consensus on examination techniques and population-based standards for interpreting quantitative IMT measures.
        iii. Overall strength of evidence for prediction of CVD events by use of IMT was rated as poor
        iv. Effects on treatment decisions based on IMT independent of LDL levels unclear
        v. Usefulness of IMT in cardiac risk assessment needs to be validated in prospective, population-based cohort studies that use appropriate methods to measure other risk factors and examine the added predictive ability of carotid IMT in persons classified as intermediate-risk by the Framingham risk score.
        vi. Recommendation: The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of using the nontraditional risk factors (includes IMT) studied to screen asymptomatic men and women with no history of CHD to prevent CHD events. (I statement).
    b. **Goff 2013**, ACC/AHA Guideline on the Assessment of Cardiovascular Risk
        i. Routine measurement of CIMT is not recommended in clinical practice for risk assessment for a first ASCVD event.
            1. NHLBI Grade: N (No recommendation for or against); ACC/AHA COR III: No Benefit, LOE: B
2) HERC staff recommendation: **Non-covered List**
    a. USPSTF and ACC/AHA recommend against use in clinical practice

Original Article

Percutaneous Image-Guided Cryoablation of Painful Metastases Involving Bone

Multicenter Trial

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BACKGROUND: This study sought to describe the results of a single-arm multicenter clinical trial using image-guided percutaneous cryoablation for the palliation of painful metastatic tumors involving bone. **METHODS:** Over a 44-month period, 61 adult patients with 1 or 2 painful bone metastases with a score of 4 or more on a scale of 0 to 10 ($\geq$4/10) worst pain in a 24-hour period who had failed or refused conventional treatment were treated with percutaneous image-guided cryoablation. Patient pain and quality of life was measured using the Brief Pain Inventory prior to treatment, 1 and 4 days after the procedure, weekly for 4 weeks, and every 2 weeks thereafter for a total of 6 months. Patient analgesic use was also recorded at these same follow-up intervals. Complications were monitored. Analysis of the primary endpoint was undertaken via paired comparison procedures. **RESULTS:** A total of 69 treated tumors ranged in size from 1 to 11 cm. Prior to cryoablation, the mean score for worst pain in a 24-hour period was 7.1/10 with a range of 4/10 to 10/10. At 1, 4, 8, and 24 weeks after treatment, the mean score for worst pain in a 24-hour period decreased to 5.1/10 ($P < .0001$), 4.0/10 ($P < .0001$), 3.6/10 ($P < .0001$), and 1.4/10 ($P < .0001$), respectively. One of 61 (2%) patients had a major complication with osteomyelitis at the site of ablation. **CONCLUSIONS:** Percutaneous cryoablation is a safe, effective, and durable method for palliation of pain due to metastatic disease involving bone. ***Cancer* 2013;119:1033-41.** © *2012 American Cancer Society.*

KEYWORDS: palliation, image-guided, cryoablation, metastases, skeletal.

The bony skeleton is the most common metastatic site from cancer after lungs and liver, with prostate, breast, lung, kidney, and thyroid malignancies accounting for approximately 80% of skeletal metastases. Of the patients who develop skeletal metastases, approximately 50% of patients will develop poorly controlled pain during the course of their disease.[1-4] Unfortunately, it is often difficult to achieve adequate pain relief, and the quality of life for these patients is poor. Current standard-of-care treatments for patients who develop painful bone metastases are primarily palliative. For focal palliative pain treatment, external beam radiation and surgery are most commonly offered, whereas for diffuse metastases, a systemic approach is used including chemotherapy, hormonal therapy, radiopharmaceuticals, and bisphosphonates.

Because many patients fail to derive adequate pain relief despite use of current conventional therapies, multiple minimally invasive focal therapies have been explored as new strategies for the palliation of painful metastatic disease. Of these methods, radiofrequency (RF) ablation has been most thoroughly evaluated with results from 2 multicenter studies demonstrating significantly reduced focal pain in patients who are unresponsive to standard treatments.[5,6] Although effective at reducing a patient's pain, RF ablation has a critical limitation, which is the nonvisualization of the ablation margin with computed tomography (CT) monitoring. In contrast to RF ablation, the ablation zone of cryoablation is readily identified with CT imaging as a low-attenuation ice ball beyond which tissues are safe from thermal injury.[7] Another advantage of cryoablation relative to RF ablation is that patients treated with cryoablation do not experience increased pain during the procedure or in the immediate posttreatment period.[8]

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National Institute for Health and Clinical Excellence

Cryotherapy for the treatment of liver metastases

Issued: December 2010

NICE interventional procedure guidance 369

www.nice.org.uk/ipg369

NHS Evidence has accredited the process used by the NICE Interventional Procedures Programme to produce interventional procedures guidance. Accreditation is valid for three years from January 2010 and applies to guidance produced since January 2009 using the processes described in the 'Interventional Procedures Programme: Process guide, January 2009' and the 'Interventional Procedures Programme: Methods guide, June 2007'

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1 Guidance

1.1 Current evidence on the safety of cryotherapy for the treatment of liver metastases appears adequate in the context of treating patients whose condition has such a poor prognosis, but the evidence on efficacy is inadequate in quality. Therefore cryotherapy for the treatment of liver metastases should only be used with special arrangements for clinical governance, consent and audit or research.

1.2 Clinicians wishing to undertake cryotherapy for the treatment of liver metastases should take the following actions.

- Inform the clinical governance leads in their Trusts.
- Ensure that patients and their carers understand that other ablative treatments are available and provide them with clear written information. In addition, the use of NICE's information for patients ('Understanding NICE guidance') is recommended.
- Audit and review clinical outcomes of all patients having cryotherapy for liver metastases (see section 3.1).

1.3 Patient selection and treatment should be carried out by a hepatobiliary multidisciplinary team with expertise in the use of ablative techniques.

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2 The procedure

2.1 Indications and current treatments

2.1.1 Liver metastases are commonly caused by colorectal cancer or other primary malignancies, such as lung and gastric cancer.

2.1.2 For a minority of patients, surgical resection of liver metastases with curative intent may be possible. For most patients however, treatment is palliative and options include systemic chemotherapy, external beam radiotherapy, other thermal ablation techniques, and selective internal radiation therapy. Multiple treatment modalities may be used for individual patients.

2.2 Outline of the procedure

2.2.1 Thermal ablation procedures including cryotherapy may be used as the primary treatment for liver metastases in patients who are not considered suitable for surgery, or to treat post-resection recurrence. They may also be used as an adjunct to hepatic resection. Cryotherapy can be delivered as part of an open resection procedure (requiring general anaesthesia and intraoperative ultrasound), or percutaneously (under local or general anaesthesia, with ultrasound, computed tomography [CT] or magnetic resonance imaging guidance).

2.2.2 Cryotherapy probes (single or multiple, depending on the size of the tumour) deliver coolant at subzero temperatures directly to the tumour in freeze-thaw cycles with the aim of destroying tumour cells.

2.2.3 The maximum recommended hepatic tumour size for cryotherapy is 4 cm. Cryotherapy for larger tumours requires multiple probes and is associated with increased morbidity.

2.2.4 Various devices can be used for this procedure.

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Sections 2.3 and 2.4 describe efficacy and safety outcomes from the published literature that the Committee considered as part of the evidence about this procedure. For more detailed information on the evidence, see the overview.

2.3 Efficacy

2.3.1 A randomised controlled trial (RCT) of 123 patients treated by cryotherapy and chemotherapy or by surgical resection and chemotherapy reported disease-free survival in 14% (9/63) and 5% (3/60) of patients respectively at 10-year follow-up (significance not stated).

2.3.2 A non-randomised controlled trial of 415 patients treated by surgical resection and cryotherapy (for remnant lesions) or resection alone reported median survival of 29 and 34 months respectively (p = 0.206).

2.3.3 A case series of 326 patients reported median overall survival of 29 months after cryotherapy of colorectal liver metastases, with tumour recurrence in 42% (136/326) of patients at a median of 32 months. In a subset of 280 patients who had CT imaging at baseline and during follow-up, complete response was reported in 15% (41/280), partial response in 41% (115/280), stable disease in 24% (68/280), and progressive disease in 20% (56/280) (follow-up not stated).

2.3.4 The Specialist Advisers listed key efficacy outcomes as tumour destruction with small margins of additional tissue destruction, and survival.

2.4 Safety

2.4.1 A non-randomised controlled study of 223 patients reported no significant difference in perioperative mortality between the cryotherapy group (2% [1/55]) and the surgical resection group (5% [8/168]) (p = 0.30). However overall perioperative morbidity was significantly lower in the cryotherapy group (11% [6/55] than in the resection group (26% [44/168]) (p = 0.01).

2.4.2 A case series of 326 patients reported mortality in 2% (7/326) of patients (follow-up period and details of causes were not reported, but 1 patient died from 'cryoshock' syndrome and 1 from liver failure).

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2.4.3 A case report described death of a patient from hepatic failure related to portal vein thrombosis and intra-hepatic MRSA sepsis, 2 months after a second cryotherapy course via an open approach.

2.4.4 Infections directly related to cryotherapy were reported after 8% (12/158) of procedures in the case series of 150 patients (most occurred within 3 weeks of treatment). Fever over 38°C was reported in 33% (108/326) of patients in the case series of 326 patients (timing of events not stated).

2.4.5 Haemorrhage was reported in 4% (2/55) and 1% (2/168) of patients treated with cryotherapy and surgical resection respectively in the non randomised controlled trial of 223 patients (timing of event not stated).

2.4.6 The Specialist Advisers listed anecdotal adverse events as damage to bile ducts and structures outside the liver. The Specialist Advisers also cited reports of major haemorrhage, the rare but fatal complication of cryoshock syndrome, and a high local recurrence rate; they stated that cryotherapy is therefore no longer widely used.

2.5 Other comments

2.5.1 The Committee noted possible differences in the safety of this procedure when used intraoperatively, compared with the percutaneous approach that uses narrower probes. Evidence on the percutaneous approach is limited in quantity.

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3 Further information

3.1 This guidance requires that clinicians undertaking the procedure make special arrangements for audit. NICE has identified relevant audit criteria and has developed an audit tool (which is for use at local discretion).

3.2 For related NICE guidance see our website.

Information for patients

NICE has produced information on this procedure for patients and carers ('Understanding NICE guidance'). It explains the nature of the procedure and the guidance issued by NICE, and has been written with patient consent in mind.

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4 About this guidance

NICE interventional procedure guidance makes recommendations on the safety and efficacy of the procedure. It does not cover whether or not the NHS should fund a procedure. Funding decisions are taken by local NHS bodies after considering the clinical effectiveness of the procedure and whether it represents value for money for the NHS. It is for healthcare professionals and people using the NHS in England, Wales, Scotland and Northern Ireland, and is endorsed by Healthcare Improvement Scotland for implementation by NHSScotland.

This guidance was developed using the NICE interventional procedure guidance process.

It has been incorporated into the NICE pathway on colorectal cancer, along with other related guidance and products.

We have produced a summary of this guidance for patients and carers. Tools to help you put the guidance into practice and information about the evidence it is based on are also available.

Changes since publication

2 January 2012: minor maintenance.

Your responsibility

This guidance represents the views of NICE and was arrived at after careful consideration of the available evidence. Healthcare professionals are expected to take it fully into account when exercising their clinical judgement. This guidance does not, however, override the individual responsibility of healthcare professionals to make appropriate decisions in the circumstances of the individual patient, in consultation with the patient and/or guardian or carer.

Implementation of this guidance is the responsibility of local commissioners and/or providers. Commissioners and providers are reminded that it is their responsibility to implement the guidance, in their local context, in light of their duties to avoid unlawful discrimination and to have regard to promoting equality of opportunity. Nothing in this guidance should be interpreted in a way which would be inconsistent with compliance with those duties.

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Cryotherapy for liver metastases (Review)

Bala MM, Riemsma RP, Wolff R, Kleijnen J

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2013, Issue 6

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[Intervention Review]

Cryotherapy for liver metastases

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ABSTRACT

Background

Primary liver tumours and liver metastases from colorectal carcinoma are the two most common malignant tumours to affect the liver. The liver is second only to the lymph nodes as the most common site for metastatic disease. More than half of the patients with metastatic liver disease will die from metastatic complications. In cryoablation, liquid nitrogen or argon gas is delivered to the liver tumour, guided by ultrasound using a specially designed probe. Ice crystal formation during the rapid freezing process causes destruction of cellular structure and kills the tumour cells.

Objectives

To study the beneficial and harmful effects of cryotherapy compared with no intervention, other ablation methods, or systemic treatments in patients with liver metastases.

Search methods

We searched the Cochrane Hepato-Biliary Group Controlled Trials Register, The Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library*, MEDLINE, EMBASE, Science Citation Index Expanded, LILACS, and CINAHL up to December 2012.

Selection criteria

We included all randomised clinical trials assessing the beneficial and harmful effects of cryotherapy and its comparators, irrespective of the location of the primary tumour.

Data collection and analysis

We extracted relevant information on participant characteristics, interventions, study outcomes, and data on the outcomes for our review, as well as information on the design and methodology of the trials. Bias risk assessment of and data extraction from the trials fulfilling the inclusion criteria were done by one author and checked by a second author.

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Cryotherapy for hepatocellular carcinoma (Review)

Awad T, Thorlund K, Gluud C

THE COCHRANE COLLABORATION®

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[Intervention Review]

Cryotherapy for hepatocellular carcinoma

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ABSTRACT

Background

Hepatocellular carcinoma is the most common primary malignant cancer of the liver. Evidence for the role of cryotherapy in the treatment of hepatocellular carcinoma is controversial.

Objectives

The aim of this review is to evaluate the potential benefits and harms of cryotherapy for the treatment of hepatocellular carcinoma.

Search strategy

We searched *The Cochrane Hepato-Biliary Group Controlled Trials Register,* the *Cochrane Central Register of Controlled Trials (CENTRAL)* in *The Cochrane Library, MEDLINE, EMBASE,* and *LILACS* until June 2009. We identified further studies by searching national and topic-specific databases, bibliographies, conference abstracts, journals, and grey literature. Furthermore, we reviewed the reference lists and contacted the principal authors of the identified studies.

Selection criteria

Randomised clinical trials (irrespective of language or publication status) comparing cryotherapy with or without co-intervention(s) to placebo, no treatment, or other control interventions were considered for the review. Due to the absence of randomised clinical trials, we searched for quasi-randomised studies as well as prospective cohort studies and retrospective cohort studies.

Data collection and analysis

Two authors independently identified and assessed studies for their fulfilment of the inclusion criteria. Dichotomous data were expressed as risk ratio (RR) with 95% confidence intervals (CI). We performed the review following the recommendations of The Cochrane Collaboration.

Main results

We were unable to identify any randomised clinical trials. We were also unable to identify quasi-randomised trials. Instead, we identified two prospective cohort studies and two retrospective cohort studies. However, only one of these studies could be included for the assessment of benefit as the study results were stratified according to both the type of hepatic malignancy (primary or secondary) and the intervention group. This retrospective study compared percutaneous cryotherapy with percutaneous radiofrequency. The remaining

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studies were excluded for the analyses of benefit but included for the assessment of harm. Both severe and non-severe adverse events were reported, but the true nature and extent of harm was difficult to asses.

Authors' conclusions

At present, there is no evidence to recommend or refute cryotherapy for patients with hepatocellular carcinoma. Randomised clinical trials with low-risk of bias may help in defining the role of cryotherapy in the treatment of hepatocellular carcinoma.

PLAIN LANGUAGE SUMMARY

No evidence to evaluate the potential benefits and harms of cryotherapy in the treatment of hepatocellular carcinoma

Liver cell cancer (hepatocellular carcinoma) is the most common primary cancer of the liver. Liver cancer is regarded as the third most common cause of death from cancer. Only a minority of the patients are candidates for liver surgery or liver transplantation. Cold therapy (cryotherapy), where rapid freezing causes cancerous cells' destruction and death, is one of several 'minimally invasive treatment techniques' that have been recently developed. Currently, no large well-planned clinical investigations have been conducted to establish the potential benefits and harms associated with treating liver cell cancer patients with cryotherapy. Nor have any such investigations been undertaken to establish the superiority or inferiority of cryotherapy compared with other treatments for liver cell cancer. At present, there is no evidence to support or refute the alleged and potential benefits of cryotherapy. Large well-designed randomised clinical trials are feasible and are necessary to define the role of cryotherapy in the treatment of hepatocellular carcinoma.

BACKGROUND

In the year 2002, liver cancer represented the sixth most common cancer world-wide in regard to numbers of patients living with cancer (within 5 years of diagnosis) (626,000 patients or 5.7% of new cancer patients) (Parkin 2005). However, due to the poor prognosis of liver cancer, the number of deaths is almost the same (598,000) (Parkin 2005). Liver cancer is regarded as the third most common cause of death from cancer (Parkin 2005). Hepatocellular carcinoma is the most common primary malignant cancer of the liver, accounting for 80% to 90% of all liver cancers. Furthermore, the incidence of hepatocellular carcinoma has been rising in a number of countries (Taylor-Robinson 1997; Deuffic 1998; Stroffolini 1998; El-Serag 2003; Bosch 2004; Parkin 2005).

Hepatocellular carcinoma is known to be associated with a range of risk factors, mainly cirrhosis of any aetiology (eg, hepatitis C and B virus infection, haemochromatosis, alcohol, and primary biliary cirrhosis) (Regan 1989; Tiribelli 1989; Fattovich 2004; Kumar 2007). Aflatoxin B1 is also regarded as a major factor in the development of hepatocellular carcinoma in Southern China and Sub-Saharan Africa (Yeh 1989; Aguilar 1993).

The diagnosis of hepatocellular carcinoma includes radiology, biopsy, and alpha-fetoprotein serology. According to the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL), the type and sequence of the tests used to diagnose hepatocellular carcinoma depend on the size and the location of the cancer (Bruix 2001; Bruix 2005).

Surgical resection, liver transplantation, and percutaneous ablation of the liver cancer are considered to be potentially curative treatments. Despite many attempts in the prevention and screening for hepatocellular carcinoma, only 20% to 30% of patients present at an early stage, and therefore, are amenable to potentially curative treatments (Llovet 2003; Schwartz 2007). Although transarterial embolisation and chemoembolisation are recommended as first-line non-curative therapy (Bruix 2005), a recent Cochrane systematic review suggests that the available evidence is insufficient to support such recommendations (Oliveri 2009).

Percutaneous ablation seems to be a promising treatment option for patients with early stage hepatocellular carcinoma who are not suitable for liver resection or transplantation (Bruix 2005). Cancerous cells destruction can be achieved by several techniques; the injection of chemical substances (eg, ethanol injection), by modifying the temperature (eg, cryotherapy), or radiofrequency ablation. However, the indications and results for percutaneous

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Transversus Abdominis Plane Block to Ameliorate Postoperative Pain Outcomes After Laparoscopic Surgery: A Meta-Analysis of Randomized Controlled Trials

Gildasio S. De Oliveira Jr, MD, MSCI, Lucas Jorge Castro-Alves, MD, Autoun Nader, MD, Mark C. Kendall, MD, and Robert J. McCarthy, PharmD

BACKGROUND: Transversus abdominis plane (TAP) block has been used as a multimodal strategy to optimize postoperative pain outcomes; however, it remains unclear which type of surgical procedures can benefit from the administration of a TAP block. Several studies have examined the effect of the TAP block on postoperative pain outcomes after laparoscopic surgical procedures and generated conflicting results. Our main objective in the current investigation was to evaluate the effect of TAP block on postoperative analgesia outcomes for laparoscopic surgical procedures.

METHODS: A search was performed to identify randomized controlled trials that evaluated the effects of the TAP block compared with an inactive group (placebo or "no treatment") on postoperative pain outcomes in laparoscopic surgical procedures. Primary outcomes included early (0–4 hours) and late (24 hours) postoperative pain at rest and on movement and postoperative opioid consumption (up to 24 hours). Meta-analysis was performed using a random-effects model. Publication bias was evaluated by examining the presence of asymmetric funnel plots using Egger regression test. Meta-regression analysis was performed to establish an association between the local anesthetic dose and the evaluated outcomes.

RESULTS: Ten randomized clinical trials with 633 subjects were included in the analysis. The weighted mean difference (99% confidence interval) of the combined effects favored TAP block over control for pain at rest (≤4 hours, −2.41 [−3.6 to −1.16]) and (at 24 hours, −1.33 [−2.19 to −0.48]) (0–10 numerical scale). Postoperative opioid consumption was decreased in the TAP block group compared with control, weighted mean difference (99% confidence interval) of −5.74 (−8.48 to −2.99) mg morphine IV equivalents. Publication bias was not present in any of the analysis. Preoperative TAP block administration resulted in greater effects on early pain and opioid consumption compared with postoperative administration. Meta-regression analysis revealed an association between local anesthetic dose and the TAP block effect on late pain at rest and postoperative opioid consumption. None of the studies reported symptoms of local anesthetic toxicity.

CONCLUSIONS: TAP block is an effective strategy to improve early and late pain at rest and to reduce opioid consumption after laparoscopic surgical procedures. In contrast, the TAP block was not superior compared with control to reduce early and late pain during movement. Preoperative administration of a TAP block seems to result in greater effects on postoperative pain outcomes. We also detected a local anesthetic dose response on late pain and postoperative opioid consumption. (Anesth Analg 2014;118:454–63)

The advance in surgical techniques and the development of anesthetics with low side effect profiles have enabled the current rapid growth of outpatient surgical procedures.[1,2] Recently, larger procedures such as prostatectomy and gynecological cancer surgeries have been performed laparoscopically allowing a better and faster postoperative recovery to patients.[3,4] Nevertheless, postoperative pain seems to remain a very important factor that can deteriorate the overall quality of recovery after laparoscopic procedures.[5,6]

Transversus abdominis plane (TAP) block has been used as a multimodal strategy to optimize postoperative pain outcomes. However, in recent reviews evaluating the clinical effectiveness of TAP block, the investigators were not able to identify the surgical procedures, dosing, techniques, and timing that provide optimal analgesia after TAP block.[7,8] Since then, evidence suggested an effective role for TAP block to minimize postoperative pain in certain procedures such as cesarean deliveries that do not include

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Reprints will not be available from the authors.

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Review

Analgesic effectiveness of transversus abdominis plane blocks after hysterectomy: a meta-analysis

Rita Champaneria [a,*], Laila Shah [a], James Geoghegan [b], Janesh K. Gupta [c], Jane P. Daniels [a,c]

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ABSTRACT

To determine the effectiveness of transversus abdominis plane blocks in gynecological surgery by systematic review and meta-analysis. Embase, MEDLINE and the Cochrane Library (CENTRAL) bibliographic databases were searched using a Cochrane Library search strategy modified for gynecological surgery. We included randomized controlled trials comparing transversus abdominis plane block with no block or placebo block. We retrieved 681 citations from which we included five published studies (225 randomized participants) which fulfilled our inclusion criteria, and identified a further six ongoing studies. Quality was assessed across six risk of bias domains: randomization sequence generation, allocation concealment, blinding, missing outcome data, selective reporting and other biases. Data were meta-analyzed where possible and presented as mean differences with 95% confidence intervals. Study quality was moderate. Compared with no block or saline placebo, transversus abdominis plane block provided significantly less postoperative pain at rest on a 10 cm visual analog scale at 2 h (mean difference −2.14 cm, 95% confidence interval (CI) −3.57 to −0.71) but not at 24 h postoperatively (−0.52 cm, 95% CI −1.49 to 0.45). Pain on movement showed similar results. Transversus abdominis plane block resulted in significantly less postoperative requirement for morphine use at 24 h (−11.76 mg, 95% CI −18.77 to −4.75) but not at 48 h (−16.01 mg, 95% CI −39.40 to 7.39). Evidence exists for the short-term efficacy (within 24 h) of transversus abdominis plane blocks during hysterectomy in terms of reported pain and morphine consumption, which may not be sustained at 48 h. Updates to this review should be undertaken periodically, and until further robust evidence is available, anesthetists should not rush to adopt this procedure into routine practice.

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1. Introduction

Pain experienced after abdominal surgery, resulting from both the incision made to the abdominal wall and trauma to the internal viscera, can be considerable and is often inadequately controlled [1]. In addition to the negative emotional and physical suffering of the patient, postoperative complications can include respiratory difficulties, delays in mobilization and prolonged hospital stay

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BJA

REVIEW ARTICLES

Transversus abdominis plane block for postoperative analgesia after Caesarean delivery performed under spinal anaesthesia? A systematic review and meta-analysis

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Editor's key points

- The utility of transversus abdominis plane (TAP) block in Caesarean delivery was assessed by analysing results of previous studies.
- TAP block reduced i.v. morphine consumption and pain scores in the first day after surgery.
- TAP block can provide effective analgesia after Caesarean delivery when intrathecal morphine has not been used.

Summary. The transversus abdominis plane (TAP) block is a field block that provides postoperative analgesia for abdominal surgery. Its analgesic utility after Caesarean delivery (CD) remains controversial. This systematic review and meta-analysis examines whether TAP block can reduce i.v. morphine consumption in the first 24 h after CD. The authors retrieved randomized controlled trials comparing TAP block with placebo in CD. Postoperative i.v. morphine consumption during the first 24 h was selected as a primary outcome. Pain scores and both maternal and neonatal opioid-related side-effects were secondary outcomes. Where possible, meta-analytic techniques and random effects modelling were used to combine data. Trials were stratified based on whether or not spinal morphine was used as part of the analgesic regimen. Five trials including 312 patients were identified. TAP block reduced the mean 24 h i.v. morphine consumption by 24 mg [95% confidence interval (CI) −39.65 to −7.78] when spinal morphine was not used. TAP block also reduced visual analogue scale pain scores (10 cm line where 0 cm, no pain, and 10 cm, worst pain) by 0.8 cm (95% CI −1.53 to −0.05, P=0.01), and decreased the incidence of opioid-related side-effects. The differences in primary and secondary outcomes were not significant when spinal morphine was used. TAP block provides superior analgesia compared with placebo and can reduce the first 24 h morphine consumption in the setting of a multimodal analgesic regimen that excludes spinal morphine. TAP block can provide effective analgesia when spinal morphine is contraindicated or not used.

Keywords: acute pain, novel techniques; anaesthesia, obstetric; anaesthetic blocks, regional; analgesia, postoperative; regional blockade

Inadequate postoperative pain relief after Caesarean delivery (CD) can negatively impact ambulation, breastfeeding, and even maternal bonding,[1] while effective analgesia improves the amount of breastfeeding and infant weight gain.[2] Neuraxial anaesthesia has become the anaesthetic technique of choice in CD because of its safety and reduction in maternal mortality.[3]

The transversus abdominis plane (TAP) block, a field block[4] whose analgesic efficacy in several abdominal surgeries has been confirmed,[5–7] has also been proposed for postoperative analgesia in parturients undergoing elective CD under spinal anaesthesia.[8] However, the analgesic utility of TAP block remains controversial; some trials comparing it with placebo reported significant advantages,[8 9] while others found no analgesic benefit.[10 11] Reviews examining the analgesic effects of TAP block in various surgeries have not provided definitive answers regarding the specific role of TAP block in CD. A Cochrane review examining the efficacy of TAP block in abdominal surgeries excluded CD.[12] A recent meta-analysis supporting TAP block for its effective pain relief included only one trial in the setting of CD.[13] A 2012 qualitative systematic review[14] examined the role of TAP block across all abdominal surgeries and raised questions about its role in the setting of multimodal analgesia but stopped short of conducting any further analysis specific to CD. The purpose of this systematic review was to determine whether or not TAP block is effective in providing pain relief after CD. The primary outcome was morphine consumption in the first 24 h, an important issue for the breastfeeding woman.

Methods

The authors followed the PRISMA[15] recommendations in preparing this review.

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Risk Factors Used in the Modified Gail Model (BSCR-B)
Assessment Category Definitions (BSCR-C)

Clinical Trials: NCCN believes that the best management for any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

To find clinical trials online at NCCN member institutions, click here: nccn.org/clinical_trials/physician.html.

NCCN Categories of Evidence and Consensus: All recommendations are Category 2A unless otherwise specified.

See NCCN Categories of Evidence and Consensus.

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Updates in Version 1.2014 of the NCCN Guidelines for Breast Cancer Screening from Version 2.2013 include:

BSCR-1
- **4th column, 3rd bullet modified: "Lobular carcinoma in situ (LCIS) or** *ADH/ALH*" **(Also for BSCR-2).**
- **5th bullet modified: "Prior thoracic RT** *for patients younger than* **30 y (eg, mantle)."**
- **"Pedigree suggestive of or known genetic predisposition": prior link to BSCR-3 deleted and replaced with a link to the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian.**
- **"Adequate breast exams include the following: upright and supine position during exam, appearance of breast, and palpation of all components of the breast" is a new footnote to the page corresponding to 6th column, 1st bullet, "Clinical breast exam every 1-3 y."**
- **For women with average-risk (≥40 y) screening mammography is now a category 1 recommendation.**
- **"Screening" was added before mammogram for clarification. (Also for BSCR-2).**

BSCR-2
- **Footnote "j": "Randomized trials comparing clinical breast exam versus no screening have not been performed. Rationale for recommending clinical breast exam every 6-12 mo is the concern for interval breast cancers," is new to the page and corresponds to,** *"Annual screening mammogram + clinical breast exam every 6-12 mo."*
- **"Consider beginning at age of first-degree relative's diagnosis of cancer if younger than age 30" is a new footnote to the page and corresponds to the 2nd column, 4th bullet, 1st sub-bullet,** *"beginning at age 30 y."*
- **Footnote "l": High-quality breast MRI limitations include having: a need for a dedicated breast coil, the ability to perform biopsy under MRI guidance, experienced radiologists in breast MRI, and regional availability. "Breast MRI is performed preferably days 7-15 of menstrual cycle for premenopausal women.** *MRI should be integrated with other breast imaging modalities,"* **is new to the page and corresponds to the 7th bullet which has been modified:** *Recommend* ~~**consider**~~ **annual breast MRI.**
- **8th bullet is new to the page: "Referral to genetic counseling if not already done."**

BSCR-3
- **3rd column, 5th bullet,** *Skin ulcers* **is new to the page.**

BSCR-4
- **"***Diagnostic***" was added before mammogram throughout the guidelines for clarity.**
- **2nd column, under Initial Evaluation modified:** *Diagnostic mammogram ± ultrasound as needed.*
- **4th column,** ~~**Ultrasound**~~ **was deleted after BI-RADS® category 1-3.**

BSCR-5
- **5th column, top branch off of "Increase in size," added "***Tissue biopsy* **before (See BSCR-7)"**
- **Modified footnote "u": FNA and core (needle or vacuum-assisted) biopsy are both valuable. FNA requires cytologic expertise. Surgical excision is appropriate if unable to perform core needle biopsy** *or if strongly desired by patient.*
- **The following reference was deleted:** ~~**Stavros A, Thickman D, Rapp C, et al. Solid breast nodules: Use of sonography to distinguish between benign and malignant lesions. Radiology 1995;196:123-124.**~~
- ~~**Routine**~~ **was deleted before Screening (See BSCR-1) throughout the guidelines.**

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Updates in Version 1.2014 of the NCCN Guidelines for Breast Cancer Screening from Version 2.2013 include:

BSCR-10

- **2nd column, top branch off Palpable mass age <30 y was modified: Ultrasound (preferred) ± diagnostic mammogram ~~(preferred)~~ "Simple cyst" BI-RADS® category 2 we previously sent the reader to ~~Ultrasound Findings~~, now** *Screening* **(See BSCR-1).**

BSCR-11

- **4th column under Follow-up Evaluation, modified: Observe every 3-6 mo ± imaging for 1-2 y to assess stability** *if low clinical suspicion.*

BSCR-12

- **3rd column, 2nd branch off Age ≥40 y, 1st bullet modified:** *Diagnostic* **mammogram,** *if not done recently.*
- **3rd column, bottom branch modified: Age ≥30 y** *Diagnostic* **mammogram + ultrasound.**

BSCR-14

- **2nd column, bottom branch off Skin changes modified: Clinical suspicion of Paget's disease** *or other manifestations of Breast cancer.*
- **3rd bullet is new to the page:** *Skin ulceration.*

BSCR-A

- **8th bullet modified to include:** *Important outcomes are not yet established for supplemental screening; some states have passed legislation mandating patient notification of breast density.*
- **9th bullet modified: Early studies show promise for tomosynthesis mammography. ~~Currently, there is insufficient evidence to recommend routine use for screening or diagnosis at this time.~~** *Two large trials showing a combined use of digital mammograpy and tomosynthesis resulted in improved cancer detection and decreased call back rates; of note, this is double the dose of radiation and is a factor in recommending this modality. Definitive studies are still pending.*

BSCR-C

- **Global update for BI-RADS® Mammography and Ultrasound to the ACR-BI-RADS®--5th Edition.**

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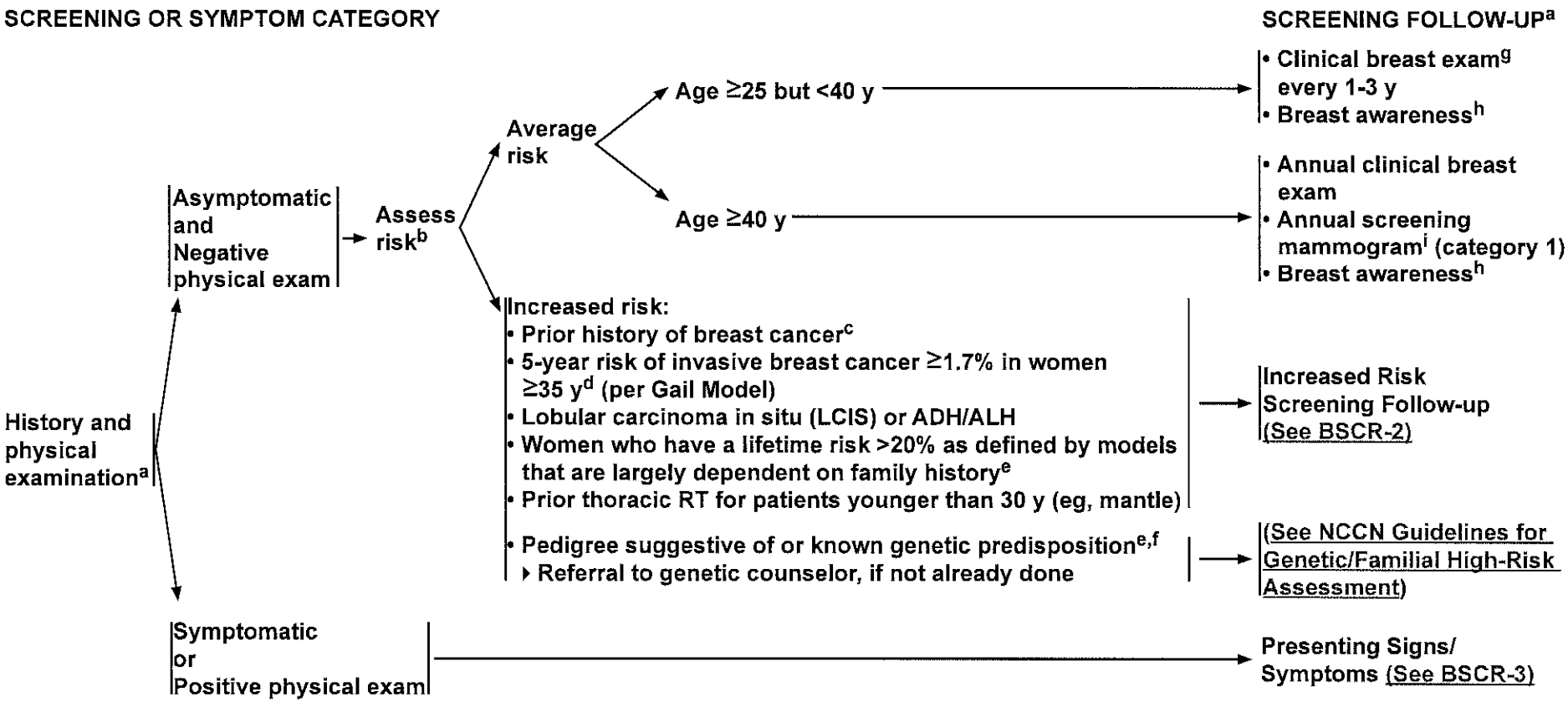

[a]See Breast Screening Considerations (BSCR-A).
[b]Refer to the NCCN Guidelines for Breast Cancer Risk Reduction for a detailed qualitative and quantitative assessment.
[c]See NCCN Guidelines for Breast Cancer - Surveillance Section.
[d]See Risk Factors Used in the Modified Gail Model (BSCR-B).
[e]Risk models that are largely dependent on family history (eg, Claus, BRCAPRO, BOADICEA, Tyrer-Cuzick). See NCCN Guidelines for Breast Cancer Risk Reduction.
[f]There is variation in recommendations for initiation of screening for different genetic syndromes. See NCCN Guidelines for Genetic/Familial High-Risk Assessment.
[g]Adequate breast exams include the following: upright and supine position during exam, appearance of breast, and palpation of all components of the breast.
[h]Women should be familiar with their breasts and promptly report changes to their health care provider. Periodic, consistent breast self exam (BSE) may facilitate breast self awareness. Premenopausal women may find BSE most informative when performed at the end of menses.
[i]See Mammographic Evaluation (BSCR-15).

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

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SCREENING OR SYMPTOM CATEGORY	SCREENING FOLLOW-UP
Increased Risk:	
Prior history of breast cancer	→ See NCCN Guidelines for Breast Cancer - Surveillance Section
Women ≥35 y with 5-year risk of invasive breast cancer ≥1.7%[d] OR LCIS (begin screening at diagnosis) OR ADH/ALH	→ • Annual screening mammogram[i] + clinical breast exam every 6-12 mo[j] • Breast awareness[h] • Consider risk reduction strategies (See NCCN Guidelines for Breast Cancer Risk Reduction)
Women who have a lifetime risk >20% as defined by models that are largely dependent on family history[e]	→ • Annual screening mammogram[i] + clinical breast exam every 6-12 mo[j] ▸ beginning at age 30 y[k] • Breast awareness[h] • Consider risk reduction strategies (See NCCN Guidelines for Breast Cancer Risk Reduction) • Recommend annual breast MRI[l] ▸ beginning at age 30 y • Referral to genetic counseling if not already done
Prior thoracic RT between the ages of 10 and 30 y: Age <25 y	→ • Annual clinical breast exam ▸ beginning 8 to 10 y after RT • Breast awareness[h]
Prior thoracic RT between the ages of 10 and 30 y: Age ≥25 y	→ • Annual screening mammogram[i] + clinical breast exam every 6-12 mo[j] ▸ Begin 8-10 y after RT or age 40, whichever comes first • Recommend annual breast MRI[l] • Breast awareness[h]

[d] See Risk Factors Used in the Modified Gail Model (BSCR-B).

[e] Risk models that are largely dependent on family history (eg, Claus, BRCAPRO, BOADICEA, Tyrer-Cuzick). See NCCN Guidelines for Breast Cancer Risk Reduction.

[h] Women should be familiar with their breasts and promptly report changes to their health care provider. Periodic, consistent breast self exam (BSE) may facilitate breast self awareness. Premenopausal women may find BSE most informative when performed at the end of menses.

[i] See Mammographic Evaluation (BSCR-15).

[j] Randomized trials comparing clinical breast exam versus no screening have not been performed. Rationale for recommending clinical breast exam every 6-12 mo is the concern for interval breast cancers.

[k] Consider beginning at age of first-degree relative's diagnosis of cancer if younger than age 30.

[l] High-quality breast MRI limitations include having: a need for a dedicated breast coil, the ability to perform biopsy under MRI guidance, experienced radiologists in breast MRI, and regional availability. Breast MRI is performed preferably days 7-15 of menstrual cycle for premenopausal women. MRI should be integrated with other breast imaging modalities.

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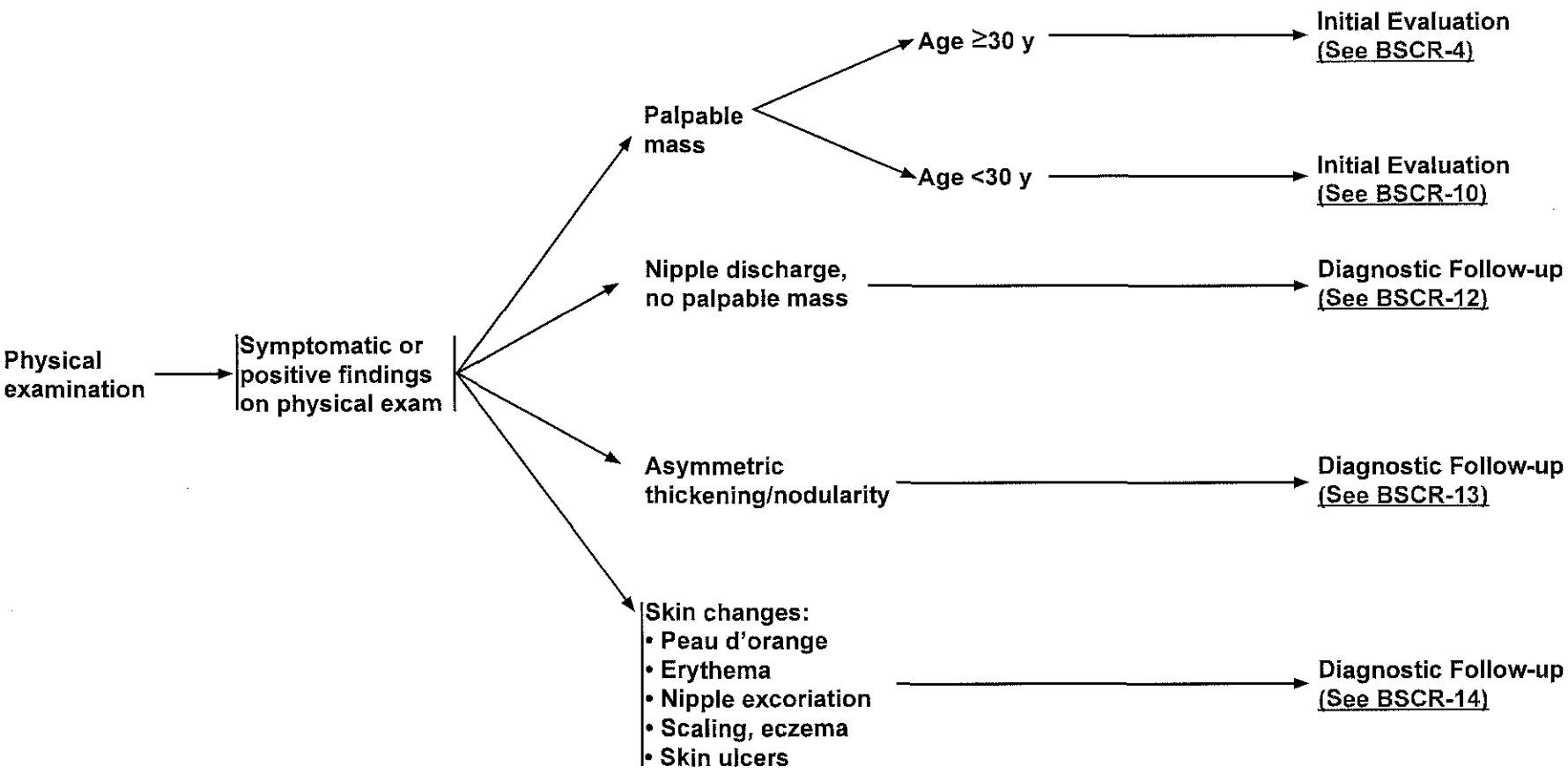

Note: All recommendations are category 2A unless otherwise indicated.
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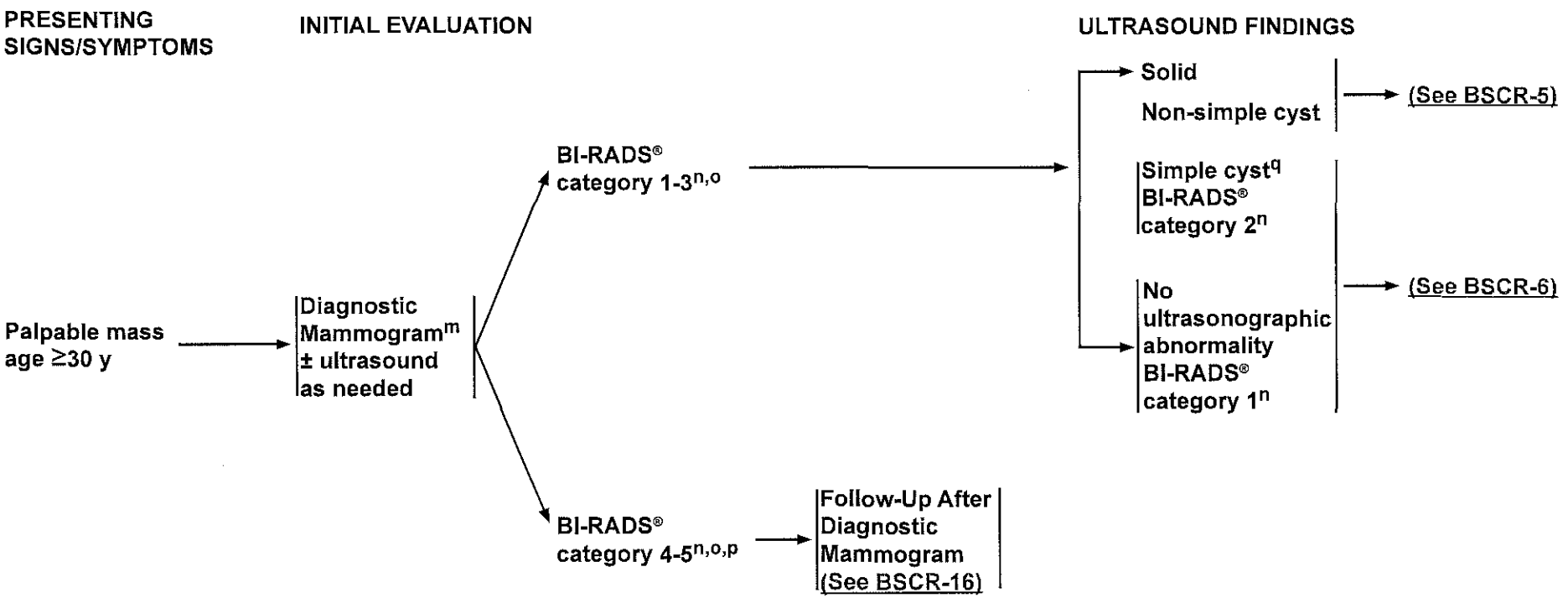

[m]There are some clinical circumstances such as mass with low clinical suspicion or suspected simple cyst, in which ultrasound would be preferred and may suffice for women 30-39 years of age. See Discussion section.
[n]See Assessment Category Definitions (BSCR-C).
[o]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).
[p]Assess geographic correlation between clinical and imaging findings. If there is a lack of correlation, return to Category 1-3 for further workup of palpable lesion. If imaging findings correlate with the palpable finding, workup of the imaging problem will answer the palpable problem.
[q]Concordance is needed between clinical exam and ultrasound results. Consider therapeutic aspiration for persistent clinical symptoms.

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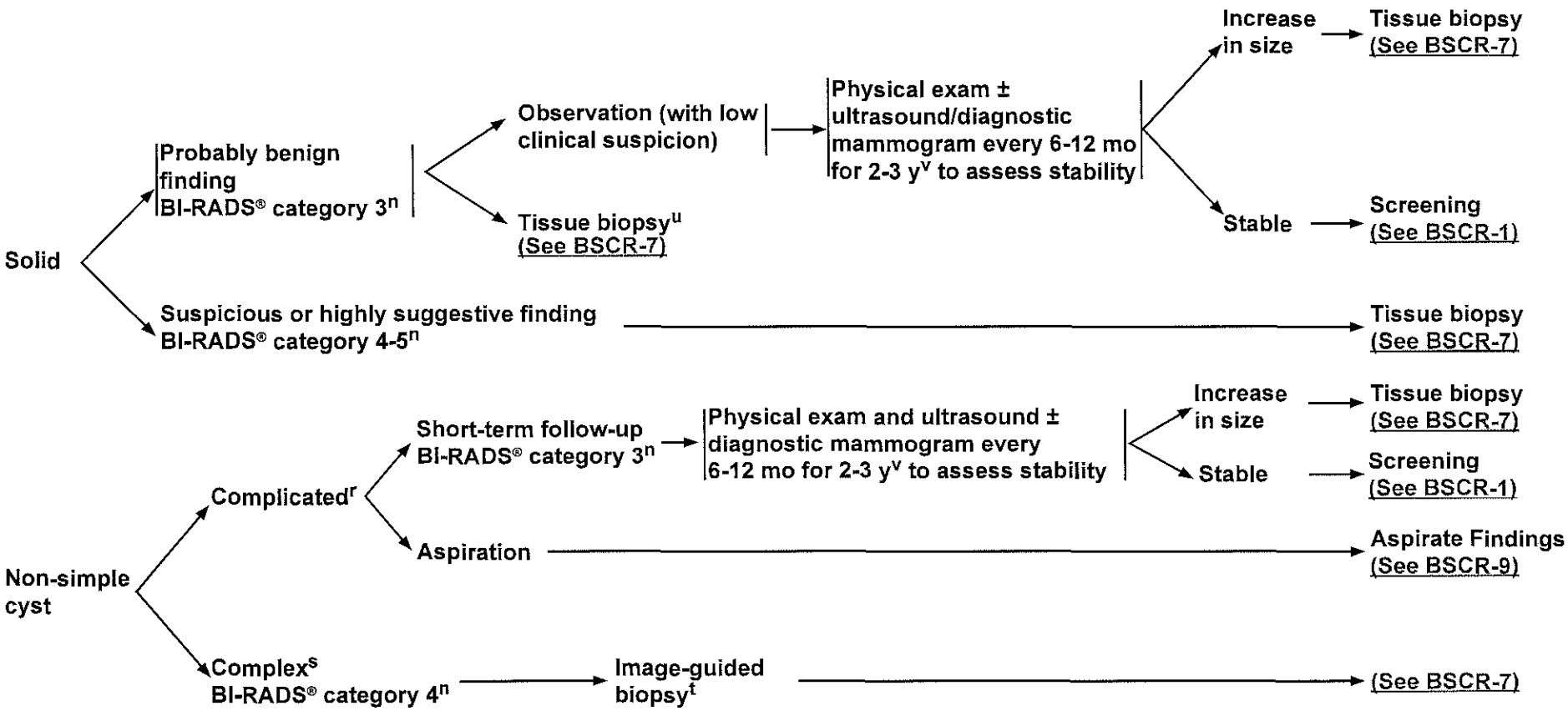
ULTRASOUND FINDINGS/PALPABLE MASS



[n]See Assessment Category Definitions (BSCR-C).
[r]Round, circumscribed mass containing low-level echoes without vascular flow, fulfilling most but not all criteria for simple cyst.
[s]A complex cyst has both cystic and solid components.
[t]Surgical excision if image-guided/core needle biopsy is not possible.
[u]FNA and core (needle or vacuum-assisted) biopsy are both valuable. FNA requires cytologic expertise. Surgical excision is appropriate if unable to perform core needle biopsy or if strongly desired by patient.
[v]There may be variability on the follow-up interval based on the level of suspicion.

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ULTRASOUND FINDINGS/PALPABLE MASS

Simple cyst[q]
BI-RADS® category 2[n] → **Screening** (See BSCR-1)

For age ≥30 y
No ultrasonographic abnormality
BI-RADS® category 1[n] →

Observe (for low clinical suspicion) every 3-6 mo ± imaging for 1-2 y to assess stability
- **Progression or enlargement on clinical exam** → (See BSCR-7)
- **Stable** → **Screening** (See BSCR-1)

or

Tissue biopsy → (See BSCR-7)

[n]See Assessment Category Definitions (BSCR-C).
[q]Concordance is needed between clinical exam and ultrasound results. Consider therapeutic aspiration for persistent clinical symptoms.

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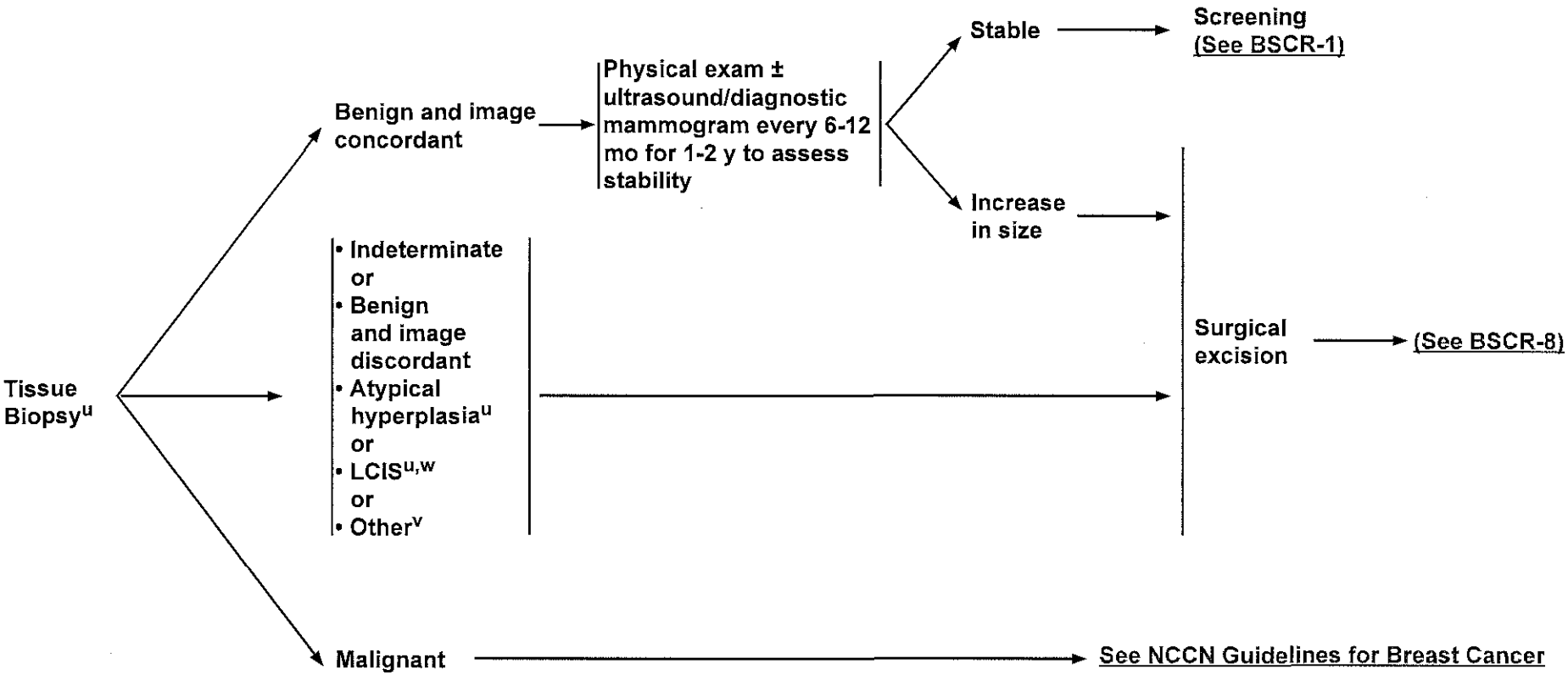
PALPABLE MASS

FOLLOW-UP EVALUATION



[u]FNA and core (needle or vacuum-assisted) biopsy are both valuable. FNA requires cytologic expertise. Surgical excision is appropriate if unable to perform core needle biopsy or if strongly desired by patient.
[w]Select patients may be suitable for monitoring in lieu of surgical excision (eg, ALH, LCIS, papillomas, fibroepithelial lesions, radial scars).
[x]Other histologies that may require additional tissue: mucin-producing lesions, potential phyllodes tumor, papillary lesions, radial scar, or histologies of concern to pathologist.
[y]Multifocal/extensive LCIS involving >4 terminal ductal lobular units on a core biopsy may be associated with increased risk of invasive cancer on surgical excision.

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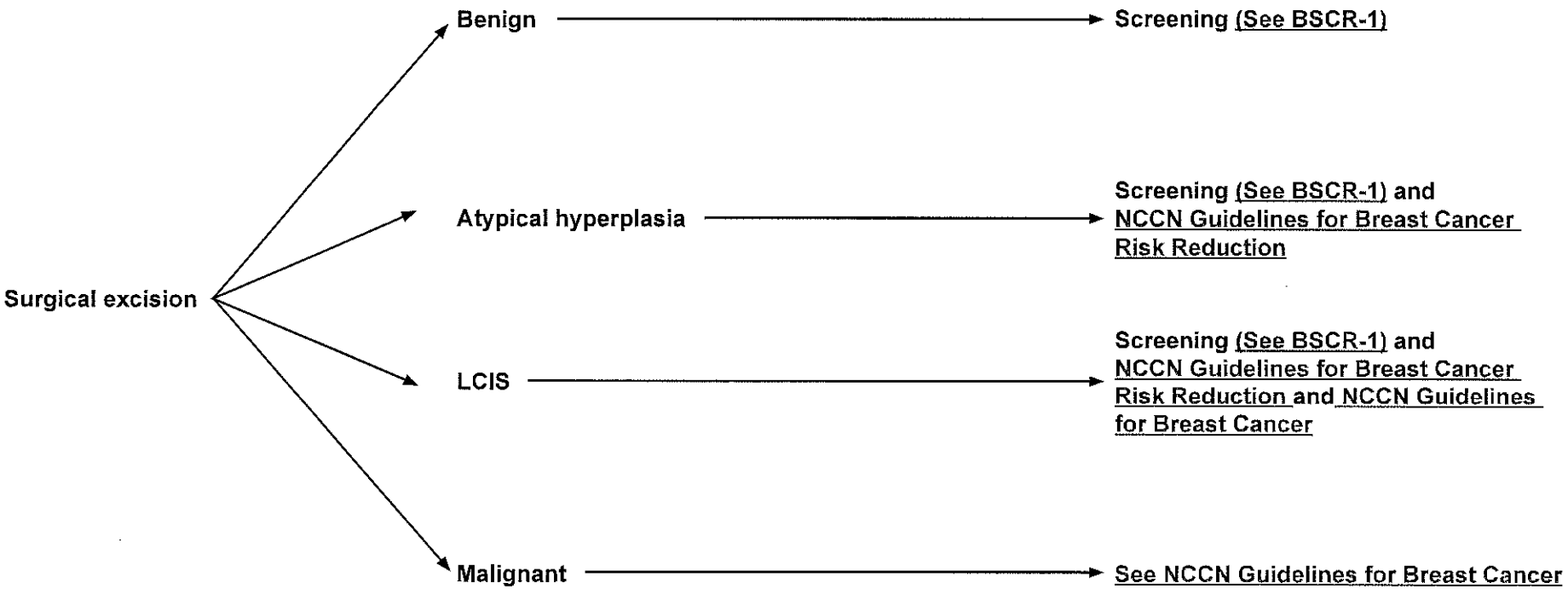
FOLLOW-UP EVALUATION



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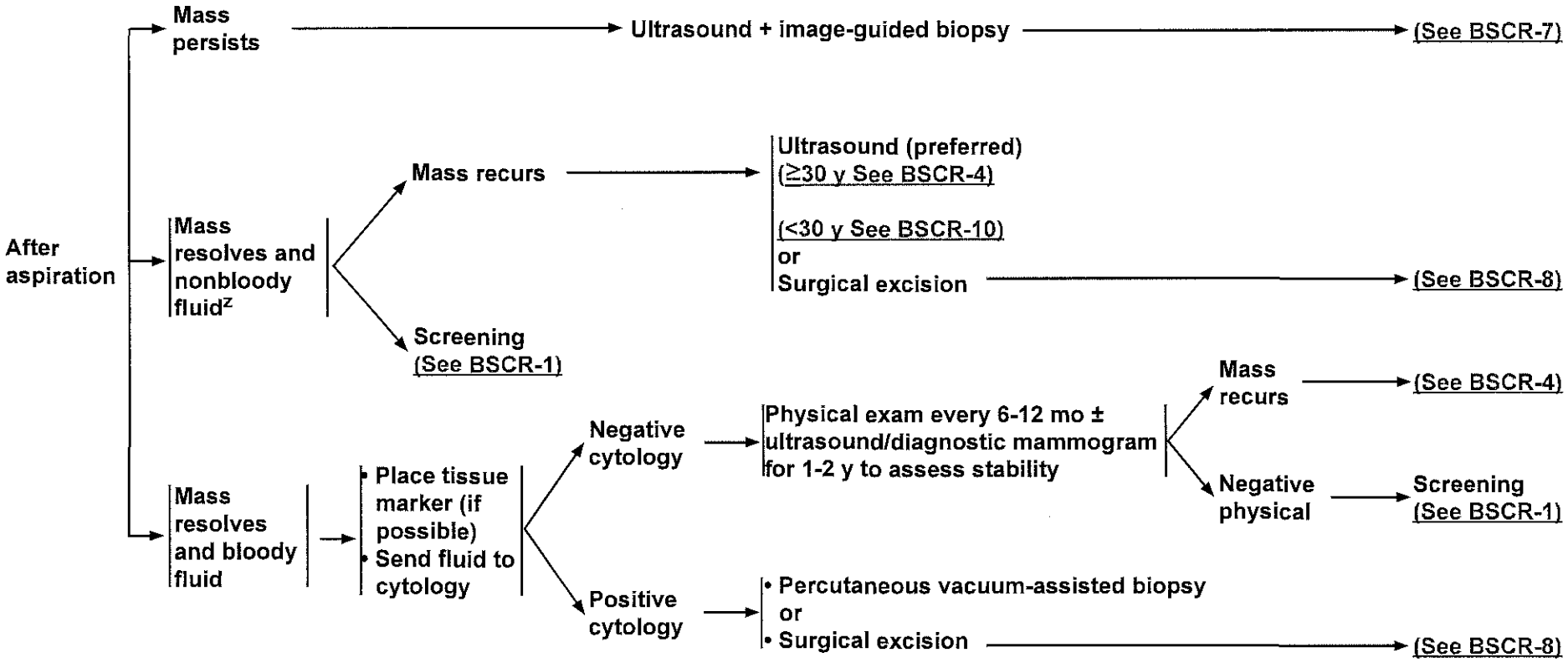
ASPIRATE FINDINGS/PALPABLE MASS | **FOLLOW-UP EVALUATION**



[z]Routine cytology not recommended.

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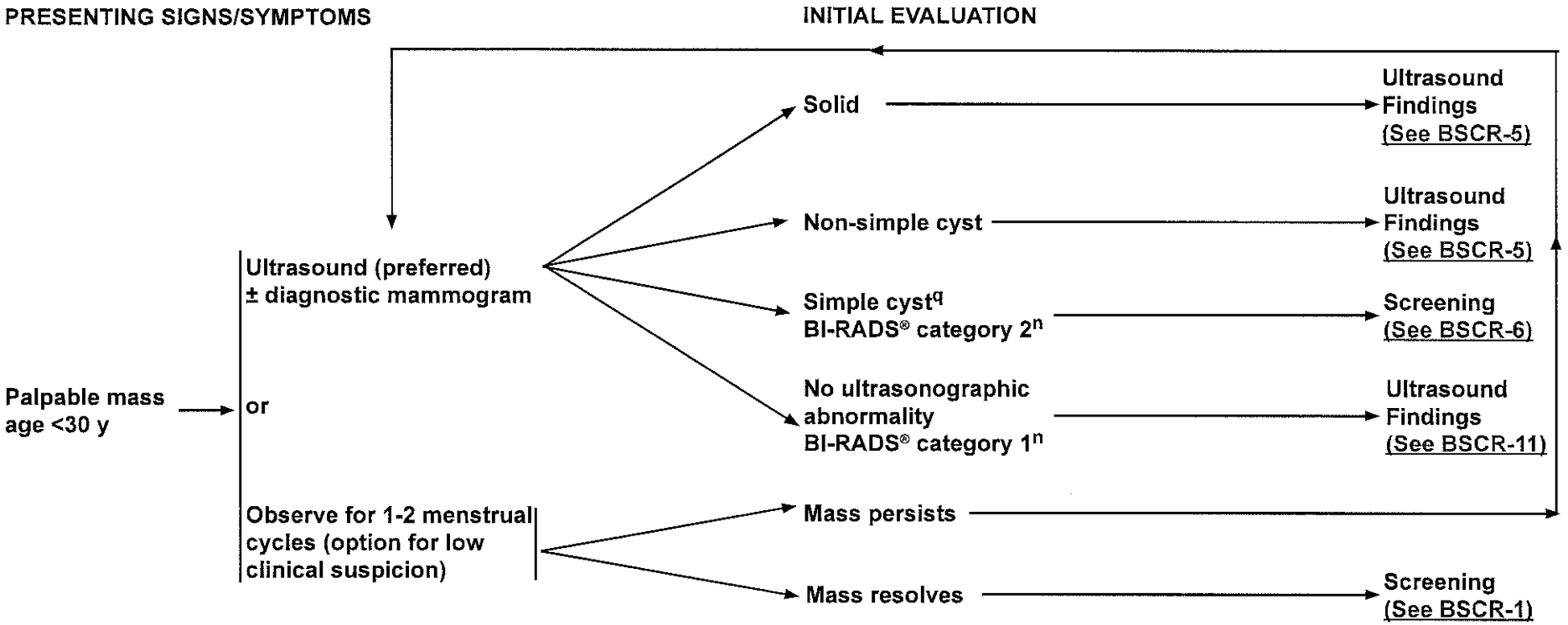

[n]See Assessment Category Definitions (BSCR-C).
[q]Concordance is needed between clinical exam and ultrasound results. Consider therapeutic aspiration for persistent clinical symptoms.

Note: All recommendations are category 2A unless otherwise indicated.
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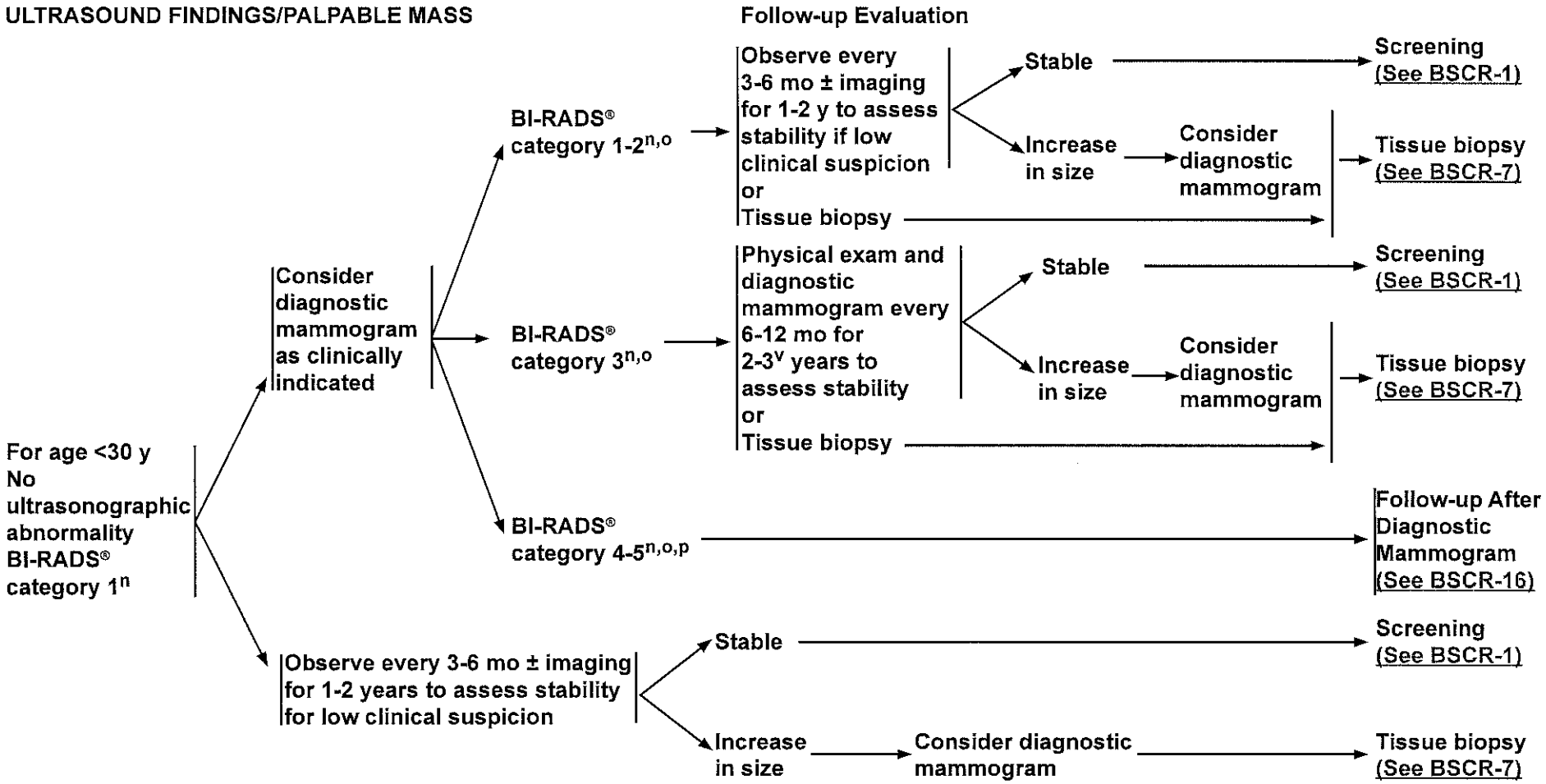

[n] See Assessment Category Definitions (BSCR-C).

[o] Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

[p] Assess geographic correlation between clinical and imaging findings. If there is a lack of correlation, return to Category 1-3 for further workup of palpable lesion. If imaging findings correlate with the palpable finding, workup of the imaging problem will answer the palpable problem.

[v] There may be variability on the follow-up interval based on the level of suspicion.

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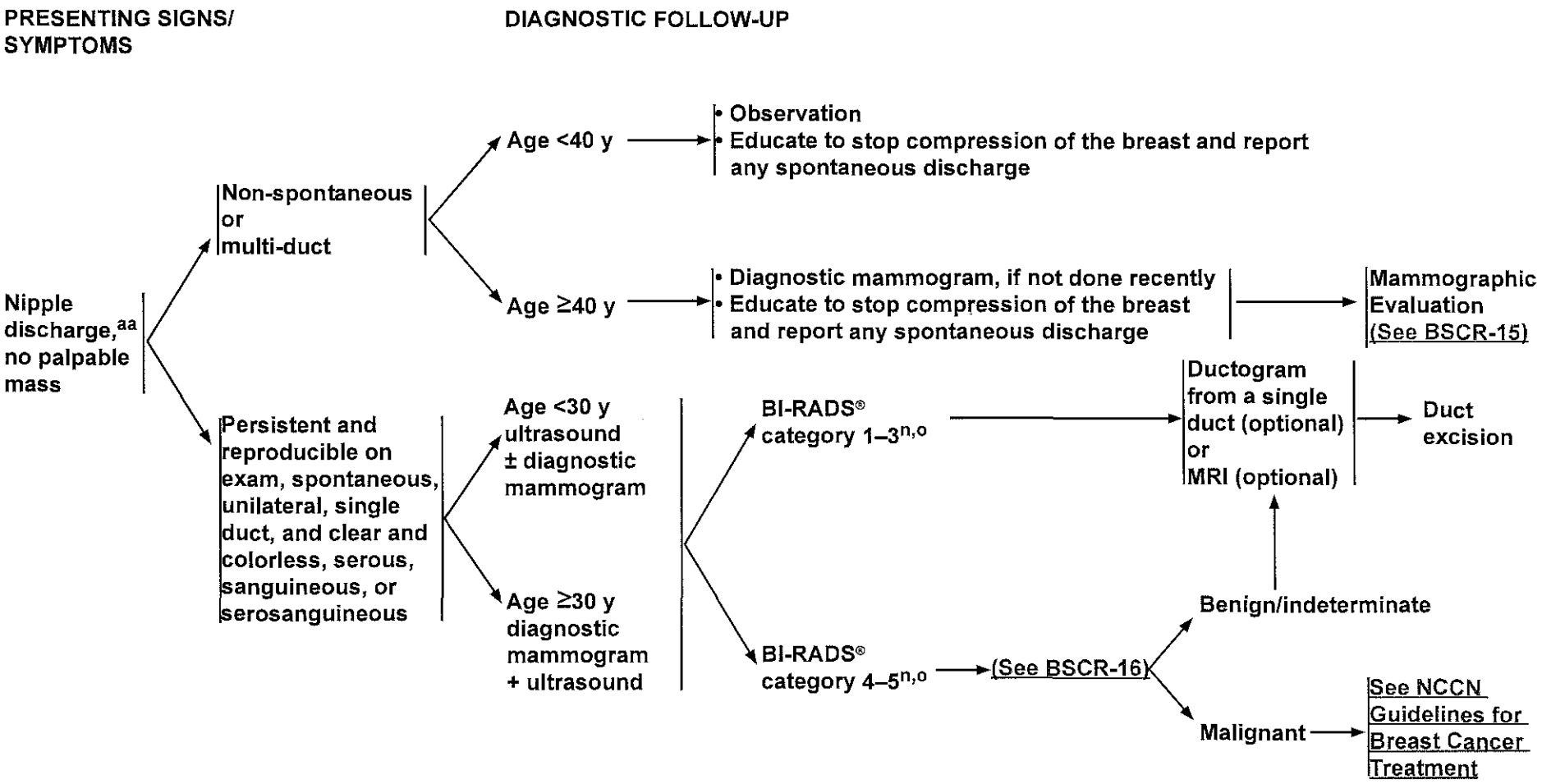

[n]See Assessment Category Definitions (BSCR-C).
[o]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).
[aa]A list of drugs that can cause nipple discharge (not all-inclusive): Psychoactive drugs, antihypertensive medications, opiates, oral contraceptives, and estrogen.

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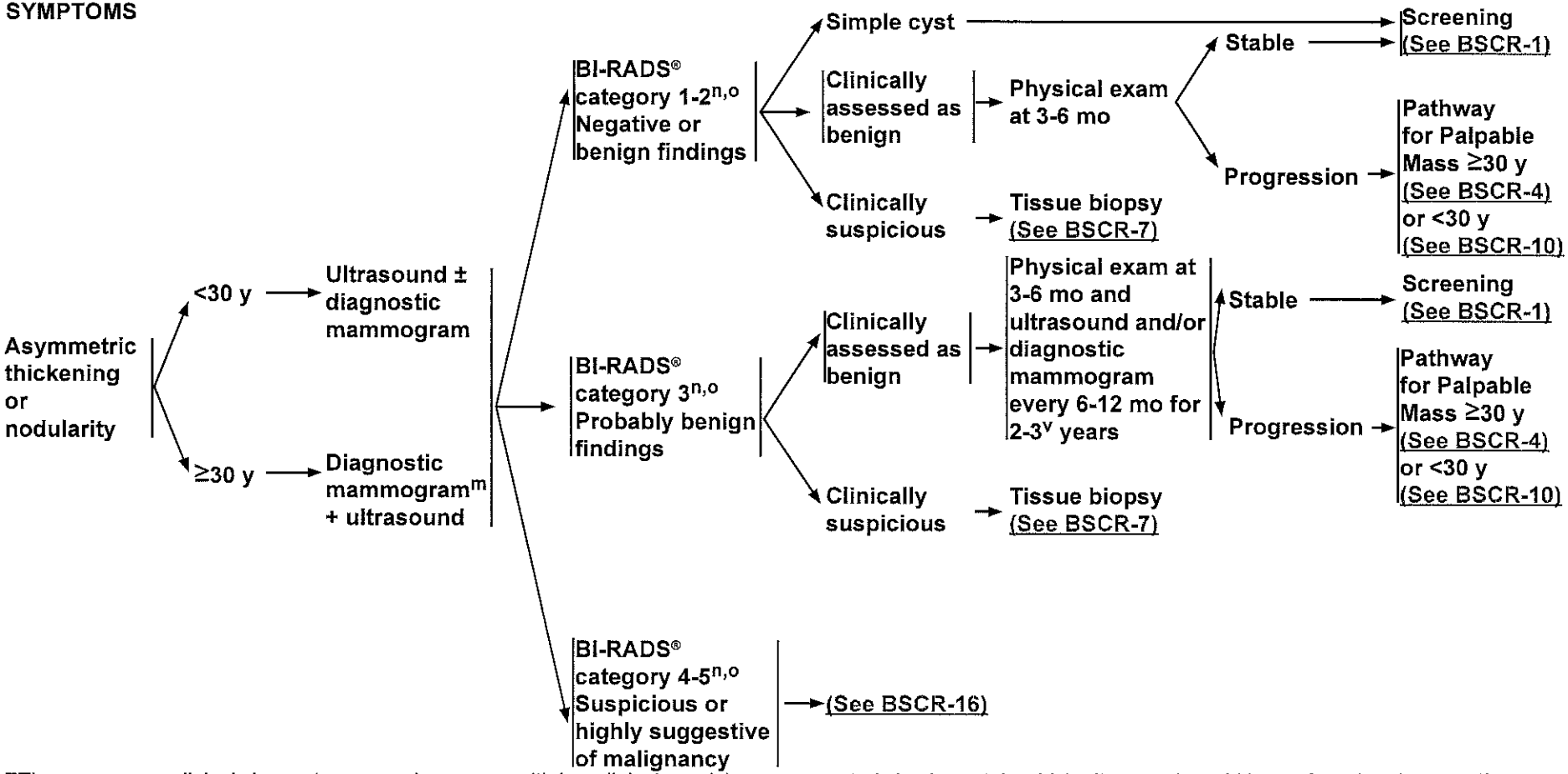

[m]There are some clinical circumstances such as mass with low clinical suspicion or suspected simple cyst, in which ultrasound would be preferred and may suffice for women 30-39 years of age. See Discussion section.

[n]See Assessment Category Definitions (BSCR-C).

[o]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

[v]There may be variability on the follow-up interval based on the level of suspicion.

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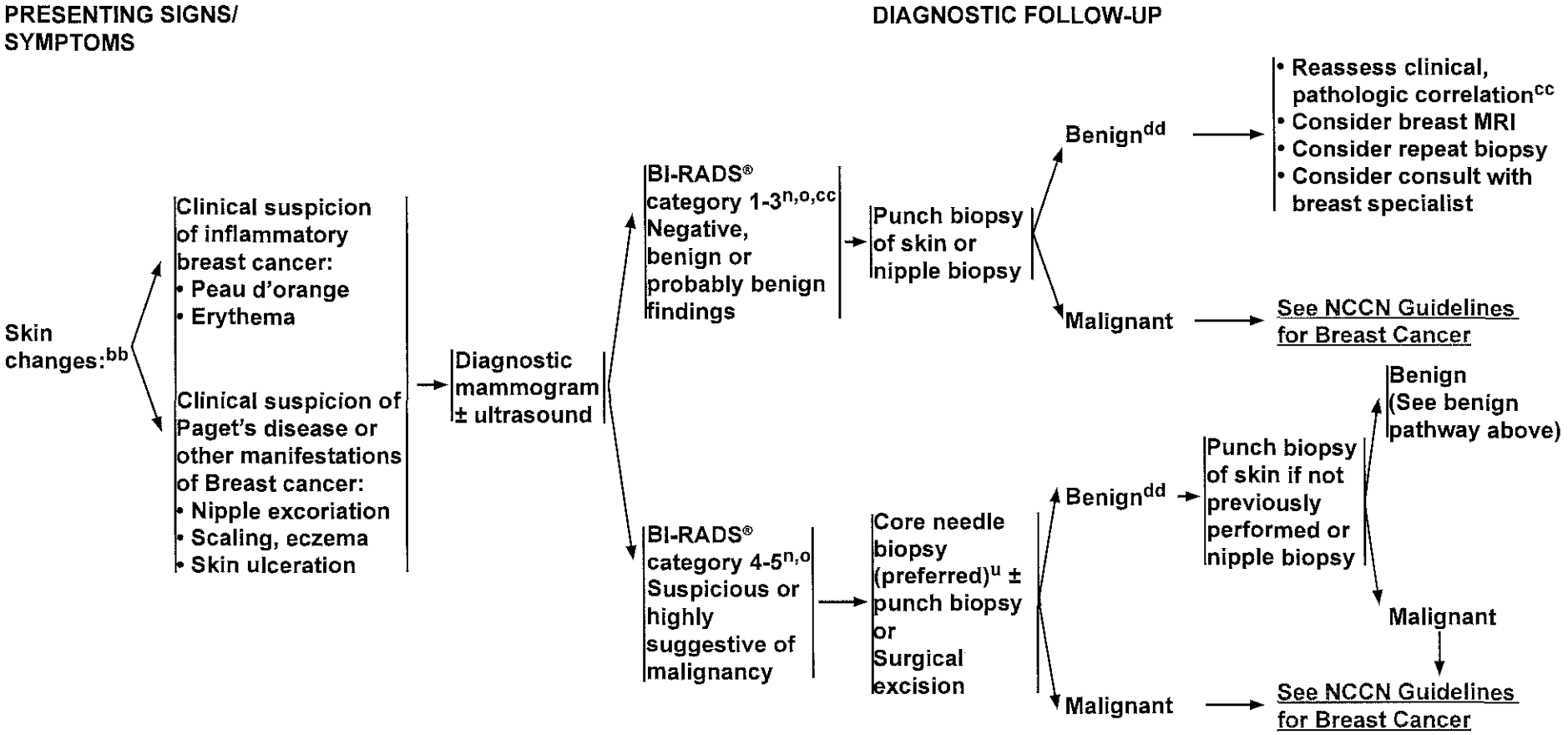

[n] See Assessment Category Definitions (BSCR-C).
[o] Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).
[u] FNA and core (needle or vacuum-assisted) biopsy are both valuable. FNA requires cytologic expertise. Surgical excision is appropriate if unable to perform core needle biopsy or if strongly desired by patient.
[bb] This may represent serious disease of the breast and needs evaluation.
[cc] If clinically of low suspicion, a short trial (7-10 days) of antibiotics for mastitis may be indicated.
[dd] A benign skin punch biopsy in a patient with a clinical suspicion of inflammatory breast cancer does not rule out malignancy. Further evaluation is recommended.

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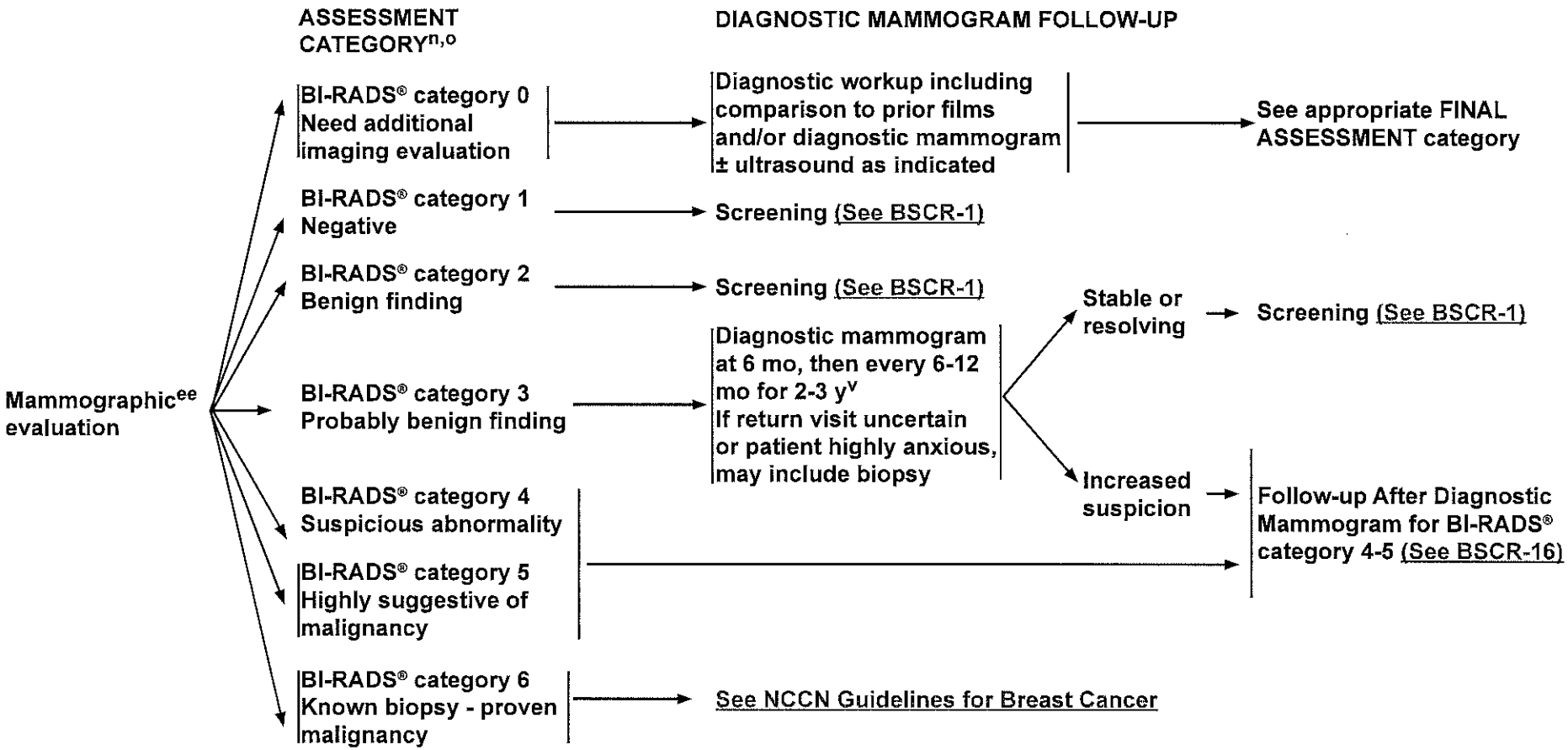

[n]See Assessment Category Definitions (BSCR-C).
[o]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards:Final Rule. Federal Register.1997;62:55988).
[v]There may be variability on the follow-up interval based on the level of suspicion.
[ee]Mammogram considerations: Specify if mammogram is screening or diagnostic and comparison should be made with prior noncopied films (original films), if obtainable.

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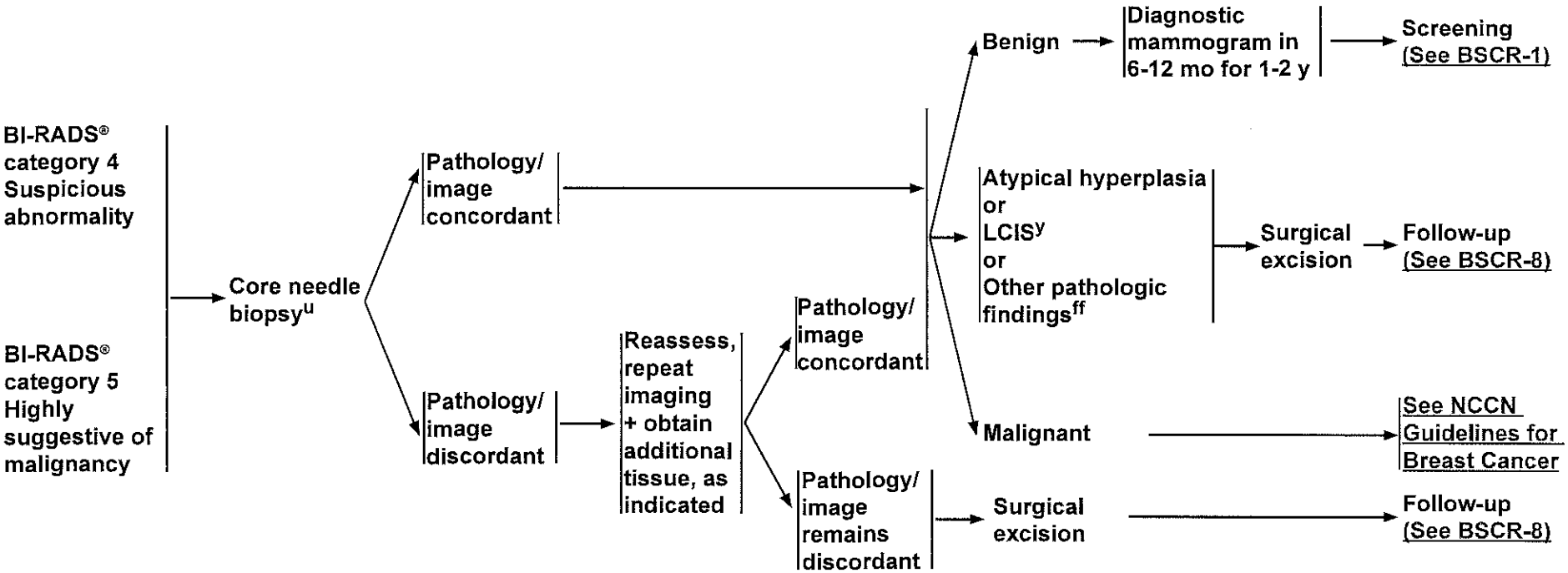
ASSESSMENT CATEGORY[n,o]

FOLLOW-UP AFTER DIAGNOSTIC MAMMOGRAM



[n] See Assessment Category Definitions (BSCR-C).

[o] Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register.1997;62:55988).

[u] FNA and core (needle or vacuum-assisted) biopsy are both valuable. FNA requires cytologic expertise. Surgical excision is appropriate if unable to perform core needle biopsy.

[y] Multifocal/extensive LCIS involving >4 terminal ductal lobular units on a core biopsy may be associated with increased risk of invasive cancer on surgical excision.

[ff] Other histologies that may require additional tissue: mucin-producing lesions, potential phyllodes tumor, papillary lesions, radial scar, or other histologies of concern to pathologist.

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BREAST SCREENING CONSIDERATIONS

- **Women should be counseled regarding potential benefits, risks, and limitations of breast screening.**
- **Thorough clinical breast exam involves inspection and palpation of all breast tissue, including lymph node basins.**
- **Consider severe comorbid conditions limiting life expectancy and whether therapeutic interventions are planned.**
- **Upper age limit for screening is not yet established.**
- **Current evidence does not support the routine use of breast scintigraphy (eg, sestamibi scan) or ductal lavage as screening procedures.**
- **There are several studies supporting the use of ultrasound for breast cancer screening as an adjunct to screening mammography for high-risk women with dense breast tissue.[1]**
- **Digital mammography appears to benefit young women and women with dense breasts.[2]**
- **Dense breasts limit the sensitivity of mammography. Dense breasts are associated with an increased risk for breast cancer, but there is insufficient evidence to support routine supplemental screening in women with dense breasts and no other risk factors. Important outcomes are not yet established for supplemental screening; some states have passed legislation mandating patient notification of breast density.**
- **Early studies show promise for tomosynthesis mammography. Two large trials showing a combined use of digital mammography and tomosynthesis resulted in improved cancer detection and decreased call back rates; of note, this is double the dose of radiation and is a factor in recommending this modality. Definitive studies are still pending.**

[1]Berg WA, Blume JD, Cormack JB, et al. Combined screening with ultrasound and mammography vs. mammography alone in women at elevated risk of breast cancer. JAMA 2008,299(18):2151-2163.

[2]Pisano ED, Gatsonis C, Hendrick E et al for the Digital Mammographic Imaging Screening Trial (DMIST) Investigators. Diagnostic performance of digital versus film mammography for breast cancer screening. N Engl J Med 2005;353:1773-1783.

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BREAST SCREENING CONSIDERATIONS

RECOMMENDATIONS FOR BREAST MRI SCREENING AS AN ADJUNCT TO MAMMOGRAPHY[3,4]

Recommend Annual MRI Screening (Based on Evidence):[5]
- **BRCA mutation**
- **First-degree relative of BRCA carrier, but untested**
- **Lifetime risk 20% or greater, as defined by BRCAPRO or other models that are largely dependent on family history (See BSCR-2)**

Recommend Annual MRI Screening (Based on Expert Consensus Opinion):[6]
- **Radiation to chest between age 10 and 30 years**
- **Li-Fraumeni syndrome6 and first-degree relatives**
- **Cowden and Bannayan-Riley-Ruvalcaba syndromes[7] and first-degree relatives**

Insufficient Evidence to Recommend for or Against MRI Screening:[8]
- **Lifetime risk 15%–20%, as defined by BRCAPRO or other models that are largely dependent on family history**
- **LCIS or atypical lobular hyperplasia (ALH)**
- **Atypical ductal hyperplasia (ADH)**
- **Heterogeneously or extremely dense breast on mammography**
- **Women with a personal history of breast cancer,[9] including ductal carcinoma in situ (DCIS)**

Recommend Against MRI Screening (Based on Expert Consensus Opinion):
- **Women at <15% lifetime risk**

[3]Adapted with permission from John Wiley and Sons. Copyright ©2007 American Cancer Society. Saslow D, Boetes C, Burke W, et al. American Cancer Society Guidelines for Breast Cancer Screening with MRI as an Adjunct to Mammography. CA: Cancer J Clin 2007;57:75-89.
[4]Breast MRI examinations require a dedicated breast coil and breast imaging radiologists familiar with the optimal timing sequences and other technical details for image interpretation. The imaging center should have the ability to preform MRI-guided needle sampling and/or wire localization of MRI-detected findings.
[5]Evidence from nonrandomized screening trials and observational studies.
[6]Based on evidence of lifetime risk for breast cancer.
[7]There is variation in recommendations for initiation of screening for different genetic syndromes. See NCCN Guidelines for Genetic/Familial High-Risk Assessment.
[8]Payment should not be a barrier. Screening decisions should be made on a case-by-case basis, as there may be particular factors to support MRI. More data on these groups is expected to be published soon.
[9]Brennan S, Liberman L, Dershaw DD, et al. Breast MRI screening of women with a personal history of breast cancer. AJR 2010;195:510-516.

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RISK FACTORS USED IN THE MODIFIED GAIL MODEL[1]

- **Current age**
- **Age at menarche**
- **Age at first live birth or nulliparity**
- **Number of first-degree relatives with breast cancer**
- **Number of previous benign breast biopsies**
- **Atypical hyperplasia in a previous breast biopsy**
- **Race[2]**

For calculation of risk, based on the modified Gail Model, see http://www.cancer.gov/bcrisktool/Default.aspx

[1]For detailed information, see http://www.cancer.gov/bcrisktool/Default.aspx.
[2]The current Gail Model may not accurately assess breast cancer risk in non-Caucasian women.

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MAMMOGRAPHIC ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - MAMMOGRAPHY FINDINGS

A. Assessment Is Incomplete:

Category 0: Incomplete - Need Additional Imaging Evaluation and/or Prior Mammograms For Comparison:

There is a finding for which additional evaluation is needed. This is almost always used in a screening situation. Under certain circumstances this assessment category may be used in a diagnostic mammography report, such as when US equipment or personnel are not immediately available, or when the patient is unable or unwilling to wait for completion of a full diagnostic examination. A recommendation for additional imaging evaluation includes, the use of spot compression (with or without magnification), special mammographic views, and ultrasound. Category 0 should not be used for diagnostic breast imaging findings that warrant further evaluation with MRI. Rather, the interpreting physician should issue a final assessment in a report that is made before the MRI examination is performed. In most circumstances and when feasible, if a mammography examination is not assessed as negative or benign, the current examination should be compared with prior examination(s). The interpreting physician should use judgment on how vigorously to attempt obtaining prior examinations, given the likelihood of success of such an endeavor and the likelihood that comparison will affect the final assessment. In this context, it is important to note that comparison with previous examination(s) may be irrelevant when a finding is inherently suspicious for malignancy.

Category 0 should be used for prior image comparison only when such comparison is required to make a final assessment. When category 0 is used in the context of awaiting prior examinations for comparison, there should be in place a tracking procedure guaranteeing with 100% reliability that a final assessment will be made within 30 days (preferably sooner) even if prior examinations do not become available. Some mammography practices may reasonably choose never to use category 0 in the context of awaiting prior examinations simply because they do not have a 100% reliable tracking procedure. If a mammography examination is assessed as category 0 in the context of awaiting prior examinations and then the prior examinations do become available, an addendum to the initial mammography report should be issued, including a revised assessment. For auditing purposes, the revised assessment should replace the initial assessment.

[1]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

[2]Terminology in this table is reflective of the American College of Radiology (ACR). ACR-BI-RADS®--5th Edition. ACR Breast Imaging Reporting and Data System, Breast Imaging Atlas; BI-RADS. Reston VA. American College of Radiology, 2014. For more information, see www.acr.org.
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MAMMOGRAPHIC ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - MAMMOGRAPHY FINDINGS

B. Assessment Is Complete - Final Assessment Categories:

Category 1: Negative:
There is nothing to comment on. This is a normal examination.

Category 2: Benign:
Like Category 1, this is a "normal" assessment, but here, the interpreter chooses to describe a benign finding in the mammography report. Involuting, calcified fibroadenomas, skin calcifications, metallic foreign bodies (such as core biopsy and surgical clips),and fat-containing lesions (such as oil cysts, lipomas, galactoceles, and mixed-density hamartomas) all have characteristically benign appearances, and may be described with confidence. The interpreter may also choose to describe intramammary lymph nodes, vascular calcifications, implants, or architectural distortion clearly related to prior surgery while still concluding that there is no mammographic evidence of malignancy. On the other hand, the interpreter may choose not to describe such findings, in which case the examination should be assessed as negative (category 1).

Note that both category 1 and category 2 assessments indicate that there is no mammographic evidence of malignancy. Both should be followed by the management recommendation for routine mammography screening. The difference is that category 2 should be used when describing one or more specific benign mammographic findings in the report, whereas category 1 should be used when no such findings are described (even if such findings are present).

[1]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

[2]Terminology in this table is reflective of the American College of Radiology (ACR). ACR-BI-RADS®--5th Edition. ACR Breast Imaging Reporting and Data System, Breast Imaging Atlas; BI-RADS. Reston VA. American College of Radiology, 2014. For more information, see www.acr.org.
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MAMMOGRAPHIC ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - MAMMOGRAPHY FINDINGS

Category 3: Probably Benign:

A finding assessed using this category should have a ≤2% likelihood of malignancy, but greater than the essentially 0% likelihood of malignancy of a characteristically benign finding. A probably benign finding is not expected to change over the suggested period of imaging surveillance, but the interpreting physician prefers to establish stability of the finding before recommending management limited to routine mammography screening.

There are several prospective clinical studies demonstrating the safety and efficacy of periodic mammographic surveillance instead of biopsy for specific mammographic findings.

Three specific findings are validated as being probably benign (the noncalcified circumscribed solid mass, the focal asymmetry, and solitary group of punctate calcifications). All the previously cited studies emphasize the need to conduct a complete diagnostic imaging evaluation before making a probably benign (category 3) assessment; hence, it is recommended not to render such an assessment in interpreting a screening mammography examination. The practice of rendering category 3 assessments directly from screening examination also has been shown to result in adverse outcomes: 1) unnecessary follow-up of many lesions that could have been promptly assessed as benign, and 2) delayed diagnosis of a small number of cancers that otherwise may have been smaller in size and less likely to be advanced in stage. Also, all the previously cited studies exclude palpable lesions, so the use of a probably benign assessment for a palpable lesion is not supported by robust scientific data, although there are two single-institution studies that do report successful outcomes for palpable lesions. Finally, because evidence from previously cited studies indicates the need for biopsy rather than continued surveillance when a probably benign finding increases in size or extent, it is not prudent to render a category 3 assessment when a finding that otherwise meets "probably benign" imaging criteria is either new or has increased in size or extent.

While the vast majority of probably benign findings are managed with an initial short-interval follow-up (6 mo) examination followed by additional examinations until long-term (2- or 3-year) stability is demonstrated, there may be occasions in which a biopsy is done instead (patient preference or overriding clinical concern).

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MAMMOGRAPHIC ASSESSMENT Category Definitions[1,2]

BI-RADS® - MAMMOGRAPHY FINDINGS

Category 4: Suspicious:
This category is reserved for findings that do not have the classic appearance of malignancy but are sufficiently suspicious to justify a recommendation for biopsy. The ceiling for category 3 assessment is a 2% likelihood of malignanacy and the floor for category 5 assessment is 95%, so category 4 assessments cover the wide range of likelihood of malignancy in between. Thus, almost all recommendations of breast interventional procedures will come from assessments made using this category. By subdividing category 4 into 4A, 4B, and 4C, as recommended in Guidance chapter and using the cut point indicated therein, it is hoped that patients and referring clinicians will more readily make informed decisions on the ultimate course of action.

Category 5: Highly Suggestive of Malignancy:
These assessments carry a very high probability (≥95%) of malignancy. This category initially was established to involve lesions for which 1-stage surgical treatment was considered without preliminary biopsy, in an era when preoperative wire localization was the primary breast interventional procedure. Nowadays, given the widespread acceptance of imaging-guided percutaneous biopsy, 1-stage surgery is rarely, if ever, performed. Rather, current oncologic management almost always involves tissue diagnosis of malignancy via percutaneous tissue sampling to facilitate treatment options, such as when sentinel node biopsy is included in surgical management or when neoadjuvant chemotherapy is administered prior to surgery. Therefore, the current rationale for using a category 5 assessment is to identify lesions for which any non-malignant percutaneous tissue diagnosis is automatically considered discordant, resulting in the recommendation for repeat (usually surgical) biopsy.

Category 6: Known Biopsy - Proven Malignancy:
This category is reserved for examinations performed after biopsy proof of malignancy (imaging performed after percutaneous biopsy but prior to complete surgical excision), in which there are no mammographic abnormalities other than the known cancer that might need additional evaluation.

[1]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

[2]Terminology in this table is reflective of the American College of Radiology (ACR). ACR-BI-RADS®--5th Edition. ACR Breast Imaging Reporting and Data System, Breast Imaging Atlas; BI-RADS. Reston VA. American College of Radiology, 2014. For more information, see www.acr.org.
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ULTRASOUND ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - ULTRASOUND FINDINGS

A. Assessment is Incomplete:

Category 0: Incomplete - Need Additional Imaging Evaluation:

There is a finding for which additional imaging evaluation is needed. The is almost always used in a screening situation. In this context, additional imaging evaluation includes the recording of (nonstandard) ultrasound images to supplement the standard images recorded for a screening examination. Note that this does not include repeat real-time scanning by the interpreting physician and/or colleague as long as additional images are not recorded. This respects the unique real-time nature of ultrasound and does not penalize its use.

Under certain circumstances, assessment category 0 may be used in a diagnostic ultrasound report, such as when equipment or personnel are not immediately available to perform a needed concurrent diagnostic mammography examination, or when the patient is unable or unwilling to wait for completion of a full diagnostic examination. Category 0 should not be used for diagnostic breast imaging findings that warrant further evaluation with MRI. Rather, the interpreting physician should issue a final assessment in a report that is made before the MRI examination is performed.

In most circumstances and when feasible, if a screening ultrasound examination is not assessed as negative or benign, the current examination should be compared to prior examination(s), if any exist. The interpreting physician should use judgement on how vigorously to attempt obtaining prior examinations, given the likelihood of success of such an endeavor and the likelihood that comparison will affect the final assessment. In this context, it is important to note that comparison to previous examination(s) may be irrelevant when a finding is inherently suspicious for malignancy.

Category 0 should be used for prior image comparison only when such comparison is required to make a final assessment. When category 0 is used in the context of awaiting prior examinations for comparison, there should be in place a tracking system guaranteeing with 100% reliability that a final assessment will be made within 30 days (preferably sooner), even if prior examinations do not become available. Some breast imaging practices may reasonably choose never to use category 0 in the context of awaiting prior examinations simply because they do not have a 100% reliable tracking system. If an ultrasound examination is assessed as category 0 in the context of awaiting prior examinations and then the prior examinations do become available, an addendum to the initial ultrasound report should be issued, including a revised assessment. For auditing purposes, the revised assessment should replace the initial assessment.

A need for previous studies to determine appropriate management might also temporarily defer a final assessment.

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ULTRASOUND ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - ULTRASOUND FINDINGS

B. Assessment is Complete — Final Categories:

Category 1: Negative:

There is nothing to comment on. This is a normal examination.

Category 2: Benign:

As with category 1, this is a "normal" assessment, but here the interpreter chooses to describe a benign finding in the ultrasound report. For example, the interpreter may choose to describe one or more simple cysts, intramammary lymph nodes, postsurgical fluid collections, breast implants, or complicated cysts/probable fibroadenomas that are unchanged for at least 2 or 3 years, while still concluding that there is no sonographic evidence of malignancy. On the other hand, the interpreter may choose not to describe such findings, in which case the examination should be assessed as negative (category 1).

Note that both category 1 and category 2 assessments indicate that there is no sonographic evidence of malignancy. Both should be followed by the management recommendation for routine age-appropriate screening. The difference is that category 2 should be used when describing one or more specific benign sonographic findings in the report, whereas category 1 should be used when no such findings are described (even if such findings are present).

[1]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

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ULTRASOUND ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - ULTRASOUND FINDINGS

Category 3: Probably Benign:

Assessment category 3, probably benign, is not an indeterminate category for use simply when the radiologist is unsure whether to render a benign (BI-RADS® category 2) or suspicious (BI-RADS® category 4) assessment, but one that is reserved for specific imaging findings known to have >0% but ≤2% likelihood of malignancy. For ultrasound, there is robust evidence that a solid mass with a circumscribed margin, oval shape, and parallel orientation (most commonly fibroadenoma), and an isolated complicated cyst have a likelihood of malignancy in the defined (≤2%), probably benign range, for which short-interval (6-month) follow-up sonography and then periodic sonographic surveillance may represent appropriate management. Similar data have been reported for clustered microcysts, but these data are less strong because they involve much fewer cases. The use of assessment category 3 for sonographic findings other than these three should be considered only if the radiologist has personal experience to justify a watchful-waiting approach, preferably involving observation of a sufficient number of cases of an additional sonographic finding to suggest a likelihood of malignancy within the defined (≤2%), probably benign range.

This edition of the BI-RADS® Atlas also emphasizes the recommendation that a category 3 assessment should not be made at screening; rather, this should be done only after completion of full diagnostic breast imaging examination. This recommendation is appropriate for screening mammography, for which batch interpretation usually is utilized, because in this setting there is no opportunity to complete the diagnostic workup before interpreting the screening examination. However, screening ultrasound almost always is interpreted online, so a full diagnostic examination also is completed while the patient remains in the breast imaging facility, and a single breast imaging report may be issued that combines the findings of both screening and diagnostic components of the examination. Hence, there is no purpose in recommending against category 3 assessment at screening ultrasound because the diagnostic workup would be completed simultaneously. Note that for auditing purposes, the screening component of a category 3-assessed screening ultrasound examination will be audit-positive, not only because additional nonstandard (diagnostic) images will be recorded but also because a category 3 assessment at screening is defined as being audit-positive.

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ULTRASOUND ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - ULTRASOUND FINDINGS

For category 3 assessments, the initial short-term follow-up interval is usually 6 months, involving the breast(s) containing the probably benign finding(s). Assuming stability at this 6-month examination, a category 3 assessment again is rendered with a management recommendation for a second short-interval follow-up examination in 6 months. Again assuming stability at this second short-interval follow-up, the examination is once more assessed as category 3, but now the recommended follow-up interval usually is lengthened to 1 year due the already-observed 12-month stability. Note that although the 1-year follow-up coincides with the routine screening interval in the United States, a category 3 assessment is rendered, to indicate that the period of imaging surveillance is still underway. As with surveillance using mammography, after 2 to 3 years of stability, the final assessment category should be changed to benign (BI-RADS® category 2). A benign evaluation may also be rendered before completion of category 3 analysis if, in the opinion of the interpreter, the finding has no chance of malignancy and is thus a category 2.

Category 4: Suspicious:
This category is reserved for findings that do not have the classic appearance of malignancy but are sufficiently suspicious to justify a recommendation for biopsy. The ceiling for category 3 assessment is a 2% likelihood of malignancy, and the floor for category 5 assessment is 95%, so category 4 assessments cover the wide range of likelihood of malignancy in between. Thus, almost all recommendations for breast interventional procedures will come from assessments made using this category. By subdividing category 4 into 4A, 4B, and 4C, it is hoped that patients and referring clinicians will more readily make informed decisions on the ultimate course of action. An example of separating the BI-RADS® assessment category from the management recommendation occurs when a simple cyst, correctly assessed as BI-RADS® 2, undergoes cyst aspiration for pain control.

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ULTRASOUND ASSESSMENT CATEGORY DEFINITIONS[1,2]

BI-RADS® - ULTRASOUND FINDINGS

Category 5: Highly Suggestive of Malignancy:
These assessments carry a very high probability (≥95%) of malignancy. This category initially was established to involve lesions for which 1-stage surgical treatment could be considered without preliminary biopsy in an era when preoperative wire localization was the primary breast interventional procedure. Nowadays, given the widespread acceptance of imaging-guided percutaneous biopsy, 1-stage surgery rarely, if ever, is performed. Rather, current oncologic management almost always involves tissue diagnosis of malignancy via percutaneous tissue sampling to facilitate treatment options, such as when sentinel node imaging is included in surgical management or when neoadjuvant chemotherapy is administered prior to surgery. Therefore, the current rationale for using a category 5 assessment is to identify lesions for which any nonmalignant percutaneous tissue diagnosis is considered discordant, resulting in the recommendation for repeat (usually vacuum-assisted or surgical) biopsy. Also note that whereas the fourth edition simply indicated that "appropriate action should be taken" as management for category 5 assessments, the fifth edition provides the more directed management recommendation that "biopsy should be performed in the absence of clinical contraindication." This new text unequivocally specifies tissue diagnosis as the interpreting physician's management recommendation for category 5 assessments, appropriately and effectively transferring the burden of establishing a contraindication to this recommendation to the referring clinician.

Category 6: Known Biopsy-Proven Malignancy:
This category is reserved for examinations performed after biopsy proof of malignancy (imaging performed after percutaneous biopsy but prior to surgical excision), in which there are no abnormalities other than the known cancer that might need additional evaluation.

[1]Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards: Final Rule. Federal Register. 1997;62:55988).

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Discussion

This discussion is being updated to correspond with the newly updated algorithm. Last updated 07/03/14

NCCN Categories of Evidence and Consensus

Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All recommendations are category 2A unless otherwise noted.

Discussion update in progress

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Overview

The average lifetime risk of breast cancer for a woman in the United States has been estimated at 12.3% (ie, 1 in 8 women).[1] In 2013, the American Cancer Society (ACS) estimates, 64,640 cases of female carcinoma in situ of the breast and 234,580 cases of invasive breast cancer (232,340 women and 2240 men) will be diagnosed in the United States.[2] About 40,030 deaths are estimated in 2013.[2] The good news is that mortality from breast cancer has dropped slightly. This decrease has, in part, been attributed to mammographic screening.[3]

The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology® (NCCN Guidelines®) for Breast Cancer Screening and Diagnosis are for facilitating clinical decision-making. The general public and health care providers need to be aware that mammography or any other imaging modality is not a stand-alone procedure. Neither the current technology of mammography or other imaging tests nor the subsequent interpretation of such tests is foolproof. Clinical judgment is needed to ensure appropriate management. The patient's concerns and physical findings must be taken into account along with imaging results and histologic assessment.

Breast Screening

Breast screening is performed in women without any signs or symptoms of breast cancer so that disease can be detected as early as possible. The components of a breast screening evaluation are dependent on patient age and other factors such as medical and family history, and can include breast awareness (ie, patient familiarity with her breasts), physical examination, risk assessment, screening mammography, and in selected cases, screening breast magnetic resonance imaging (MRI).

A diagnostic breast evaluation differs from breast screening in that it is used to evaluate an existing problem (eg, palpable mass, discharge from the nipple). Although there is preliminary evidence that breast ultrasonography can be a useful screening adjunct to mammography in the evaluation of high-risk women with dense breasts,[4] its use as a screening test is not recommended at this time. These guidelines include ultrasonography in the diagnostic work-up of selected women only based on specific positive findings (see section on "Breast Ultrasonography" on MS-12). Current evidence does not support the routine use of breast scintigraphy (eg, sestamibi scan) or ductal lavage as screening procedures.

History and physical examination

The starting point of these guidelines for screening and evaluating breast abnormalities is a complete medical history followed by the clinical breast examination (CBE). Inspection of the breasts should be performed with the patient in upright and supine positions. Positioning may be done so as to elicit any subtle shape or contour changes in the breast.[5]

Women should be familiar with their breasts and promptly report any change to their health care provider.[6,7] This does not need to be in any specific formalized education program. Data from a large randomized trial of breast self-examination (BSE) screening has shown that instruction in BSE has no effect on reducing breast cancer mortality. In this study, 266,064 women were randomly assigned to either receive instruction in BSE or not.[8] Compliance was encouraged through feedback and reinforcement sessions. After 10 to 11 years of follow-up, 135 breast cancer deaths in the instruction group and 131 in the control group were observed and the cumulative breast cancer mortality rates were not significantly different between the two arms (RR, 1.04; 95% CI,

Discussion Final Progress

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0.82–1.33; $P = .72$). The number of benign breast lesions detected in the BSE instruction group was higher than that detected in the control group. Nevertheless, women should be encouraged to be aware of their breasts since this may facilitate detection of interval cancers between routine screenings. The NCCN Panel recommends that the women should be familiar with their breasts and promptly report changes to their health care provider and that periodic, consistent BSE may facilitate breast self-awareness.

Risk Assessment

If the physical examination is negative in an asymptomatic woman, the next decision point is based on risk stratification. Women can be stratified into two basic categories for the purpose of screening recommendations: those at average risk and those at increased risk. Risk assessment is outlined in the NCCN Guidelines for Breast Cancer Risk Reduction. The increased risk category consists of six groups: (1) women with a prior history of breast cancer; (2) women 35 years or older with a 5-year risk of invasive breast carcinoma ≥1.7% by per Gail model; (3) women with a lifetime risk of breast cancer > 20% based on models largely dependent on family history; (4) women who have previously received therapeutic thoracic irradiation (eg, mantle irradiation) between the ages of 10-30 years; (5) women with lobular carcinoma in situ (LCIS) and (6) women with a pedigree suggestive of or with a known genetic predisposition.

Screening Women at Average Risk

For women between ages 25 and under 40 years, the NCCN Panel recommends CBE every 1 to 3 years and breast awareness encouraged.

For women aged 40 years and older, the NCCN Panel recommends annual CBE and screening mammography, and encourages breast awareness. Although controversies persist regarding the benefits and risks of mammographic screening in certain age groups (notably women age 40-49),[9-16] most medical experts reaffirmed current recommendations supporting screening mammography (see section on "Mammographic Evaluation" on MS-8). The recommendation that women at normal risk begin annual mammographic screening at age 40 years is based on a consensus statement from the American Cancer Society (ACS) and National Cancer Institute in 1997 and is supported by the ACS guidelines for breast cancer screening published in 2003,[15] as well as the results and meta-analyses of randomized clinical trials. Women also should be informed about the evidence demonstrating the value of detecting breast cancer early, before symptoms develop. The benefits of early detection include less aggressive treatment and a wide range of treatment options. The evaluation of benefits versus risk strongly supports the value of screening and the importance of adhering to a schedule of regular mammograms.

A second consideration is the time interval of screening in women aged 50-74 years. Whether breast screening should be performed annually or every other year remains controversial.[16] The NCCN Panel believes that the benefits of yearly mammography outweigh the risks of the procedure as breast cancer mortality is lower with annual compared to biennial screening mammograms.[17] Additionally, mammograms can often detect a lesion 2 years before the lesion is discovered by CBE. To reduce mortality from breast cancer, yearly screening is thought to be more beneficial.

There are limited data regarding screening of elderly women because most clinical trials for breast screening have used a cutoff age of 65 or 70 years.[18-20] With the high incidence of breast cancer in the elderly

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population, the same screening guidelines used for women who are age 40 or older are recommended. Clinicians should always use judgment when applying screening guidelines. Mammography screening should be individualized weighing its potential benefits/risks in the context of the patients overall health and estimated longevity.[21] If a patient has severe comorbid conditions limiting her life expectancy and no intervention would occur based on the screening findings, then the patient should not undergo screening.[15,21]

Recently Bleyer and Welch published a study on screening mammography and the risk of over-diagnosis of breast cancer.[22] The NCCN panel believes that this study analysis is misleading. The authors used the period 1976 through 1978 to estimate an annual increase of 0.25% in breast-cancer incidence. In fact, 40 years of recorded data shows that the actual increase is 1% per year.[23] In addition, the study analysis did not differentiate between DCIS and invasive cancers. If their analysis had included invasive cancers alone with a valid baseline of an annual increase of 1% and then compared the results with SEER data, they would have found fewer invasive cancers than predicted.

Screening Women at Increased Risk

Women with Prior History of Breast Cancer: These women should be treated according to the surveillance and follow-up recommendations outlined in NCCN Guidelines for Breast Cancer.

Women Aged 35 Years or Older with a 5-Year Risk of Invasive Breast Carcinoma Greater Than or Equal to 1.7%: For women age 35 and older, a risk assessment tool is available to identify those who are at increased risk. The National Cancer Institute (NCI) and the National Surgical Adjuvant Breast and Bowel Project (NSABP) Biostatistics Center has developed a computerized interactive risk-assessment tool based on the modified Gail model[24-28] that can be accessed at: http://www.cancer.gov/bcrisktool/Default.aspx which provides risk projections on the basis of several risk factors for breast cancer. The modified Gail model assesses the risk of invasive breast cancer as a function of age, menarche, age at first live birth or nulliparity, number of first-degree relatives with breast cancer, number of previous benign breast biopsies, atypical hyperplasia in a previous breast biopsy, and race. The model calculates and prints 5-year and lifetime projected probabilities of developing invasive breast cancer and can be used to identify women who are at increased risk. The Gail model should not be used for women with a predisposing gene mutation, a strong family history of breast or ovarian cancer suggestive of a genetic predisposition, women with a prior history of thoracic radiation, or for those with LCIS.

The Gail model was updated using combined data from the Women's Contraceptive and Reproductive Experiences (CARE) study and the Surveillance Epidemiology and End Results (SEER) database, as well as causes of death from the National Center of Health Statistics, to provide a more accurate determination of risk for African-American women.[29] It has also been updated using the data from the Asian American Breast Cancer Study (AABCS) and the SEER database to provide a more accurate risk assessment for Asian and Pacific Islander women in the United States.[30]

Increased risk of developing breast cancer is defined by the modified Gail model for women ≥35 years of age as a 5-year risk of 1.7% or greater. This is the average risk of a 60-year-old woman, which is the median age of diagnosis of breast cancer in the U.S. The 5-year predicted risk of breast cancer required to enter the NSABP Breast Cancer Prevention Trial of tamoxifen versus placebo, as well as the Study of Tamoxifen and Raloxifene (STAR) trial, was 1.7% or greater. As previously mentioned, the modified Gail model risk assessment tool

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also provides an estimate of a woman's lifetime risk of breast cancer. However, this estimate is based on the Gail model risk criteria which differ from criteria used in risk assessment models predominantly based on family history (see below); lifetime breast cancer risk as determined by the Gail model is not used in these guidelines to determine whether a woman is eligible for screening breast MRI.

For a woman aged 35 years or older with a 5-year risk ≥1.7%, the NCCN Panel encourages breast awareness and recommends CBE every 6 to 12 months and annual mammography. In addition, according to the NCCN Panel, women in these groups should be asked to consider risk reduction strategies in accordance with the NCCN Guidelines for Breast Cancer Risk Reduction.

Women with LCIS: A diagnosis of LCIS is associated with estimated risks of 10%-20% for the subsequent development of cancer in either breast over the next 15 years, although it is not in itself considered to be a site of origin for cancer.[31,32]

For women with LCIS, the NCCN Panel encourages breast awareness and recommends CBE every 6 to 12 months and annual mammography beginning at diagnosis. In addition, according to the NCCN Panel, women in these groups should be asked to consider risk reduction strategies in accordance with the NCCN Guidelines for Breast Cancer Risk Reduction.

Women with a Lifetime Risk of Breast Cancer >20% based on models largely dependent on family history: A lifetime risk of breast cancer of >20% as assessed by models based largely on family history is another risk threshold used in the guidelines to identify a woman as a potential candidate for risk reduction strategies, as well as to direct screening strategies. According to the ACS guidelines for breast screening, MRI may be performed as an adjunct to mammography[33] in a high risk woman if her lifetime risk of breast cancer is approximately 20% or greater based on models that rely mainly on family history. A cancer genetic professional should be involved in determining the lifetime risk of the individual based on models dependent on family history. These include Claus,[34] Tyrer-Cuzick,[35] and other models[36-38]. BRCAPRO[39] and Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA)[40] are more commonly used to estimate the risk based of *BRCA* mutations. Strong genetic association between breast and ovarian cancer has been demonstrated in some families by linkage analyses.

For a woman with a >20% lifetime risk of breast cancer based on models largely dependent on family history, the NCCN Panel encourages breast awareness and beginning at age 30, the NCCN Panel recommends CBE every 6 to 12 months and annual mammography. In addition, in accordance with the ACS guidelines,[33] the NCCN Panel recommends considering annual breast MRI for women who have a lifetime risk of breast cancer >20% based on models that rely mainly on family history. According to the NCCN Panel, women in this group should be asked to consider risk reduction strategies in accordance with the NCCN Guidelines for Breast Cancer Risk Reduction.

Women Who Have Received Prior Thoracic Irradiation Between the Ages of 10 to 30 Years: Results from several studies have demonstrated that women who received thoracic irradiation in their second or third decade of life have a substantially increased risk of developing breast cancer by age 40 years.[41-46] For example, in the Late Effects Study Group trial, the overall risk of breast cancer associated with prior thoracic irradiation at a young age was found to be 56.7–fold (55.5-fold for female patients) greater than the risk of breast cancer in

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the general population.[42,45] In that study, the relative risk of female breast cancer according to follow-up interval was: 0 at 5-9 years; 71.3 at 10-14 years; 90.8 at 15-19 years; 50.9 at 20-24 years; 41.2 at 25-29 years; and 24.5 at > 29 years.[45] Results from a case-control study of women treated with thoracic radiation at a young age for Hodgkin lymphoma indicated that the estimated cumulative absolute risk of breast cancer at 55 years of age was 29.0% (95% CI, 20.2%-40.1%) for a woman treated at 25 years of age with at least 40 Gy of radiation and no alkylating agents.[47] Although there is a concern that the cumulative radiation exposure from mammography in a young woman may itself pose a risk for cancer, it is felt that the benefit of early detection of breast cancer in this high-risk group would outweigh the potential side effect. Findings from a survey of breast screening practices in this population of patients suggest that a sizable segment of this group is not undergoing regular mammographic screening.[48]

For women aged 25 years and older who have received prior thoracic irradiation, the NCCN Panel recommends encouraging breast awareness, annual mammograms, annual MRI as an adjunct to mammograms[49] and CBE every 6 to 12 months be initiated 8 to 10 years after radiation exposure or 40, whichever comes first.[50]

For women younger than 25 years who have received prior thoracic irradiation, the NCCN Panel recommends encouraging breast awareness, counseling on risk, and an annual CBE starting 8-10 years after the radiation therapy.

Women with a Pedigree Suggestive Of or With a Known Genetic Predisposition: Accurate family history information is needed to adequately assess a woman's breast cancer risk. Familial cancers share some but not all features of hereditary cancers. For example, although familial breast cancers occur in a given family more frequently than expected on the basis of statistics, they generally do not exhibit inheritance patterns or onset age consistent with hereditary cancers. Familial breast cancers may be associated with chance clustering, genetic variations in lower-penetrance genes, a shared environment, small family size, and/or other factors.

The NCCN Guidelines for Genetic/Familial High-Risk Assessment include a recommendation for referral to a cancer genetics professional for further evaluation for an individual who has either a personal history or a close family history meeting any of the following criteria (see NCCN Guidelines for Genetic/Familial High-Risk Assessment).

In the statement on Genetic Testing for Cancer Susceptibility from the American Society of Clinical Oncology (ASCO) updated in 2003, genetic counseling/testing is recommended when there is: (i) a personal or family history suggesting genetic cancer susceptibility (ii) the test can be adequately interpreted and (iii) the results will aid in the diagnosis or influence the medical or surgical management of the patient or family members at hereditary risk of cancer.[51] Additional genetic testing criteria are included in the NCCN Guidelines for Genetic/Familial High-Risk Assessment. Genetic testing should be done only in the setting of pre-and post-test genetic counseling.

The manifestations of hereditary syndromes are often variable in individuals (e.g., age of onset, tumor site, and number of primary tumors). The risk of developing breast cancer in individuals with one of these hereditary syndromes depends upon numerous variables including the gender and age of the individual. Therefore there is variation in screening recommendations for different genetic syndromes. The NCCN Guidelines for Genetic/Familial High-Risk Assessment lists screening recommendations for common hereditary

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syndromes that put the individuals at increased risk for breast and ovarian cancer.

Hereditary breast and ovarian cancer syndrome (HBOC): It has been estimated that over 90% of early onset cancers in families with both breast and ovarian cancers are caused by mutation(s) in the *BRCA1* or *BRCA2* genes.[52] Hence, the degree of clinical suspicion for breast cancer in an individual with *BRCA* mutation or someone with a family history of both breast and ovarian cancer should be very high. The emphasis on initiating screening considerably earlier than standard recommendations is a reflection of the early age of onset seen in hereditary breast/ovarian cancer.[38,53-55] The overall sensitivity of screening mammography was reported to be only 33% in a study of women with suspected or known *BRCA1/2* mutations who were more likely to be younger and to have dense breasts.[56] Other reasons for the low sensitivity of mammography in women with *BRCA1/2* mutations include an increased likelihood of developing tumors with more benign mammographic characteristics (e.g., less likely to appear as a spiculated mass).[57] The ACS recommends annual MRI as an adjunct to screening mammogram.[33]

The risk from radiation exposure due to mammography in young women with an inherited cancer predisposition is unknown, and there is some concern about whether this genetic factor may increase sensitivity to irradiation. A recent study of *BRCA1/BRCA2* mutation carriers showed that lifetime mammogram exposure was not associated with an increased risk in breast cancer when the overall group was considered; however, a small increase in risk was seen when only those women with *BRCA1* mutations were evaluated.[58] Because the lifetime risk of breast cancer in BRCA1 or BRCA2 mutation carriers is estimated to be 3-6 fold greater (40% to 80% range)[59] than in the general population, the benefit of screening may justify the radiation exposure.

For a woman with a pedigree suggestive of a genetic predisposition or who is a carrier of a *BRCA1/2* mutation, the NCCN Panel recommends encouraging breast awareness and CBE every 6-12 months starting at age 25 years. The NCCN Panel also recommends screening women with annual mammograms and breast MRI as an adjunct to mammogram (MRI performed preferably on day 7-15 of menstrual cycle for premenopausal women) starting at age 25 years or on an individualized timetable based on the earliest age of cancer onset in family members. According to the NCCN Panel, women in this group should be offered risk reduction counseling and the opportunity to consider risk reduction strategies following multidisciplinary consultation in accordance with the NCCN Guidelines for Breast Cancer Risk Reduction.

Male carriers of a *BRCA* gene mutation also have a greater risk for cancer susceptibility.[60] In one study of 26 high-risk families with at least one case of male breast cancer, 77% demonstrated a *BRCA2* mutation.[52] However, among males with breast cancer who were not selected on the basis of family history, only 4%-14% tested positive for a germline *BRCA2* mutation.[61,62] For males with a *BRCA2* mutation, the risk of breast cancer by age 80 years has been estimated at 6.9%.[63] A mutation in the *BRCA2* gene, accounts for about 1 in10 breast cancers in men. *BRCA1* mutations can also cause breast cancer in men, but the risk is not as high as it is for mutations in the *BRCA2* gene. In contrast, for men without such a mutation, the lifetime risk of breast cancer has been estimated at about 1/10th of 1% (1 in 1,000).[64]

The NCCN Panel recommendations for men positive for a *BRCA1/2* mutation include breast awareness and a CBE every 6-12 months starting at age 35. Baseline mammography should be considered at age 40 years, followed by annual mammography for those men with

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gynecomastia or parenchymal/glandular breast density on baseline study.

Mammographic Screening

A screening mammogram typically involves 2 x-ray images of each breast (ie, one taken from the top [craniocaudal] of the breast and the other from the side [mediolateral oblique]). Randomized clinical trials have demonstrated that screening mammography lowers the rate of death from breast cancer,[5,65] with a reported overall sensitivity of about 75%.[66] Nevertheless, the overall sensitivity of screening mammography was reported to be only 50% in a study of women with at least heterogeneous dense tissue,[67] and 33% in a study of women with suspected or known BRCA mutations who were more likely to be younger and to have dense breasts.[56]

Technical aspects of mammography can affect the quality of screening results. Digital mammography differs from conventional film mammography in that the former generates an electronic image of the breast and allows for computer storage and manipulation. Four large scale trials have compared these two procedures although the designs and findings of these trials differ.[68-73] In a study of 49,528 women who underwent both film and digital mammography, no difference was seen in the overall accuracy of the two procedures.[68,69] However, digital mammography was significantly more accurate in younger women with dense breasts, and there was a nonsignificant trend toward improved accuracy of film mammography in women aged 65 years and older. In another trial of women aged 45 to 69 years randomly assigned to film or digital screening mammography, the latter procedure was shown to result in a higher rate of cancer detection.[70] Other outstanding issues related to these two procedures include possible differences in recall rates, and cost and availability issues.

Dense breast tissue as measured by mammography is increasingly recognized as an important risk factor for breast cancer.[74,75] Although there are some studies supporting the use of ultrasound for breast cancer screening as an adjunct to mammography for high risk women with dense breast tissue,[67] the NCCN Panel however cautions that there is insufficient evidence to support routine supplemental screening in women with dense breasts and no other risk factors.

Mammographic Assessment Category Definitions:
Mammographic results are mandated to be reported using Final Assessment Categories [Breast Imaging Reporting and Data System (BI-RADS®)] categories developed by the American College of Radiology [Mammography Quality Standards Act, 1997][76] The purpose of the Final Assessment Category definitions is to create a uniform system of reporting mammography results with a recommendation associated with each category. The fourth edition of BI-RADS® is adopted in these guidelines. In this edition, substantive changes have been incorporated and category 6 has been added.[77]
BI-RADS® assessment categories apply to an individual imaging method if only one type of imaging is done (eg, mammography), but if multiple imaging modalities are used (e.g. additional ultrasonography and MRI), the BI-RADS® categories represent the cumulative findings of the examinations that were performed. Therefore, the overall BI-RADS® assessment category can change depending on subsequent imaging findings (ie, the BI-RADS® assessment category given following a mammographic study may increase, decrease, or remain the same upon diagnostic ultrasonography or MRI). In the event that multiple abnormalities are identified on imaging, the overall final BI-RADS® assessment category is based on the most worrisome findings present. After the mammographic evaluation is completed, the results are classified according to one of the following BI-RADS® categories[76]:

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- ***Category 1 - Negative:*** This is a negative mammogram. The breasts are symmetric, and there are no masses, architectural distortion or suspicious calcification.
- ***Category 2 - Benign Finding(s):*** This is also a negative mammogram, but there may be an actual finding that is benign. The typical case scenarios include benign-appearing calcifications, such as a calcifying fibroadenoma, an oil cyst, or a lipoma. The interpreter may also choose to describe intramammary lymph nodes, vascular calcification, implants or architectural distortion clearly related to prior surgery while still concluding that there is no mammographic evidence of malignancy.
- ***Category 3 - Probably Benign Finding(s) – Short-Interval Follow-up Suggested:*** This is a mammogram that is usually benign. Close monitoring of the finding is recommended to ensure its stability. The risk of malignancy is estimated to be less than 2%.
- ***Category 4 - Suspicious Abnormality –Biopsy Should Be Considered:*** These lesions fall into the category of having a wide range of probability of being malignant but are not obviously malignant mammographically. The risk of malignancy is widely variable and is greater than that for category 3 but less than that for category 5.
- ***Category 5 - Highly Suggestive of Malignancy-Appropriate Action Should Be Taken***: These lesions have a high probability (≥ 95%) of being a cancer. They include spiculated mass or malignant-appearing pleomorphic calcifications, etc.
- ***Category 6 - Known Biopsy - Proven Malignancy-Appropriate Action Should Be Taken:*** This category is reserved for breast lesions identified on the imaging study with biopsy proof of malignancy but prior to definitive therapies.

There is also another BI-RADS® category - Category 0 – which represents an incomplete assessment.

Category 0: Needs Additional Imaging Evaluation and/or Prior Mammograms For Comparison. This category is almost always used in the context of a screening situation, if a finding requiring additional evaluation has been identified. A recommendation for additional imaging evaluation may include, but is not limited to spot compression, magnification, special mammographic views and ultrasound. Under certain circumstances, this category may be used after a full mammographic workup. Whenever possible, if the study is not negative and does not contain a typical benign finding, the current examination should be compared to previous studies. The radiologist should use judgment on how vigorously to attempt obtaining previous studies. The practice guideline for the performance of screening and diagnostic mammography from the American College of Radiology can be accessed at: http://www.acr.org/SecondaryMainMenuCategories/quality_safety/guidelines/breast/Screening_Diagnostic.aspx.

NCCN Recommendations after Mammographic Evaluation

For patients with mammograms classified as BI-RADS® categories 1 and 2, in which the mammogram is completely normal or the finding is benign mammographically, the NCCN Panel recommends routine screening, based on age and risk of breast cancer. When screening mammography reveals an abnormal finding, the radiologist should attempt to obtain any prior mammograms. This is most important for lesions that are of low suspicion mammographically. If, after a comparison of films, there is still a questionable area that is not clearly benign, then a diagnostic mammogram (see section on "Diagnostic

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Mammography" on MS-11), with or without ultrasonography (see section on "Breast Ultrasonography" on MS-12) should be performed.

For NCCN recommendations and follow-up of patients with mammograms categorized as BI-RADS® 0 and 3 or higher, see section on "Diagnostic Evaluation for Positive Findings" on MS-11.

Breast Magnetic Resonance Imaging Screening

The sensitivity of breast MRI at detecting breast cancer is higher than the sensitivity of mammography, although the specificity of the former procedure is lower, resulting in a higher rate of false-positive findings.[78] In addition, microcalcifications are not detectable with MRI,[79,80] and the issue of whether breast MRI screening impacts survival has not been addressed in randomized clinical trials. Therefore, careful patient selection for additional screening with MRI is needed. Although current evidence does not support the use of breast MRI to screen women at average risk of breast cancer, benefits of screening MRI for women with prior thoracic radiation, and those with a genetic predisposition for breast cancer have been demonstrated in several studies,[49,56,81-87] and the ACS has published guidelines recommending use of breast MRI as an adjunct to screening mammography in certain populations of women at high risk of breast cancer.[33] Nevertheless, a high false-positive rate for screening MRI was identified in several these studies. For example, in one study of high-risk women, many of whom were young and had very dense breast tissue, screening MRI led to 3 times as many benign biopsies as mammography.[54]

A single retrospective study of asymptomatic women with atypical hyperplasia or LCIS enrolled in a high-risk screening program has evaluated use of MRI in this population.[88] Approximately half of the women underwent screening with mammography and MRI whereas the other half was screened with mammography alone. For those undergoing both types of screening, breast cancer was detected by MRI in 4% of patients with LCIS who had negative mammogram results. MRI screening did not impact the rate of cancer detection in women with atypical hyperplasia. Women who underwent screening with MRI were more likely to be younger and premenopausal, and to have a stronger family history of breast cancer than those who were evaluated by mammography alone. However, only one woman with cancer detected by MRI following a negative mammography finding had reported a family history of breast cancer, and no difference was seen in the percentages of patients who ultimately developed cancer in the 2 groups.

The NCCN Panel recommends an annual MRI as an adjunct to screening mammogram and CBE for the following groups with increased risk of breast cancer: 1) Women with a pedigree suggestive of or known genetic predisposition for breast cancer, starting at age 25 for HBOC, or individualized based on earliest age of onset in the family and 2) Women who received with thoracic radiation therapy between ages 10 to 30 years (MS-6). MRI may be considered as an adjunct to screening mammogram for women with a >20% lifetime risk of breast cancer as defined by models largely based on family history as described in the ACS guidelines.[40]

Criteria for the performance/interpretation of high quality breast MRI include: a dedicated breast coil, radiologists experienced in breast MRI; and the ability to perform MRI-guided needle sampling and/or wire localization of MRI-detected findings. Breast MRI Guidelines from the European Society of Breast Imaging include detailed descriptions of the technical aspects of the use of breast MRI.[78] The American College of Radiology has also published guidelines for the performance of contrast-enhanced MRI of the breast.[89]

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Diagnostic Evaluation for Positive Findings

Additional evaluations in selected patients with positive findings can include diagnostic mammography, ultrasonography, diagnostic breast MRI, and tissue sampling.

Diagnostic Mammography

Screening mammography which consists of 2 standard X-ray images of each breast differs from diagnostic mammography in that the latter is used to evaluate a patient with a positive clinical finding—such as a breast lump or an abnormal screening mammogram. A diagnostic mammogram includes additional views, such as spot compression views or magnifications views, to investigate the finding in question.

NCCN Recommendations for Mammogram BI-RADS® Assessment Categories 0, 3, 4, 5 and 6

For BI-RADS® category 0 (need additional imaging evaluation), the diagnostic work-up includes comparison to prior films and/or diagnostic mammogram with or without ultrasound scan.

For BI-RADS® category 3 (probably benign), diagnostic mammograms at 6 months, then every 6 to 12 months for 2 to 3 years are appropriate. At the first 6-month follow-up, a unilateral mammogram of the index breast is performed. The 12-month study would be bilateral in women aged 40 years and older so that the contralateral breast is imaged at the appropriate yearly interval. Depending on the level of concern, the patient is then followed, either annually with bilateral mammograms or every 6 months for the breast in question, for a total of 2 to 3 years.

If the lesion remains stable or resolves mammographically, the patient resumes routine screening intervals for mammography. If, in any of the interval mammograms, the lesion increases in size or changes its benign characteristics, a biopsy is then performed. The exception to this approach of short-term follow-up is when a return visit is uncertain or the patient is highly anxious or has a strong family history of breast cancer. In those cases, initial biopsy with histologic sampling may be a reasonable option.

For BI-RADS® categories 4 and 5, tissue diagnosis using core needle biopsy (preferred) or needle localization excisional biopsy with specimen radiograph is necessary. When a needle biopsy (aspiration or core needle biopsy) is performed, concordance between the pathology report and the imaging finding must be obtained.[77,90] For example, a negative needle biopsy associated with a spiculated category 5 mass is discordant and clearly would not be an acceptable diagnosis. When the pathology and the imaging are discordant, the breast imaging should be repeated and/or additional tissue sampled or excised; surgical excision is recommended when pathology/image remain discordant. Women with a benign result exhibiting pathology/image concordance should be followed with mammography every 6-12 months for 1-2 years before returning to routine screening.

For BI-RADS® category 6 (proven malignancy), the patient should be managed according to the NCCN Guidelines for Breast Cancer.

Breast Ultrasonography

Mammography and ultrasound are complementary imaging methods for diagnosing breast cancer. However, breast ultrasonography does not detect most microcalcifications.[56,67,91-93]

Initial diagnostic imaging with breast ultrasonography is recommended as the preferred option for women aged < 30 years presenting with a palpable mass or asymmetric thickening/nodularity. Breast

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ultrasonography is recommended for women ≥ 30 years of age with a palpable mass and a diagnostic mammogram assessed as BI-RADS® 1-3, and as an adjunct to diagnostic mammography for women in this age group with a finding of asymmetric thickening/nodularity. In addition, breast ultrasonography should be considered as an adjunct to mammography for those of all ages with skin changes consistent with serious breast disease or with spontaneous nipple discharge in the absence of a palpable mass, and as a possible option for women with a BI-RADS® category 0 screening mammographic assessment. Consideration of follow-up ultrasound testing is also recommended when initial ultrasound findings of a solid mass are classified as a probably benign finding, or when biopsy results are found to be benign and image concordant. Ultrasound-guided biopsy is included in the guidelines for women with a complex cyst or a persistent mass following cyst aspiration.

Ultrasonographic Assessment Category Definitions

After the ultrasonographic evaluation is completed, the results are classified according to one of the following BI-RADS® categories:[94]

- ***Category 0 – Needs Additional Imaging Evaluation:*** This represents an incomplete assessment. A finding for which additional evaluation is needed. If ultrasound is the initial study, mammography might be indicated, or if mammography and ultrasound findings are nonspecific, MRI might be appropriate.
- ***Category 1 - Negative:*** This is a negative ultrasound. No abnormalities are detected.
- ***Category 2 - Benign Finding(s):*** This is also a negative ultrasound, but there may be an actual finding that is benign. Included in this category are simple cysts (see section below on "Breast cysts") and breast implants.
- ***Category 3 - Probably Benign Finding(s) - Short-Interval Follow-up Suggested:*** This is a ultrasound that is usually benign. Close monitoring of the finding is recommended to ensure its stability. The risk of malignancy is estimated to be less than 2%. Fibroadenomas and nonpalpable complicated cysts and clustered microcysts might be placed in this category for short-interval follow-up (see section below on "Breast cysts")
- ***Category 4 - Suspicious Abnormality –Biopsy Should Be Considered:*** These lesions fall into the category of having a wide range of probability of being malignant but are not obviously malignant ultrasonographically. The risk of malignancy is widely variable and is greater than that for category 3 but less than that for category 5. A complex cyst would be included in this group (see section below on "Breast cysts").
- ***Category 5 - Highly Suggestive of Malignancy-Appropriate Action Should Be Taken:*** These lesions have a high probability (≥ 95%) of being a cancer.
- ***Category 6 - Known Biopsy - Proven Malignancy-Appropriate Action Should Be Taken:*** This category is for breast lesions identified on the imaging study with biopsy proof of malignancy but prior to definitive therapies.

Breast Cysts

Breast cysts are either classified as simple or non-simple cysts, with the latter class being subdivided into complicated cysts and complex cysts (see Table 1 for definitions).

Simple cyst

A cyst meeting all criteria of a simple cyst is considered to be benign,[67,95] if the clinical findings and ultrasonographic results are concordant. Therapeutic fluid aspiration can be considered if clinical

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symptoms persist, and these patients can be followed with routine screening. Cytologic examination is recommended if bloody fluid is obtained.

Non-simple Cysts

A complicated non-simple cyst is associated with a low risk of malignancy (<2%).[67,96-98] Options for managing complicated cysts are either aspiration or short-term follow-up with physical examination and ultrasonography with or without mammography every 6-12 months for 2-3 years to assess stability. There may be variability on the follow-up interval based on the level of suspicion. The option of aspiration may be more strongly considered in a patient likely to be lost to follow-up. Complicated cysts which increase in size should be biopsied. As with simple cysts, cytologic analysis of fluid aspirated from a complicated cyst is required only if bloody fluid is obtained. In the event of a persistent mass, a biopsy is needed.

For cysts which resolve following aspiration but are characterized by bloody fluid, the NCCN Panel recommends placement of a tissue marker followed by cytologic evaluation of fluid. Follow-up of a positive finding includes percutaneous vacuum-assisted biopsy or excision. If findings are negative, physical examination with or without ultrasound/mammogram every 6-12 months for 1-2 years is recommended to assess stability. Repeat imaging (ultrasound with or without mammogram) is recommended for a recurrent mass whereas routine screening is the recommended strategy when follow-up examinations are negative.

Complex cysts have a relatively high risk of malignancy (eg, 14% and 23% in 2 studies).[67,97-100] Hence, these cysts should be evaluated by tissue biopsy.

Diagnostic Breast MRI

MRI can also play a role in the diagnostic setting. For patients with skin changes consistent with serious breast disease, consideration of breast MRI is included in the guidelines for those with benign biopsy of skin or nipple following BI-RADS® category 1-3 assessment. Since a benign skin punch biopsy in a patient with a clinical suspicion of inflammatory breast cancer (IBC) does not rule out malignancy, further evaluation is recommended. There is evidence that certain MRI features may facilitate diagnosis of IBC.[101]

Breast Tissue Biopsy

Breast biopsy is recommended if diagnostic mammogram and/or ultrasound findings are suspicious or highly suggestive of malignancy.

Fine Needle Aspiration (FNA) Biopsy

An FNA biopsy involves use of a smaller-bore needle to obtain cytologic samples from a breast mass. Advantages of FNA biopsy include its minimally invasive methodology and low cost,[102,103] whereas the need for pathologists with specific expertise in the interpretation of test results and the necessity of performing a follow-up tissue biopsy when atypia or malignancy is identified are disadvantages of the procedure. FNA of nonpalpable lesions can be performed under imaging guidance (eg, ultrasound), although there is evidence to indicate that both core-needle biopsy and excisional biopsy are more accurate than FNA in the evaluation of nonpalpable breast lesions.[104,105]

Core Needle Biopsy

A core needle biopsy, also called percutaneous core breast biopsy, is an automated procedure that typically involves obtaining multiple cores of solid tissue using standard techniques.[106,107] It can be performed under imaging guidance (eg, stereotactic [mammographic] or ultrasound). Advantages of breast core needle biopsy include increased

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accuracy over FNA when the procedure is performed in situations where no mass is palpable and an ability to obtain tissue samples of sufficient size so as to eliminate the need for a follow-up biopsy to confirm malignancy.[108] In some situations, the core needle biopsy is performed under vacuum assistance which can facilitate collection of adequate tissue from a breast lesion without the need for multiple needle insertions.[109,110] Clip placement is done at the time of core needle biopsy so that the radiologist can identify the location of the lesion in the event that it is entirely removed or disappears during neoadjuvant treatment of a breast cancer.[111] With a few exceptions, core needle biopsy is preferred in the NCCN Guidelines over surgical excision when tissue biopsy is required. According to the NCCN panel, surgical excision is appropriate if unable to perform core needle biopsy.

Excisional Biopsy

An excisional biopsy involves removal of the entire breast mass or suspicious area of the breast by a surgeon in an operating room setting. Needle or wire localization is done by the radiologist immediately prior to an excisional biopsy of a nonpalpable mammographic or sonographic finding to direct surgical excision. The wire localization may bracket a lesion that had a clip placed in it at the time of the core needle biopsy.[111]

Excisional biopsy is included in the NCCN Guidelines as an option when tissue biopsy is required. Although excisional biopsy is a more invasive method than core needle biopsy and requires needle localization when lesions are not palpable, there are situations where larger tissue samples may be needed. In most cases, excisional biopsy is recommended following diagnosis by core biopsy of an indeterminate lesion, atypical hyperplasia, LCIS, or a benign and image discordant lesion. Other histologies that may require additional tissue include mucin-producing lesions, potential phyllodes tumor, papillary lesions, radial scars or other histologies of concern to the pathologist.[100,103,108,112]

Support for this recommendation includes results of studies demonstrating an underestimation of cancer when atypical hyperplasia and LCIS are diagnosed by CNB.[113-118] However, there are situations (eg, select cases of LCIS, ALH, papillomas, fibroepithelial lesions, radial scars) where close observation may be substituted for excisional biopsy in select patients.[103,112,119-125]

Physical Examination

Symptomatic or positive findings on physical examination include: palpable mass in the breast, nipple discharge without a palpable mass, asymmetric thickening or nodularity, and skin changes

NCCN Recommendations for Positive Findings on Physical Exam

Palpable Mass in the Breast

A palpable mass is a discrete lesion that can be readily identified during a physical exam. The guidelines separate the evaluation of women with the palpable mass into two age groups: women aged 30 years or older and women under 30 years of age.

Women with palpable mass aged 30 years or older:

The main difference in the guidelines for evaluating a palpable mass in women age 30 or older compared with younger women is the increased degree of suspicion of breast cancer. The initial evaluation begins with a bilateral diagnostic mammogram. Observation without further evaluation is not an option in these women. There are some clinical circumstances such as mass with low clinical suspicion or suspected simple cyst, in which ultrasound would be preferred and may suffice for women 30-39 years of age.[126] After the mammographic assessment, the abnormality is placed in one of the six BI-RADS® categories.

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For women with BI-RADS® categories 4 and 5, assessment of the geographic correlation between clinical and imaging findings is indicated. If the imaging findings correlate with the palpable findings, the NCCN Panel recommends tissue evaluation through core needle biopsy. The NCCN Panel notes that fine needle aspiration (FNA) and core needle biopsy are both valuable. However, FNA requires cytologic expertise. When a core needle biopsy is utilized, concordance between the pathology report and imaging finding must be obtained. If there is a lack of geographic correlation between clinical and imaging findings, further evaluation is as recommended for BI-RADS® categories 1, 2, or 3.

For BI-RADS® categories 1, 2, and 3, the next step is to obtain an ultrasound and the findings are discussed below under "Ultrasound findings".

Ultrasound findings:

If the solid lesion found on the ultrasound is suspected to be probably benign (ie, BI-RADS® 3), the options are: observation, or core needle biopsy. Observation may be elected only if there is low clinical suspicion, in which case a physical examination with or without ultrasound or mammogram is recommended every 6 months for 2-3 years to assess stability. If the option of core needle biopsy is elected, and the result is benign and is concordant with the imaging results, the NCCN Panel recommends a physical examination every 6 to 12 months, with or without ultrasound or mammogram, for 1 to 2 years to ensure that the lesion is stable. Routine breast screening is recommended if the lesion is stable. If the solid lesion increases in size, the NCCN Panel recommends surgical excision. If the diagnosis by core biopsy is an indeterminate lesion, atypical hyperplasia, LCIS, or a benign and image discordant lesion, the NCCN Panel recommends surgical excision. Mucin-producing lesions, potential phyllodes tumor, papillary lesions, radial scars or other histologies of concern to the pathologist may also require excisional biopsy. Select patients (ie, some patients with atypical hyperplasia, LCIS, fibroepithelial lesions, radial scars etc) may be suitable for monitoring in lieu of surgical excision.

If the ultrasound evaluation reveals the mass to be consistent with an asymptomatic simple cyst (ie, BI-RADS® 2), the NCCN Panel recommends routine screening. However, it is important that there is concordance between the CBE and the ultrasound results before recommending routine screening. Therapeutic aspiration of such a simple cyst can be performed if persistent clinical symptoms are present.

If the cyst on the ultrasound is classified as a complicated non-simple cyst, options include aspiration or short-term follow-up (BI-RADS® 3). For short term follow-up, the NCCN Panel recommends physical examination and ultrasound with or without mammography every 6-12 months for 2-3 years to assess stability. A tissue biopsy should be performed for a complicated cyst which increases in size during follow-up.

Alternatively, aspiration may be performed. If blood-free fluid is obtained on aspiration, the mass resolves, and cytology results are negative, the NCCN Panel recommends that the patient should return to routine screening. If the mass first resolves after aspiration and then recurs, then repeat assessment with ultrasound or surgical excision if warranted. If the mass persists after aspiration, the NCCN Panel recommends ultrasound with image-guided biopsy. Surgical excision is appropriate if unable to perform core needle biopsy.

For cysts that resolve following aspiration but are characterized by bloody fluid, the NCCN Panel recommends placement of a tissue

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marker followed by cytologic evaluation of fluid. Follow-up of a positive finding includes percutaneous vacuum-assisted biopsy or excision. If findings are negative, physical examination with or without ultrasound/mammogram every 6-12 months for 1-2 years is recommended to assess stability. Repeat imaging is recommended for a recurrent mass whereas routine screening is the recommended strategy when follow-up examinations are negative. The NCCN Panel recommends a tissue biopsy for cysts classified as complex (BI-RADS® 4).

If the ultrasound with image-guided biopsy findings are benign and image concordant (BI-RADS® 1), physical exam with or without ultrasound or mammogram every 6-12 months for 1-2 years is recommended. If the mass increases in size, surgical excision should be repeated, with a routine breast screening recommended if the mass remains stable. If the ultrasound and image guided biopsy findings are interpreted as benign and image discordant or indeterminate or atypical hyperplasia or LCIS or other (ie, mucin-producing lesions, potential phyllodes tumor, papillary lesions, radial scar or other histologies of concern to the pathologist), surgical excision is recommended, although select patients (ie, some patients with atypical hyperplasia, LCIS, fibroepithelial lesions, radial scars, etc.) may be suitable for monitoring in lieu of surgical excision (see section on "Excisional Biopsy" on MS-14). Multifocal/extensive LCIS involving 4 terminal ductal lobular units on a core biopsy may be associated with increased risk of invasive cancer on surgical excision.

If the mass has been surgically excised and proven to be benign, the patient undergoes routine screening. If the mass is classified as atypical hyperplasia or LCIS, routine breast screening along with risk reduction therapy according to the NCCN Guidelines for Breast Cancer Risk Reduction is recommended.

If no ultrasonographic abnormality is detected (BI-RADS® 1), tissue biopsy (core needle biopsy or excision) or observation at 3-6 months intervals for 1-2 years should be considered to assess stability. If the lesion increases in size, tissue sampling should be repeated, whereas routine breast screening is recommended if the lesion remains stable.

Malignant findings either on ultrasound with image guided biopsy or surgical excision should be treated according to the NCCN Guidelines for Breast Cancer.

Women with palpable mass under 30 years of age:
The preferred option for initial evaluation of a palpable mass is to proceed directly to ultrasound with or without mammogram.[126] From this point, the decision tree for women under 30 years of age is almost identical to the pathway for older women. The main difference is consideration of a diagnostic mammogram in only some situations for the younger women. Because the degree of suspicion in women who are under the age of 30 is low, observation of the mass for one or two menstrual cycles is also an option in cases with low clinical suspicion. If observation is elected and the mass resolves after one or two menstrual cycles, the patient may return to routine screening. If the mass persists, ultrasound should be performed. Needle sampling prior to imaging is not recommended.

If no ultrasonographic abnormality is found (BI-RADS® 1), a mammogram is recommended in cases where there is high clinical suspicion or those at higher risk due to known genetic mutation or family history. Based on the mammogram results, from this point, the management is identical to the pathway for older women. Whereas if the clinical suspicion is low, observation every 3-6 months for 1-2 years is recommended. If the mass increases in size during the observation period, mammogram may be considered followed by

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tissue biopsy. If the mass remains stable, routine breast screening is recommended.

Nipple Discharge without a Palpable Mass

Nipple discharge is common, and, in many cases, unrelated to breast pathology.[127-133] For example, non-spontaneous discharge from multiple breast ducts in a non-lactating woman can occur during pregnancy, following breast stimulation, in women with certain thyroid conditions, and in those taking certain medications, such as estrogen, oral contraceptives, opiates, and particular antihypertensive agents.[127] Suspicion of underlying pathology (eg, papilloma, ductal ectasia) is raised when nipple discharge is persistent and reproducible on examination, spontaneous, unilateral, from a single duct with fluid characterized as clear and colorless, serous, sanguineous, or serosanguineous.[134]

In patients with a nipple discharge but no palpable mass, an evaluation of the characteristics of the nipple discharge is the first step. The appropriate follow-up of a non-spontaneous, multiple-duct discharge in women under age 40 is observation, coupled with education of the patient to stop compression of the breast and to report the development of any spontaneous discharge. In women aged 40 years or older, screening mammography and a further workup based upon the BI-RADS® category along with education similar to that for younger women is recommended.. Evaluation of this type of nipple discharge is based on the overall BI-RADS® category of the diagnostic mammogram with or without adjunctive ultrasound.

Mammary ductoscopy is useful in evaluating patients who have nipple discharge, for accurate visualization, analysis, and excision of intraductal abnormalities.[135-137] Magnetic resonance imaging (MRI) may play an adjunctive role, aiding in the differentiation of benign ductal abnormalities from malignant ones. Preliminary studies have shown that breast MRI aids in the diagnosis of suspected ductal disease and is an alternative to ductoscopy when the latter cannot be used.[138,139]

According to the NCCN Panel, for an overall BI-RADS® assessment category 1, 2, or 3, either a ductogram or MRI (optional) is recommended to guide the duct excision. Ductal excision is indicated for diagnosis of an abnormal nipple discharge, even if the ductogram is negative.[140] However, the ductogram is useful to exclude multiple lesions and to localize the lesions prior to surgery.

For an overall BI-RADS® assessment category 4 or 5, the NCCN Panel recommends a tissue biopsy. If the findings are benign or indeterminate, a ductogram is optional, but surgical duct excision would still be necessary. If findings are indicative of malignancy, the patient should be treated according to the NCCN Guidelines for Breast Cancer.

Asymmetric Thickening or Nodularity

Thickening, nodularity, or asymmetry is distinct from a palpable mass in that the finding is ill defined and often vague on physical breast examination. Factors to consider include whether the thickening is a new or previous finding, and whether or not it appears to be representative of normal asymmetry. If the patient is under the age of 30 years and has no high risk factors, ultrasound evaluation is appropriate followed by consideration of diagnostic mammography. Diagnostic mammograms for this age group are fairly low in yield because of the density of the breast and low risk of breast cancer. In a woman aged 30 years or older, a bilateral diagnostic mammogram, and an ultrasound evaluation should be obtained.

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If a clinically suspicious change is noted or the overall imaging findings are classified as BI-RADS® assessment category 4-5 a tissue biopsy is recommended.

If the overall imaging findings are classified as BI-RADS® category 1-3 *and* the clinical assessment is benign, the patient should be reexamined in 3 to 6 months to assess stability. For BI-RADS® category 3, the physical exam is followed by ultrasound and/or mammogram every 6-12 months for 2-3 years. If the findings on physical and/or imaging is stable, routine screening can be resumed. If the finding shows clinical progression, it should be investigated as previously described for palpable mass.

Skin Changes

Any type of unusual skin changes around the breast may represent serious disease and needs evaluation. Inflammatory breast cancer (IBC) should be considered when dermal edema (peau d'orange) and breast erythema are present, and nipple excoriation, scaling, and eczema should increase clinical suspicion of Paget's disease. IBC is a rare, aggressive form of breast cancer estimated to account for 1%-6% of breast cancer cases in the U.S. IBC is a clinical diagnosis that requires erythema and dermal edema of a third or more of the skin of the breast with a palpable border to the erythema.[141,142] Paget's disease of the breast is a rare manifestation of breast cancer characterized by neoplastic cells in the epidermis of the nipple areolar complex. It most commonly presents with eczema of the areola, bleeding, ulceration, and itching of the nipple. The diagnosis is often delayed because of the rare nature of the condition and confusion with other dermatologic conditions.[143,144]

The management of patients with IBC or Paget's disease is outlined in NCCN Guidelines for Breast Cancer.

The initial evaluation of a patient with breast skin changes begins with a bilateral diagnostic mammogram with or without ultrasound imaging. If the imaging results are abnormal, the evaluation proceeds on the basis of the imaging findings. If the breast imaging results are normal, further workup is still needed.

Punch biopsy of skin or nipple biopsy should be performed following imaging findings consistent with an overall BI-RADS® assessment category 1-3. Antibiotics may or may not be given, depending on the clinical scenario, but should not delay diagnostic evaluation. If biopsy results are benign, clinical and pathological correlation should be reassessed. In addition, a breast MRI, a repeat biopsy, and consultation with a breast specialist should be considered. If the skin biopsy is malignant, the patient should be treated according to the NCCN Guidelines for Breast Cancer.

A tissue biopsy should be performed if imaging findings are consistent of an overall BI-RADS® assessment category 4-5. According to the NCCN Panel, core needle biopsy is the preferred option with or without punch biopsy although surgical excision is also an option. A benign biopsy result should be followed by a punch biopsy of skin if not previously performed or nipple biopsy, with reassessment as described above for BI-RADS® category 1-3. A biopsy showing a malignant finding should be managed according to the NCCN Guidelines for Breast Cancer.

Summary

The intent of these guidelines is to give health care providers a practical, consistent framework for screening and evaluating a spectrum of breast lesions. Clinical judgment should always be an important component of the optimal management of the patient.

Discussion

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If the physical breast examination, radiologic imaging, and pathologic findings are not concordant, the clinician should carefully reconsider the assessment of the patient's problem. Incorporating the patient into the health care team's decision-making empowers the patient to determine the level of breast cancer risk that is personally acceptable in the screening/follow-up settings.

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Table 1: Breast Cysts - Types and Definitions

Simple cyst	Anechoic (cystic), well circumscribed, round or oval with well-defined imperceptible wall and posterior enhancement.
Non-simple cyst	Has one or more characteristics not found in a simple cyst.
• complicated	Has most but not all elements of a simple cyst. Complicated cysts do not contain solid elements, intracystic masses, thick walls, or thick septa. This type of cyst may contain low-level echoes or intracystic debris, and can be described as a round, circumscribed mass containing low level echoes without vascular flow, fulfilling most but not all criteria of a simple cyst.
• complex	Has some discrete solid component which may include thick walls, thick septa, and/or intracystic mass. Complex cysts have both anechoic (cystic) and echogenic (solid) components.
References	95-100,103,145

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References

1. American Cancer Society. Breast Cancer Facts and Figures 2009-2010. Atlanta: American Cancer Society, Inc. Available at: http://www.cancer.org/Research/CancerFactsFigures/BreastCancerFactsFigures/breast-cancer-facts--figures-2009-2010.

2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/>23335087.

3. Humphrey LL, Helfand M, Chan BK, Woolf SH. Breast cancer screening: a summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2002;137:347-360. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12204020.

4. Bevers TB. Ultrasound for the screening of breast cancer. Curr Oncol Rep 2008;10:527-528. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18928668.

5. Barton MB, Harris R, Fletcher SW. The rational clinical examination. Does this patient have breast cancer? The screening clinical breast examination: should it be done? How? JAMA 1999;282:1270-1280. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10517431.

6. Bevers TB. Breast awareness: a shift in the paradigm of breast self-examination. J Natl Compr Canc Netw 2009;7:1042-1043. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1993097.

7. Bevers T. Breast Self-examination. In: Singletary SE, Robb GL, Hortobagyi GN, eds. Advanced Therapy of Breast Disease. 2nd ed. New York: B.C. Decker, Inc; 2004.

8. Thomas DB, Gao DL, Ray RM, et al. Randomized trial of breast self-examination in Shanghai: final results. J Natl Cancer Inst 2002;94:1445-1457. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12359854.

9. Armstrong K, Moye E, Williams S, et al. Screening mammography in women 40 to 49 years of age: a systematic review for the American College of Physicians. Ann Intern Med 2007;146:516-526. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17404354.

10. Buist DSM, Porter PL, Lehman C, et al. Factors contributing to mammography failure in women aged 40-49 years. J Natl Cancer Inst 2004;96:1432-1440. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15467032.

11. Fletcher SW, Elmore JG. Clinical practice. Mammographic screening for breast cancer. N Engl J Med 2003;348:1672-1680. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12711743.

12. Gotzsche PC, Nielsen M. Screening for breast cancer with mammography. Cochrane Database Syst Rev 2006. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17054145.

13. Moss SM, Cuckle H, Evans A, et al. Effect of mammographic screening from age 40 years on breast cancer mortality at 10 years' follow-up: a randomised controlled trial. Lancet 2006;368:2053-2060. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17161727.

14. van Schoor G, Broeders MJM, Paap E, et al. A rationale for starting breast cancer screening under age 50. Ann Oncol 2008;19:1208-1209. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18448450.

15. Smith RA, Saslow D, Sawyer KA, et al. American Cancer Society guidelines for breast cancer screening: update 2003. CA Cancer J Clin 2003;53:141-169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12809408.

16. Screening for Breast Cancer: U.S. Preventive Services Task Force Recommendation Statement. Annals of Internal Medicine 2009;151:716-726. Available at: http://dx.doi.org/10.7326/0003-4819-151-10-200911170-00008.

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17. Mandelblatt JS, Cronin KA, Bailey S, et al. Effects of mammography screening under different screening schedules: model estimates of potential benefits and harms. Ann Intern Med 2009;151:738-747. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19920274.

18. Badgwell BD, Giordano SH, Duan ZZ, et al. Mammography before diagnosis among women age 80 years and older with breast cancer. J Clin Oncol 2008;26:2482-2488. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18427152.

19. Mandelblatt JS, Silliman R. Hanging in the balance: making decisions about the benefits and harms of breast cancer screening among the oldest old without a safety net of scientific evidence. J Clin Oncol 2009;27:487-490. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19075258.

20. van Dijck J, Verbeek A, Hendriks J, et al. Mammographic screening after the age of 65 years: early outcomes in the Nijmegen programme. Br J Cancer 1996;74:1838-1842. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8956803.

21. Walter LC, Covinsky KE. Cancer screening in elderly patients: a framework for individualized decision making. JAMA 2001;285:2750-2756. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11386931.

22. Bleyer A, Welch HG. Effect of three decades of screening mammography on breast-cancer incidence. N Engl J Med 2012;367:1998-2005. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23171096.

23. Garfinkel L, Boring CC, Heath CW, Jr. Changing trends. An overview of breast cancer incidence and mortality. Cancer 1994;74:222-227. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8004590.

24. Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. J Natl Cancer Inst 1999;91:1541-1548. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10491430.

25. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81:1879-1886. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2593165.

26. Gail MH, Costantino JP. Validating and improving models for projecting the absolute risk of breast cancer. J Natl Cancer Inst 2001;93:334-335. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11238688.

27. Rockhill B, Spiegelman D, Byrne C, et al. Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. J Natl Cancer Inst 2001;93:358-366. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11238697.

28. Spiegelman D, Colditz GA, Hunter D, Hertzmark E. Validation of the Gail et al. model for predicting individual breast cancer risk. J Natl Cancer Inst 1994;86:600-607. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8145275.

29. Gail MH, Costantino JP, Pee D, et al. Projecting individualized absolute invasive breast cancer risk in African American women. J Natl Cancer Inst 2007;99:1782-1792. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18042936.

30. Matsuno RK, Costantino JP, Ziegler RG, et al. Projecting Individualized Absolute Invasive Breast Cancer Risk in Asian and Pacific Islander American Women. J Natl Cancer Inst 2011. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21562243.

31. Arpino G, Laucirica R, Elledge RM. Premalignant and in situ breast disease: biology and clinical implications. Ann Intern Med 2005;143:446-457. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16172443.

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32. Lakhani SR. In-situ lobular neoplasia: time for an awakening. Lancet 2003;361:96. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12531570.

33. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007;57:75-89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17392385.

34. Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. Cancer 1994;73:643-651. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8299086.

35. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med 2004;23:1111-1130. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15057881.

36. Amir E, Evans DG, Shenton A, et al. Evaluation of breast cancer risk assessment packages in the family history evaluation and screening programme. J Med Genet 2003;40:807-814. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14627668.

37. Evans DG, Howell A. Breast cancer risk-assessment models. Breast Cancer Res 2007;9:213. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17888188.

38. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007;57:75-89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17392385.

39. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet 1998;62:145-158. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9443863.

40. Antoniou AC, Cunningham AP, Peto J, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. Br J Cancer 2008;98:1457-1466. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18349832.

41. Bhatia S, Robison LL, Oberlin O, et al. Breast cancer and other second neoplasms after childhood Hodgkin's disease. N Engl J Med 1996;334:745-751. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8592547.

42. Bhatia S, Yasui Y, Robison LL, et al. High risk of subsequent neoplasms continues with extended follow-up of childhood Hodgkin's disease: report from the Late Effects Study Group. J Clin Oncol 2003;21:4386-4394. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14645429.

43. Hancock SL, Tucker MA, Hoppe RT. Breast cancer after treatment of Hodgkin's disease. J Natl Cancer Inst 1993;85:25-31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8416252.

44. Metayer C, Lynch CF, Clarke EA, et al. Second cancers among long-term survivors of Hodgkin's disease diagnosed in childhood and adolescence. J Clin Oncol 2000;18:2435-2443. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10856104.

45. van Leeuwen FE, Klokman WJ, Stovall M, et al. Roles of radiation dose, chemotherapy, and hormonal factors in breast cancer following Hodgkin's disease. J Natl Cancer Inst 2003;95:971-980. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12837833.

46. Yahalom J, Petrek JA, Biddinger PW, et al. Breast cancer in patients irradiated for Hodgkin's disease: a clinical and pathologic analysis of 45 events in 37 patients. J Clin Oncol 1992;10:1674-1681. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1403050.

47. Travis LB, Hill D, Dores GM, et al. Cumulative absolute breast cancer risk for young women treated for Hodgkin lymphoma. J Natl

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Cancer Inst 2005;97:1428-1437. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16204692.

48. Oeffinger KC, Ford JS, Moskowitz CS, et al. Breast cancer surveillance practices among women previously treated with chest radiation for a childhood cancer. JAMA 2009;301:404-414. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19176442.

49. Ng AK, Garber JE, Diller LR, et al. Prospective study of the efficacy of breast magnetic resonance imaging and mammographic screening in survivors of hodgkin lymphoma. J Clin Oncol 2013;31:2282-2288. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23610104.

50. Children's Oncology Group Long-term Follow-up Guidelines. Version 3. 2008. Available at: www.survivorshipguidelines.org.

51. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003;21:2397-2406. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12692171.

52. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998;62:676-689. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9497246.

53. Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. JAMA 2004;292:1317-1325. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15367553.

54. Leach MO, Boggis CR, Dixon AK, et al. Screening with magnetic resonance imaging and mammography of a UK population at high familial risk of breast cancer: a prospective multicentre cohort study (MARIBS). Lancet 2005;365:1769-1778. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15910949.

55. Stoutjesdijk MJ, Boetes C, Jager GJ, et al. Magnetic resonance imaging and mammography in women with a hereditary risk of breast cancer. J Natl Cancer Inst 2001;93:1095-1102. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11459871.

56. Kuhl CK, Schrading S, Leutner CC, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. J Clin Oncol 2005;23:8469-8476. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16293877.

57. Tilanus-Linthorst M, Verhoog L, Obdeijn IM, et al. A BRCA1/2 mutation, high breast density and prominent pushing margins of a tumor independently contribute to a frequent false-negative mammography. Int J Cancer 2002;102:91-95. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12353239.

58. Goldfrank D, Chuai S, Bernstein JL, et al. Effect of mammography on breast cancer risk in women with mutations in BRCA1 or BRCA2. Cancer Epidemiol Biomarkers Prev 2006;15:2311-2313. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17119064.

59. Rebbeck TR, Domchek SM. Variation in breast cancer risk in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 2008;10:108-108. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18710587.

60. Liede A, Karlan BY, Narod SA. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. J Clin Oncol 2004;22:735-742. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14966099.

61. Couch FJ, Farid LM, DeShano ML, et al. BRCA2 germline mutations in male breast cancer cases and breast cancer families. Nat Genet 1996;13:123-125. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8673091.

62. Friedman LS, Gayther SA, Kurosaki T, et al. Mutation analysis of BRCA1 and BRCA2 in a male breast cancer population. Am J Hum

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Genet 1997;60:313-319. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9012404.

63. Thompson D, Easton D. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. Am J Hum Genet 2001;68:410-419. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11170890.

64. What are the key statistics about breast cancer in men? 2012. Available at: http://www.cancer.org/Cancer/BreastCancerinMen/DetailedGuide/breast-cancer-in-men-key-statistics.

65. Nemec CF, Listinsky J, Rim A. How should we screen for breast cancer? Mammography, ultrasonography, MRI. Cleve Clin J Med 2007;74:897-904. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18183840.

66. Carney PA, Miglioretti DL, Yankaskas BC, et al. Individual and combined effects of age, breast density, and hormone replacement therapy use on the accuracy of screening mammography. Ann Intern Med 2003;138:168-175. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12558355.

67. Berg WA, Blume JD, Cormack JB, et al. Combined screening with ultrasound and mammography vs mammography alone in women at elevated risk of breast cancer. JAMA 2008;299:2151-2163. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18477782.

68. Pisano ED, Gatsonis C, Hendrick E, et al. Diagnostic performance of digital versus film mammography for breast-cancer screening. N Engl J Med 2005;353:1773-1783. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16169887.

69. Pisano ED, Hendrick RE, Yaffe MJ, et al. Diagnostic accuracy of digital versus film mammography: exploratory analysis of selected population subgroups in DMIST. Radiology 2008;246:376-383. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18227537.

70. Skaane P, Hofvind S, Skjennald A. Randomized trial of screen-film versus full-field digital mammography with soft-copy reading in population-based screening program: follow-up and final results of Oslo II study. Radiology 2007;244:708-717. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17709826.

71. Skaane P, Skjennald A. Screen-film mammography versus full-field digital mammography with soft-copy reading: randomized trial in a population-based screening program--the Oslo II Study. Radiology 2004;232:197-204. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15155893.

72. Skaane P, Young K, Skjennald A. Population-based mammography screening: comparison of screen-film and full-field digital mammography with soft-copy reading--Oslo I study. Radiology 2003;229:877-884. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14576447.

73. Lewin JM, Hendrick RE, D'Orsi CJ, et al. Comparison of full-field digital mammography with screen-film mammography for cancer detection: results of 4,945 paired examinations. Radiology 2001;218:873-880. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11230669.

74. Chiu SY, Duffy S, Yen AM, et al. Effect of baseline breast density on breast cancer incidence, stage, mortality, and screening parameters: 25-year follow-up of a Swedish mammographic screening. Cancer Epidemiol Biomarkers Prev 2010;19:1219-1228. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20406961.

75. Vachon CM, Sellers TA, Scott CG, et al. Longitudinal breast density and risk of breast cancer [abstract]. Cancer Research 2011;70:Abstract 4828. Available at: http://cancerres.aacrjournals.org/cgi/content/meeting_abstract/70/8_MeetingAbstracts/4828.

76. Food and Drug Administration. Quality mammography standards; correction—FDA: final rule; correction. 62 Federal Register 60613-60632 (1997).

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77. American College of Radiology (ACR) Breast Imaging Reporting and Data System Atlas (BI-RADS® Atlas). Reston, Va: © American College of Radiology; 2003. Available at: http://www.acr.org/secondarymainmenucategories/quality_safety/birads atlas/biradsatlasexcerptedtext/biradsmammographyfourthedition.aspx.

78. Lord SJ, Lei W, Craft P, et al. A systematic review of the effectiveness of magnetic resonance imaging (MRI) as an addition to mammography and ultrasound in screening young women at high risk of breast cancer. Eur J Cancer 2007;43:1905-1917. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17681781.

79. Mann RM, Kuhl CK, Kinkel K, Boetes C. Breast MRI: guidelines from the European Society of Breast Imaging. Eur Radiol 2008;18:1307-1318. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18389253.

80. Schnall M, Orel S. Breast MR imaging in the diagnostic setting. Magn Reson Imaging Clin N Am 2006;14:329-337, vi. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17098174.

81. Kriege M, Brekelmans CTM, Boetes C, et al. Differences between first and subsequent rounds of the MRISC breast cancer screening program for women with a familial or genetic predisposition. Cancer 2006;106:2318-2326. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16615112.

82. Kriege M, Brekelmans CTM, Obdeijn IM, et al. Factors affecting sensitivity and specificity of screening mammography and MRI in women with an inherited risk for breast cancer. Breast Cancer Res Treat 2006;100:109-119. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16791481.

83. Robson ME, Offit K. Breast MRI for women with hereditary cancer risk. JAMA 2004;292:1368-1370. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15367560.

84. Warner E. The role of magnetic resonance imaging in screening women at high risk of breast cancer. Top Magn Reson Imaging 2008;19:163-169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18941396.

85. Warner E, Messersmith H, Causer P, et al. Systematic review: using magnetic resonance imaging to screen women at high risk for breast cancer. Ann Intern Med 2008;148:671-679. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18458280.

86. Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. JAMA 2004;292:1317-1325. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15367553.

87. Lehman CD, Smith RA. The role of MRI in breast cancer screening. J Natl Compr Canc Netw 2009;7:1109-1115. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19930977.

88. Port ER, Park A, Borgen PI, et al. Results of MRI screening for breast cancer in high-risk patients with LCIS and atypical hyperplasia. Ann Surg Oncol 2007;14:1051-1057. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17206485.

89. ACR practise guideline for the performance of contrast-enhanced magnetic resonance imaging (MRI) of the breast 2012. Available at: http://www.acr.org/Search?q=guidelines%20for%20contrast-enhanced %20MRI%20of%20the%20breast.

90. Bassett L, Winchester DP, Caplan RB, et al. Stereotactic core-needle biopsy of the breast: a report of the Joint Task Force of the American College of Radiology, American College of Surgeons, and College of American Pathologists. CA Cancer J Clin 1997;47:171-190. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9152175.

91. Buchberger W, DeKoekkoek-Doll P, Springer P, et al. Incidental findings on sonography of the breast: clinical significance and

Discussion
Updates
Proof

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diagnostic workup. AJR Am J Roentgenol 1999;173:921-927. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10511149.

92. Corsetti V, Houssami N, Ferrari A, et al. Breast screening with ultrasound in women with mammography-negative dense breasts: evidence on incremental cancer detection and false positives, and associated cost. Eur J Cancer 2008;44:539-544. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18267357.

93. Kaplan SS. Clinical utility of bilateral whole-breast US in the evaluation of women with dense breast tissue. Radiology 2001;221:641-649. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11719658.

94. American College Radiology Ultrasound BI-RADS Final Assessment Categories. Available at: http://www.acr.org/Quality-Safety/Resources/BIRADS/Ultrasound.

95. Booi RC, Carson PL, O'Donnell M, et al. Characterization of cysts using differential correlation coefficient values from two dimensional breast elastography: preliminary study. Ultrasound Med Biol 2008;34:12-21.

96. Daly CP, Bailey JE, Klein KA, Helvie MA. Complicated breast cysts on sonography: is aspiration necessary to exclude malignancy? Acad Radiol 2008;15:610-617. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18423318.

97. Huff JG. The sonographic findings and differing clinical implications of simple, complicated, and complex breast cysts. J Natl Compr Canc Netw 2009;7:1101-1104; quiz 1105. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19930976.

98. Venta LA, Kim JP, Pelloski CE, Morrow M. Management of complex breast cysts. AJR Am J Roentgenol 1999;173:1331-1336. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10541113.

99. Doshi DJ, March DE, Crisi GM, Coughlin BF. Complex cystic breast masses: diagnostic approach and imaging-pathologic correlation. Radiographics 2007;27 Suppl 1:53-64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18180235.

100. Tea M-KM, Grimm C, Fink-Retter A, et al. The validity of complex breast cysts after surgery. Am J Surg 2009;197:199-202. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18649871.

101. Renz DM, Baltzer PAT, Bottcher J, et al. Inflammatory breast carcinoma in magnetic resonance imaging: a comparison with locally advanced breast cancer. Acad Radiol 2008;15:209-221. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18206620.

102. Abati A, Simsir A. Breast fine needle aspiration biopsy: prevailing recommendations and contemporary practices. Clin Lab Med 2005;25:631-654. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16308084.

103. Levine P, Simsir A, Cangiarella J. Management issues in breast lesions diagnosed by fine-needle aspiration and percutaneous core breast biopsy. Am J Clin Pathol 2006;125 Suppl:S124-134. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16830962.

104. Pijnappel RM, van den Donk M, Holland R, et al. Diagnostic accuracy for different strategies of image-guided breast intervention in cases of nonpalpable breast lesions. Br J Cancer 2004;90:595-600. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14760370.

105. Pisano ED, Fajardo LL, Caudry DJ, et al. Fine-needle aspiration biopsy of nonpalpable breast lesions in a multicenter clinical trial: results from the radiologic diagnostic oncology group V. Radiology 2001;219:785-792. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11376270.

106. Fishman JE, Milikowski C, Ramsinghani R, et al. US-guided core-needle biopsy of the breast: how many specimens are necessary?

Discussion

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Radiology 2003;226:779-782. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12601206.

107. Yeow KM, Lo YF, Wang CS, et al. Ultrasound-guided core needle biopsy as an initial diagnostic test for palpable breast masses. J Vasc Interv Radiol 2001;12:1313-1317. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11698631.

108. Verkooijen HM. Diagnostic accuracy of stereotactic large-core needle biopsy for nonpalpable breast disease: results of a multicenter prospective study with 95% surgical confirmation. Int J Cancer 2002;99:853-859. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12115488.

109. Pandelidis S, Heiland D, Jones D, et al. Accuracy of 11-gauge vacuum-assisted core biopsy of mammographic breast lesions. Ann Surg Oncol 2003;10:43-47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12513959.

110. Sigal-Zafrani B, Muller K, El Khoury C, et al. Vacuum-assisted large-core needle biopsy (VLNB) improves the management of patients with breast microcalcifications - analysis of 1009 cases. Eur J Surg Oncol 2008;34:377-381. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17604937.

111. Burbank F, Forcier N. Tissue marking clip for stereotactic breast biopsy: initial placement accuracy, long-term stability, and usefulness as a guide for wire localization. Radiology 1997;205:407-415. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9356621.

112. Egyed Z, Pentek Z, Jaray B, et al. Radial scar-significant diagnostic challenge. Pathol Oncol Res 2008;14:123-129. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18409019.

113. Cangiarella J, Guth A, Axelrod D, et al. Is surgical excision necessary for the management of atypical lobular hyperplasia and lobular carcinoma in situ diagnosed on core needle biopsy?: a report of 38 cases and review of the literature. Arch Pathol Lab Med 2008;132:979-983. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18517282.

114. Elsheikh TM, Silverman JF. Follow-up surgical excision is indicated when breast core needle biopsies show atypical lobular hyperplasia or lobular carcinoma in situ: a correlative study of 33 patients with review of the literature. Am J Surg Pathol 2005;29:534-543. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15767810.

115. Frouge C, Tristant H, Guinebretiere JM, et al. Mammographic lesions suggestive of radial scars: microscopic findings in 40 cases. Radiology 1995;195:623-625. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7753984.

116. Margenthaler JA, Duke D, Monsees BS, et al. Correlation between core biopsy and excisional biopsy in breast high-risk lesions. Am J Surg 2006;192:534-537. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16978969.

117. Parker SH, Burbank F, Jackman RJ, et al. Percutaneous large-core breast biopsy: a multi-institutional study. Radiology 1994;193:359-364. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7972743.

118. Linell F, Pinder SE, Ellis IO. Precursor lesions of breast carcinoma. The Breast 1993;2:220-223. Available at: http://www.sciencedirect.com/science/article/pii/096097769390003X.

119. Middleton LP, Grant S, Stephens T, et al. Lobular carcinoma in situ diagnosed by core needle biopsy: when should it be excised? Mod Pathol 2003;16:120-129. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12591964.

120. Sohn VY, Arthurs ZM, Kim FS, Brown TA. Lobular neoplasia: is surgical excision warranted? Am Surg 2008;74:172-177. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18306873.

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121. Bowman K, Munoz A, Mahvi DM, Breslin TM. Lobular neoplasia diagnosed at core biopsy does not mandate surgical excision. J Surg Res 2007;142:275-280.

122. Kopans DB. LCIS found at core needle biopsy may not need surgical excision. AJR Am J Roentgenol 2008;191:W152; author reply W153.

123. O'Neil M, Madan R, Tawfik OW, et al. Lobular carcinoma in situ/atypical lobular hyperplasia on breast needle biopsies: does it warrant surgical excisional biopsy? A study of 27 cases. Ann Diagn Pathol 2010;14:251-255. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20637429.

124. Rendi MH, Dintzis SM, Lehman CD, et al. Lobular in-situ neoplasia on breast core needle biopsy: imaging indication and pathologic extent can identify which patients require excisional biopsy. Ann Surg Oncol 2012;19:914-921. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21861212.

125. Nagi CS, O'Donnell JE, Tismenetsky M, et al. Lobular neoplasia on core needle biopsy does not require excision. Cancer 2008;112:2152-2158. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18348299.

126. Lehman CD, Lee CI, Loving VA, et al. Accuracy and value of breast ultrasound for primary imaging evaluation of symptomatic women 30-39 years of age. AJR Am J Roentgenol 2012;199:1169-1177. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23096195.

127. Hussain AN, Policarpio C, Vincent MT. Evaluating nipple discharge. Obstet Gynecol Surv 2006;61:278-283. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16551379.

128. Jain A, Crawford S, Larkin A, et al. Management of nipple discharge: technology chasing application. Breast J 2010;16:451-452. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20443787.

129. Markopoulos C, Mantas D, Kouskos E, et al. Surgical management of nipple discharge. Eur J Gynaecol Oncol 2006;27:275-278. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16800258.

130. Fuchsjager MH, Philipp MO, Loewe C, Helbich TH. [Diagnostic management of nipple discharge]. Wien Klin Wochenschr 2003;115 Suppl 2:33-39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15518144.

131. Jardines L. Management of nipple discharge. Am Surg 1996;62:119-122. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8554189.

132. Ohuchi N, Furuta A, Mori S. Management of ductal carcinoma in situ with nipple discharge. Intraductal spreading of carcinoma is an unfavorable pathologic factor for breast-conserving surgery. Cancer 1994;74:1294-1302. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8055451.

133. Gulay H, Bora S, Kilicturgay S, et al. Management of nipple discharge. J Am Coll Surg 1994;178:471-474. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8167884.

134. Leis HP, Jr. Management of nipple discharge. World J Surg 1989;13:736-742. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2696228.

135. Kapenhas-Valdes E, Feldman SM, Boolbol SK. The role of mammary ductoscopy in breast cancer: a review of the literature. Ann Surg Oncol 2008;15:3350-3360. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18841418.

136. Simpson JS, Connolly EM, Leong WL, et al. Mammary ductoscopy in the evaluation and treatment of pathologic nipple discharge: a Canadian experience. Can J Surg 2009;52:E245-248. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20011159.

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137. Kapenhas-Valdes E, Feldman SM, Cohen JM, Boolbol SK. Mammary ductoscopy for evaluation of nipple discharge. Ann Surg Oncol 2008;15:2720-2727. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18685898.

138. Tokuda Y, Kuriyama K, Nakamoto A, et al. Evaluation of suspicious nipple discharge by magnetic resonance mammography based on breast imaging reporting and data system magnetic resonance imaging descriptors. J Comput Assist Tomogr 2009;33:58-62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19188786.

139. Ballesio L, Maggi C, Savelli S, et al. Role of breast magnetic resonance imaging (MRI) in patients with unilateral nipple discharge: preliminary study. Radiol Med 2008;113:249-264. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18386126.

140. Foulkes RE, Heard G, Boyce T, et al. Duct excision is still necessary to rule out breast cancer in patients presenting with spontaneous bloodstained nipple discharge. Int J Breast Cancer 2011;2011:495315. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22295227.

141. Dawood S, Cristofanilli M. What progress have we made in managing inflammatory breast cancer? Oncology (Williston Park) 2007;21:673-679. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17564325.

142. Haagensen C. Inflammatory carcinoma. In: Diseases of the breast. 3rd ed. Philadelphia: WB Saunders; 1986. Available at:

143. Sakorafas GH, Blanchard DK, Sarr MG, Farley DR. Paget's disease of the breast: a clinical perspective. Langenbecks Arch Surg 2001;386:444-450. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11735019.

144. Sakorafas GH, Blanchard K, Sarr MG, Farley DR. Paget's disease of the breast. Cancer Treat Rev 2001;27:9-18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11237774.

145. Berg WA, Campassi CI, Ioffe OB. Cystic lesions of the breast: sonographic-pathologic correlation. Radiology 2003;227:183-191. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12668745.

Discussion
Update in Progress

Use of Digital Breast Tomosynthesis with Mammography for Breast Cancer Screening or Diagnosis

Assessment Program
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Executive Summary

Background

Digital breast tomosynthesis was developed to improve the accuracy of mammography by capturing three-dimensional (3D) images of the breast to clarify areas of overlapping tissue. Digital breast tomosynthesis produces a 3D image by taking multiple low-dose images per view along an arc over the breast. During breast tomosynthesis, the compressed breast remains stationary while the x-ray tube moves approximately 1 degree for each image in a 15- to 50-degree arc, acquiring 11–49 images. These images are projected as cross-sectional "slices" of the breast, with each slice typically 1 mm thick. By reducing problems caused by overlapping tissue, the amount of compression needed for digital breast tomosynthesis can be reduced by up to 50% compared with mammography, thereby improving patient satisfaction.

Objective

To compare the sensitivity and specificity, as well as the recall rate, of mammography and digital breast tomosynthesis for screening or diagnosis.

Search Strategy

A search was conducted in June 2013 of the MEDLINE® (via PubMed) and EMBASE databases, limited to studies in humans, published in English, and using the following terms: ((breast OR mammary) AND tomosynthesis) OR ("Mammography"[MeSH] AND (3D OR "3-D" OR "3-dimensional" OR "three-dimensional")). The Cochrane database was also searched for applicable studies. In addition, relevant journals and newsletters were monitored subsequent to the search to identify new publications.

Selection Criteria

- The population of women represented at least part of the spectrum of findings encountered in clinical practice; for example, studies that only include women with cancer would be excluded.
- The study used digital breast tomosynthesis in addition to mammography for screening. For diagnosis, breast tomosynthesis was compared with mammographic spot views, with or without results from other tests such as ultrasound.
- Two views of mammography (craniocaudal [CC] and mediolateral-oblique [MLO]) were compared with mammography plus the same two views of breast tomosynthesis.
- The reference standard was histopathology or long-term (minimum of 6 months) follow-up for women with negative findings, i.e., no indication of possible cancer.
- The equipment used was similar to the U.S. Food and Drug Administration (FDA) -approved Hologic system.

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Review

Overview of the evidence on digital breast tomosynthesis in breast cancer detection

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ABSTRACT

Digital breast tomosynthesis (DBT, or 3D-mammography), a three-dimensional derivative of digital mammography (DM), reduces the effect of tissue superimposition and may improve mammographic interpretation. In this review, we examined the evidence on the accuracy of DBT in clinical studies. Published studies of DBT were relatively small studies, mostly test-set observer (reader) studies or clinical series that included symptomatic and screen-recalled cases, and were generally enriched with cancers. With these limitations in mind, the evidence showed some consistent findings, summarized as follows: two-view DBT has at least equal or better accuracy than standard two-view DM, whereas one-view DBT does not have better accuracy than standard DM; the *addition* of DBT to standard mammography (for mammographic interpretation or for assessment or triage of screen-recalled abnormalities) increases accuracy; improved accuracy from using DBT (relative to, or added to, DM) may be due to increased cancer detection or due to reduced false positive recalls, or both; and subjective interpretation of cancer conspicuity consistently found that cancers were equally or more conspicuous on DBT relative to DM.

Preliminary data from population screening trials suggest that the integration of DBT with conventional DM (screen-reading using combined 2D + 3D mammography) may substantially improve breast cancer detection, although final results are not yet available, and many logistical issues need further evaluation to determine the potential implications and cost of combined 2D + 3D mammographic screening. At present, there is insufficient evidence to justify a change from standard DM to DBT however the available data strongly support investment in new large-scale population screening trials. These trials need to avoid the 'double' acquisitions required for 2D + 3D mammograms, and should therefore focus on evaluating integrated 2Dsynthetic + 3D mammography (where 2D-images are reconstructed from the DBT acquisition), and should consider using a randomized design.

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Introduction

The evolution of conventional two-view mammography from film-screen to full-field digital mammography (FFDM or DM), has paved the way for digital breast tomosynthesis (DBT), a 3-dimensional transformation of FFDM. A proposed advantage of this derivative digital mammographic technology is its potential to improve breast imaging by providing a series of images (thin slices) through the breast which reduces the effect of tissue superimposition. Consequently, this may allow better visualization of malignancy (by reducing tissue overlap and the masking effect of tissue density), and may also facilitate discrimination between normal tissue structures and lesions. Thus, in theory, DBT could lead to improvement in sensitivity and/or specificity relative to standard mammography. At present, DBT is undergoing further technological refinement and is also the focus of much interest through clinical research, with several large-scale screening studies in progress or nearing completion; these trials will potentially provide evidence to guide the future application of DBT. In the context of breast imaging, DBT is in essence 'at a translational crossroads' as aptly said by Dobbins.[1]

DBT technology

The technologic derivation of DBT from FFDM platforms, and description of both prototype and state-of-the art DBT systems and

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Assessing Radiologist Performance Using Combined Digital Mammography and Breast Tomosynthesis Compared with Digital Mammography Alone: Results of a Multicenter, Multireader Trial[1]

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Purpose: To compare radiologists' diagnostic accuracy and recall rates for breast tomosynthesis combined with digital mammography versus digital mammography alone.

Materials and Methods: Institutional review board approval was obtained at each accruing institution. Participating women gave written informed consent. Mediolateral oblique and craniocaudal digital mammographic and tomosynthesis images of both breasts were obtained from 1192 subjects. Two enriched reader studies were performed to compare digital mammography with tomosynthesis against digital mammography alone. Study 1 comprised 312 cases (48 cancer cases) with images read by 12 radiologists; study 2, 312 cases (51 cancer cases) with 15 radiologists. Study 1 readers recorded only that an abnormality requiring recall was present; study 2 readers had additional training and recorded both lesion type and location. Diagnostic accuracy was compared with receiver operating characteristic analysis. Recall rates of noncancer cases, sensitivity, specificity, and positive and negative predictive values determined by analyzing Breast Imaging Reporting and Data System scores were compared for the two methods.

Results: Diagnostic accuracy for combined tomosynthesis and digital mammography was superior to that of digital mammography alone. Average difference in area under the curve in study 1 was 7.2% (95% confidence interval [CI]: 3.7%, 10.8%; $P < .001$) and in study 2 was 6.8% (95% CI: 4.1%, 9.5%; $P < .001$). All 27 radiologists increased diagnostic accuracy with addition of tomosynthesis. Recall rates for noncancer cases for all readers significantly decreased with addition of tomosynthesis (range, 6%–67%; $P < .001$ for 25 readers, $P < .03$ for all readers). Increased sensitivity was largest for invasive cancers: 15% and 22% in studies 1 and 2 versus 3% for in situ cancers in both studies.

Conclusion: Addition of tomosynthesis to digital mammography offers the dual benefit of significantly increased diagnostic accuracy and significantly reduced recall rates for noncancer cases.

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Comparison of Digital Mammography Alone and Digital Mammography Plus Tomosynthesis in a Population-based Screening Program[1]

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Purpose: To assess cancer detection rates, false-positive rates before arbitration, positive predictive values for women recalled after arbitration, and the type of cancers detected with use of digital mammography alone and combined with tomosynthesis in a large prospective screening trial.

Materials and Methods: A prospective, reader- and modality-balanced screening study of participants undergoing combined mammography plus tomosynthesis, the results of which were read independently by four different radiologists, is under way. The study was approved by a regional ethics committee, and all participants provided written informed consent. The authors performed a preplanned interim analysis of results from 12631 examinations interpreted by using mammography alone and mammography plus tomosynthesis from November 22, 2010, to December 31, 2011. Analyses were based on marginal log-linear models for binary data, accounting for correlated interpretations and adjusting for reader-specific performance levels by using a two-sided significance level of .0294.

Results: Detection rates, including those for invasive and in situ cancers, were 6.1 per 1000 examinations for mammography alone and 8.0 per 1000 examinations for mammography plus tomosynthesis (27% increase, adjusted for reader; $P = .001$). False-positive rates before arbitration were 61.1 per 1000 examinations with mammography alone and 53.1 per 1000 examinations with mammography plus tomosynthesis (15% decrease, adjusted for reader; $P < .001$). After arbitration, positive predictive values for recalled patients with cancers verified later were comparable (29.1% and 28.5%, respectively, with mammography alone and mammography plus tomosynthesis; $P = .72$). Twenty-five additional invasive cancers were detected with mammography plus tomosynthesis (40% increase, adjusted for reader; $P < .001$). The mean interpretation time was 45 seconds for mammography alone and 91 seconds for mammography plus tomosynthesis ($P < .001$).

Conclusion: The use of mammography plus tomosynthesis in a screening environment resulted in a significantly higher cancer detection rate and enabled the detection of more invasive cancers.

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Clinical trial registration no. NCT01248546

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Medical Policy

Subject: Digital Breast Tomosynthesis
Policy #: RAD.00060 **Current Effective Date:** 01/14/2014
Status: Reviewed **Last Review Date:** 11/14/2013

Description/Scope

Digital breast tomosynthesis (three-dimensional [3-D] mammography) (DBT) is being investigated as an adjunct and alternative to x-ray mammography for the screening and diagnosis of breast cancer. DBT combines the use of tomography and 3-D reconstruction with breast imaging to improve the visibility of breast lesions.

DBT utilizes an x-ray tube which sweeps along an arc around the breast to acquire multiple two-dimensional (2-D) projections from slightly different angles in about 10 to 20 seconds. The resulting 2-D images are digitally maneuvered to create tomograms (i.e., slices) in any plane, thus allowing for 3-D reconstruction that reflects depth. The number of slices that can be created depends on the number of 2-D projections captured during image sweeping. The tomograms can be displayed individually (similar to enhanced conventional mammograms) or in a dynamic "movie" mode. It has been theorized that providing a 3-D reconstruction of the breast will eliminate the problem of overlying tissue that might be mistaken for lesions or that might obscure small cancers.

For information related to the use of diagnostic tests for breast cancer, please see the following documents:

- RAD.00002 Positron Emission Tomography (PET) and PET/CT Fusion
- RAD.00036 MRI of the Breast

Position Statement

Investigational and Not Medically Necessary:

Digital breast tomosynthesis is considered **investigational and not medically necessary** for all indications.

Rationale

In February 2011, the Center for Devices and Radiological Health of the Food and Drug Administration (FDA) granted premarket approval for the Selenia Dimensions 3D system. According to the premarket approval letter (P080003), "the device is indicated to generate digital mammographic images that can be used for screening and diagnosis of breast cancer." The Selenia Dimensions 3-D system is an improvement on the existing 2-D system. The FDA approval letter also states that the Selenia Dimensions (2D or 3D) system is intended for use in the same clinical applications as 2D mammography systems for screening mammograms and may be used for additional diagnostic workup of the breast.

As part of the premarket approval process, the FDA reviewed results from two studies where board-certified radiologists were asked to review 2-D and 3-D images from more than 300 mammography exams. In both studies, radiologists viewing the 2-D images in conjunction with the 3-D images obtained a seven percent improvement in their ability to distinguish between cancerous and non-cancerous cases versus viewing the 2-D images alone. While the combination of the Selenia's 2-D and 3-D images approximately doubled the amount of radiation the individual was exposed to, it improved the accuracy with which radiologists detected cancers, decreasing the number of women recalled for a diagnostic workup. The Selenia Dimensions System is marketed by Hologic Inc., located in Bedford, Massachusetts.

In May 2013, the FDA expanded the scope of the premarket approval for Hologic Inc. Selenia Dimensions System to include the C-View software, which generates a synthetic 2D image using information obtained from the multiple 3D views of the breast. With the expanded application,

"each screening examination may consist of: a 2D FFDM image set; or a 2D and 3D image set, where the 2D image can be either a FFDM or a 2D image generated from the 3D image set."

Chen and colleagues (2007) carried out a small pilot study of 13 women recruited between April 2005 and November 2005 to assess the clinical feasibility of contrast enhanced (CE) DBT as an adjunct to digital mammography, and to correlate lesion enhancement characteristics and morphology obtained with CE-DBT to digital mammography, magnetic resonance (MR) and ultrasound (US). After undergoing breast imaging evaluation (including digital mammography, US, and MR) as part of the parent multimodality breast imaging study (NCI-funded grant [PO1-CA85484]) the participants were evaluated with CEDBT. Eleven of the 13 participants had pathology proven malignancies, (6 invasive ductal carcinoma, 4 ductal carcinoma in situ, and 1 invasive lobular carcinoma). Suspicious lesions were demonstrated with CE-DBT in 10 of 11 cases of proven breast malignancy. The researchers concluded that when used in conjunction with diagnostic mammography, CE-DBT may be a potential alternative tool for breast lesion morphologic and vascular characterization.

A 2007 comparative study by Poplack and colleagues assessed the image quality of DBT with that of conventional mammography and estimated the recall rate of screening when tomosynthesis is used in addition to mammography. A total of 100 consecutive women with an abnormal digital screening mammogram were included in the study. During the initial evaluation each woman underwent a DBT examination using a research prototype tomosynthesis unit (Genesis, Hologic) and screening mammography using full-field digital technology. The image quality of DBT was subjectively rated as equivalent in 52% (51/99) or superior 37% (37/99) to diagnostic mammography in 89% (88/99) of cases. In the identification of masses, DBT image quality was rated as equivalent in 26% (5/19) or superior to diagnostic mammography in 68% (13/19) of cases. Masses constituted 19% (19/99) of screening-detected findings but were 35% (13/37) of findings in which DBT had superior image quality. Calcifications usually showed better image quality on diagnostic mammography than on DBT. The authors concluded that "tomosynthesis has comparable or superior image quality to that of film-screen mammography in the diagnostic setting, and it has the potential to decrease the recall rate when used adjunctively with digital screening mammography." However, limitations of the study included, but are not limited to, the small sample size and the fact that the study was unblinded, non-randomized and uncontrolled. It also remains to be seen how these data will generalize to other populations and in the clinical setting. DBT may perform differently in a general screening population than in the study group of women who were recruited because of a breast screening abnormality. In this study group, only 5% (5/98) of women had fatty breast composition; the remainder, 95% (93/98), had higher density compositions. In a population with a greater proportion of fatty breast composition, DBT may prove to be less effective.

Good and colleagues (2008) assessed issues associated with the interpretation of images produced by DBT. The study included the breast images of 30 women who underwent digital mammography and DBT. Nine board-certified radiologists were asked to detect and rate masses and microcalcification clusters using full-field digital mammography (FFDM) images, the 11 frames mode, and the reconstructed DBT images. When compared to digital mammography, the reconstructed DBT examination took much longer to review and interpret (1.58 ± 1.07 versus 2.72 ± 1.44 minutes). Three of the nine radiologists (33%) rated the tomosynthesis frame mode to be better than digital mammography, and 6 of the 9 radiologists (66%) rated the reconstructed DBT examination to be better than digital mammography. The detection rate of malignancies was somewhat better for the reconstructed DBT examination than for digital mammography (93.9% versus 90%), and the recall rate for nonmalignancies was somewhat lower (62.6% versus 64.3%). The researchers concluded that DBT may have great potential in screening and diagnostic breast imaging practices; however, additional studies are needed before this imaging approach finds its optimal role in the clinical environment.

Gur and colleagues (2009) carried out a retrospective observer study evaluating the diagnostic performance of FFDM with that of DBT. Eight experienced radiologists interpreted images from 125 selected examinations, 35 with verified findings of cancer and 90 with negative findings. The four display conditions incorporated FFDM alone, 11 low-dose projections, reconstructed DBT images, and a combined display mode of FFDM and DBT images. The longest mean time (2.39 ± 1.65 minutes) to view and rate an examination was for the combined mode in which readers first viewed and rated the FFDM images and then viewed the corresponding DBT images together with the FFDM images and rerated the images if deemed necessary. The radiologists had the shortest mean time (1.22 ± 1.15 minutes) for viewing and rating examinations when using the FFDM image displays (FFDM alone and FFDM before viewing of the DBT images). Researchers found

that digital mammography in combination with DBT were associated with a 30% reduction in recalls for the cancer-free examinations compared with the use of digital mammography alone. DBT alone reduced recall rates by approximately 10%. DBT did not substantially improve sensitivity. The authors acknowledged that while initial results are encouraging, further work is needed before DBT finds its optimal role in the clinical environment.

Gennaro and colleagues (2010) reported the results of a study which included 200 women who had at least one breast lesion discovered by mammography and/or ultrasound classified as doubtful or suspicious or probably malignant. The study participants underwent DBT with one view (mediolateral oblique [MLO]) of both breasts at a radiation dose noted to be comparable to that of standard screen-film mammography in two views (craniocaudal [CC] and MLO). Images were rated by six radiologists using the breast imaging reporting and data system (BIRADS) score. The overall clinical performance with DBT and FFDM for malignant versus all other cases was not significantly different (AUCs 0.851 vs. 0.836, p=0.645). The authors concluded that the clinical performance of DBT in one view at the same total dose as standard screen-film mammography is not inferior to digital mammography in two views.

In another 2010 study (Teertstra, 2010), researchers assessed mammography and DBT in 513 women with an abnormal screening mammogram or with clinical symptoms. The ACR BI-RADS criteria were used to classify the cases. In 112 newly detected cancers, DBT and mammography were each false-negative in 8 cases (7%). In three cases, both mammography and DBT failed to detect the carcinoma. The sensitivity of both techniques for the detection of breast cancer was 92.9%, and the specificity of mammography was 86.1% and 84.4% for DBT. The authors concluded that DBT can be used as an additional technique to mammography in individuals referred with an abnormal screening mammogram or with clinical symptoms. They also acknowledged that the additional lesions detected by DBT are also likely to be detected by other techniques used in the clinical work-up of these individuals.

A 2012 study by Michell and colleagues examined the impact of the addition of FFDM and DBT on the diagnostic accuracy of mammography in a population of women recalled following film-screen mammography alone. Women eligible for recruitment were those recalled for further evaluation of a mammographic abnormality found on routine filmscreen mammographic screening. A total of 738 women participated in the study (93.2% recruitment rate). Participants underwent bilateral, two-view FFDM and two-view DBT. Readers rated each lesion separately for probability of malignancy on screen-film mammography, FFDM, and then DBT. The scores were compared with the presence or absence of malignancy based on the final histology report. Following assessment 204 (26.8%) were diagnosed as malignant (147 invasive and 57 in-situ tumors), 286 (37.68%) as benign, and 269 (35.4%) as normal. The diagnostic accuracy was evaluated by using receiving operating characteristic (ROC) and measurement of area under the curve (AUC). The AUC values demonstrated a significant ($p = 0.0001$) improvement in the diagnostic accuracy with the addition of DBT combined with FFDM and film-screen mammography (AUC = 0.9671) when compared to FFDM plus film-screen mammography (AUC = 0.8949) and film-screen mammography alone (AUC = 0.7882). The authors acknowledged that the equipment from a single manufacturer was used in this study but the technique and features of DBT machines vary considerably between different manufacturers and clinical performance results are, therefore, not necessarily generalizable. The authors also state that "the conclusions that can be reached from this prospective preliminary study are limited by the method; the readers were not blinded, they read and scored the DBT images with knowledge of the film-screen mammography and FFDM findings."

Skaane and colleagues (2013) reported a preplanned interim analysis of two arms of a four-arm study comparing DM and DBT in a side-by-side feature analysis for cancer detection. This single institution, prospective screening trial, was sponsored by the manufacturer of the DBT equipment (Hologic, Inc.). Cancer detection rates, false-positive rates before arbitration, positive predictive values for women recalled after arbitration, and the type of cancers detected with use of digital mammography alone and combined with tomosynthesis were reported. Of the 29,652 women invited to participate in the study, 17,960 (60.6%) attended the screening program, with 12,631 consenting to participate in the study. Independent double reading was the standard of practice and any mammogram considered suspicious for malignancy by at least one reader were discussed at a consensus-based arbitration meeting. The diagnostic work-up, including additional views, ultrasonography, magnetic resonance imaging, and needle biopsy (if indicated), was performed by the same group of radiologists. Analyses were based on marginal log-linear models for binary data, accounting for correlated interpretations and adjusting for reader-specific performance levels by using a two-sided significance level of .0294. The cancer detection rate for mammography

alone was 6.1/1000 examinations (77 of 12,631 cases) and 8.0/1000 examinations (101 of 12,621 cases) with mammography plus tomosynthesis (an increase of 31%). The false positive rate was 61.1/1000 examinations (771 of 12,621 cases) with mammography alone and 53.1/1000 examinations (670 of 12,621 cases) with mammography plus tomosynthesis (a decrease of 13%). After arbitration, positive predictive values for recalled participants with cancers verified later were comparable with 29.1% for mammography alone and 28.5%, for mammography plus tomosynthesis. Twenty-five additional invasive cancers were identified with mammography plus tomosynthesis (40% increase, adjusted for reader; $P < 0.001$). The mean interpretation time was 45 seconds for mammography alone and 91 seconds for mammography plus tomosynthesis ($P < 0.001$).

The Society of Breast Imaging (SBI) and the American College of Radiology (ACR) statement on the on Skaane et al – Tomosynthesis Breast Cancer Screening Study states the following:

> While the study results are promising, they do not provide adequate information to define the role of tomosynthesis in clinical practice… Although the cancer detection rate was higher when tomosynthesis was added to mammography alone, it is not known if an equal incremental benefit will be realized in a second screening round. This small study does not supply statistical information regarding subgroups of women that might benefit, or might not benefit from adding tomosynthesis. How the technology will affect screening accuracy among women of different ages, risk profiles and parenchymal density is uncertain. In addition, how this technology would affect reader performance among U.S. radiologists with varying practice patterns and expertise is also uncertain. Other questions include whether computer aided detection will provide any further benefit, and if reconstructed images can be used, in lieu of standard full field digital images, to reduce radiation dose (ACR, 2013).

Rafferty and colleagues (2013) compared the diagnostic accuracy and recall rates for radiologists using breast tomosynthesis combined with digital mammography versus digital mammography alone. One thousand one hundred and ninety-two (1192) participants provided mediolateral oblique and craniocaudal digital mammographic and tomosynthesis images of both breasts. At each accruing site, a breast imager read the digital mammographic images and a second breast imager trained in tomosynthesis interpretation read the tomosynthesis images for each participant presenting for screening. The breast imager was blinded to the results of the other modality and the investigational examination images of the participants presenting for biopsy were not interpreted prospectively. Study 1 consisted of 312 cases (48 cancer cases) with images read by 12 radiologists while study 2 included 312 cases (51 cancer cases) with 15 radiologists. The readers in Study 1 recorded only that an abnormality requiring recall was present; the readers in study 2 received additional training and recorded both lesion type and location. Diagnostic accuracy was compared with receiver operating characteristic analysis. Recall rates of noncancerous cases, sensitivity, specificity, and positive and negative predictive values determined by analyzing Breast Imaging Reporting and Data System scores were compared for the two methods. The authors reported the diagnostic accuracy for combined tomosynthesis and digital mammography was better than that of digital mammography alone. The average difference in area under the curve in study 1 was 7.2% (95% confidence interval [CI]: 3.7%, 10.8%; $P = 0.001$). The average difference in area under the curve in study 2 was 6.8% (95% CI: 4.1%, 9.5%; $P = 0.001$). All 27 radiologists experienced an increase in diagnostic accuracy with addition of tomosynthesis. Recall rates for noncancerous cases for all readers decreased with the addition of tomosynthesis (range, 6%–67%; $P = 0.001$ for 25 readers, $P = 0.03$ for all readers). The increase in the rate of sensitivity was greatest for invasive cancers: 15% and 22% in studies 1 and 2 versus 3% for in situ cancers in both studies. The authors concluded that the addition of tomosynthesis to digital mammography provides the dual benefit of significantly increased diagnostic accuracy and significantly reduced recall rates for noncancerous cases. Some of the limitations of the study which were acknowledged by the authors include the fact that the reader studies were enriched, and the results in true screening populations may differ. The authors also acknowledge that there was an inherent inclusion bias against tomosynthesis with regards to cancer detection in a screening population. Almost all of the cancers were acquired in participants who were scheduled for biopsy and had been detected on conventional mammograms as part of standard screening evaluation. Therefore, it is likely that the study results underestimate the potential gains in sensitivity that might occur in clinical practice. Additional studies done in the clinical environment are needed to confirm the performance of tomosynthesis combined with digital mammography versus digital mammography alone.

The American College of Obstetricians and Gynecologists guidelines on breast cancer screening

considered color Doppler ultrasonography, computer-aided detection, positron emission tomography, scintimammography, and digital breast tomosynthesis but did not include them in the recommended screening methods. ACOG acknowledged that these technologies have shown promise in selected clinical situations or as adjuncts to mammography for breast cancer diagnosis but concluded that they are not considered alternatives to routine mammography (ACOG, 2011).

According to the National Comprehensive Cancer Network's (NCCN®) clinical practice guideline for breast cancer screening and diagnosis (NCCN, 2013), though studies show promise for digital breast tomosynthesis, there is currently insufficient evidence to recommend this technology for routine screening or diagnosis at this time.

Comparative data demonstrating that DBT is equal to or better than other breast imaging technologies with regards to accuracy, sensitivity, specificity, and recall rates are lacking. The published feasibility and experimental studies also have not made it clear if DBT should be utilized for diagnostic, screening or surveillance purposes or if it should be used in lieu of or in conjunction with other imaging technologies. In addition to these issues, there remains some concern regarding radiation exposure and the learning curve for radiologist to accurately interpret the DBT results.

Background/Overview

DBT is being investigated as an adjunct and alternative to x-ray mammography for the screening and diagnosis of breast cancer. DBT utilizes a three-dimensional (3-D) breast imaging technique based on full-field digital mammography. During DBT, a woman is typically positioned for the conventional mammography. A machine takes multiple images (slices) approximately one milliliter or less as it rotates around the breast. The images are then digitally manipulated by a computer and displayed as a 3-D, high-resolution image on a workstation. Developers of this technology propose that a 3-D reconstruction of the breast will eliminate the problem of overlying tissue that might be mistaken for lesions or that might mask small cancers.

Definitions

Ductal carcinoma in situ: The presence of abnormal cells in a milk duct in the breast; considered the earliest form of breast cancer.

Full-field digital mammography (FFDM): A technology which combines the x-ray generators and tubes used in analog screen-film mammography with a detector plate that then converts the x-rays into a digital signal.

Microcalcification: A tiny deposit of calcium in the breast that cannot be felt but can be detected on a mammogram; a cluster of these very small specks of calcium may indicate that cancer is present.

Coding

The following codes for treatments and procedures applicable to this document are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement policy. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

When services are Investigational and Not Medically Necessary:|
When the code describes a procedure indicated in the Position Statement section as investigational and not medically necessary.

CPT

76499	Unlisted diagnostic radiographic procedure [when specified as digital breast tomosynthesis]

ICD-9 Diagnosis ***[For dates of service prior to 10/01/2014]***
All diagnoses

ICD-10 Diagnosis ***[For dates of service on or after 10/01/2014]***
All diagnoses

References

Peer Reviewed Publications:

1. Andersson I, Ikeda DM, Zackrisson S, et al. Breast tomosynthesis and digital mammography: a comparison of breast cancer visibility and BIRADS classification in a population of cancers with subtle mammographic findings. Eur Radiol. 2008; 18(12):2817-2825.
2. Chen S, Carton AK, Albert M, et al. Initial Clinical Experience with Contrast-Enhanced Digital Breast Tomosynthesis. Acad Radiol. 2007; 14(2): 229–238. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1850283/pdf/nihms-17424.pdf. Accessed on October 18, 2013.
3. Fornvik D, Zackrisson S, Ljungberg O, et al. Breast tomosynthesis: Accuracy of tumor measurement compared with digital mammography and ultrasonography. Acta Radiol. 2010; 51(3):240-247.
4. Gennaro G, Toledano A, di Maggio C, et al. Digital breast tomosynthesis versus digital mammography: a clinical performance study. Eur Radiol. 2010; 20(7):1545-1553.
5. Good, WF, Abrams GS, Catullo VJ, et al. Digital breast tomosynthesis: a pilot observer study. AJR Am J Roentgenol. 2008; 190(4):865-869.
6. Gur D, Abrams GS, Chough DM, Ganott MA. Digital breast tomosynthesis: observer performance study. AJR Am J Roentgenol. 2009; 193(2):586-591.
7. Michell MJ, Iqbal A, Wasan RK, et al. A comparison of the accuracy of film-screen mammography, full-field digital mammography, and digital breast tomosynthesis. Clin Radiol. 2012; 67(10):976-981.
8. Poplack SP, Tosteson TD, Kogel CA, Nagy HM. Digital breast tomosynthesis: initial experience in 98 women with abnormal digital screening mammography. AJR Am J Roentgenol. 2007; 189(3):616-623.
9. Rafferty EA, Park JM, Philpotts LE, et al. Assessing radiologist performance using combined digital mammography and breast tomosynthesis compared with digital mammography alone: results of a multicenter, multireader trial. Radiology. 2013; 266(1):104-113.
10. Skaane P, Bandos AI, Gullien R, et al. Comparison of digital mammography alone and digital mammography plus tomosynthesis in a population-based screening program. Radiology. 2013; 267(1):47-56.
11. Spangler ML, Suley ML, Sumkin JH, et al. Detection and classification of calcifications on digital breast tomosynthesis and 2D digital mammography: a comparison. Am J Roentgenol. 2011; 196(2):320-324.
12. Teertstra HJ, Loo CE., van den Bosch MA, et al. Breast tomosynthesis in clinical practice: initial results. Eur Radiol. 2010; 20(1):16-24.

Government Agency, Medical Society, and Other Authoritative Publications:

1. American College of Obstetricians-Gynecologists. Practice bulletin no. 122: Breast cancer screening. Obstet Gynecol. 2011; 118(2 Pt 1):372-382. Available at: http://www.guideline.gov/content.aspx?id=34275&search=Breast+tomosynthesis. Accessed on October 18, 2013.
2. American College of Radiology. Press Releases.ACR, SBI Statement on Skaane et al – Tomosynthesis Breast Cancer Screening Study (2013). Available at: http://www.acr.org/About-Us/Media-Center/Press-Releases/2013-Press-Releases/20130110ACR-SBI-Statement-on-Skaane-et-al. Accessed on October 18, 2013.
3. National Comprehensive Cancer Network® NCCN Clinical Practice Guidelines in Oncology™: Breast Cancer Screening and Diagnosis (V.2.2013). Revised July 2, 2013. © 2013 National Comprehensive Cancer Network, Inc. For additional information visit the NCCN website: http://www.nccn.org/index.asp. Accessed on October 18, 2013.
4. U.S. Food and Drug Administration (FDA). Center for Devices and Radiological Health (CDRH). New device approval letter March 30, 2005: Selenia Dimensions 3D System. P080003. Available at: http://www.accessdata.fda.gov/cdrh_docs/pdf8/P080003a.pdf. Accessed on October 18, 2013.
5. U.S. Food and Drug Administration (FDA). Center for Devices and Radiological Health (CDRH). Premarket Approval Application (PMA) Supplement Letter. May 16, 2013.Hologic Senia Dimensions 3D System. P080003/S001. Available at: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm353734.htm. Accessed on October 18, 2013
6. U.S. Food and Drug Administration (FDA). FDA Executive Summary. Meeting of the Radiological Devices Advisory Panel. Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee

/RadiologicalDevicesPanel/UCM226757.pdf. Accessed on October 18, 2013.
7. U.S. Food and Drug Administration (FDA) Summary of Safety and Effectiveness Data. Selenia Dimensions 3D System. P080003/S001. Rockville, MD: May 16, 2013. Available at: http://www.accessdata.fda.gov/cdrh_docs/pdf8/P080003S001b.pdf. Accessed on October 18, 2013.

Web Sites for Additional Information

1. American Cancer Society (ACS). Breast Cancer. Early Detection. Last Medical Review: 09/17/2013. Last revised 10/24/2013. Available at: http://www.cancer.org/acs/groups/cid/documents/webcontent/003165-pdf.pdf. Accessed on November 11, 2013.
2. American Cancer Society (ACS). Breast Cancer. What's New is Breast Cancer Research? Last Medical Review: 09/11/2013. Last revised 10/24/2013. Available at: http://www.cancer.org/Cancer/BreastCancer/DetailedGuide/breast-cancer-new-research. Accessed on November 11, 2013.

Index

Digital Brest Tomosynthesis (DBT)
Selenia® Dimensions® Digital Breast Tomosynthesis System
Three-D (3-D) Mammography

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete listing of all products available.

Document History

Status	Date	Action
Reviewed	11/14/2013	Medical Policy & Technology Assessment Committee (MPTAC) review.
Reviewed	08/08/2013	(MPTAC review. Updated Rationale, History and References sections.
Reviewed	08/09/2012	MPTAC review. Updated review date, History and References sections.
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Definitions
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Policy

1. Aetna considers annual mammography screening a medically necessary preventive service for women aged 40 and older. Annual screening is also considered medically necessary for younger women who are judged to be at high-risk by their primary care physician. Screening mammography for other women is considered experimental and investigational because its benefits in these other women are unproven.

2. Aetna considers screening mammography for men experimental and investigational, as the clinical benefits of such screening in men are unproven. Current guidelines from the U.S. Preventive Services Task Force and the American College of Radiology recommend such screening only for women. Aetna considers mammography medically necessary for surveillance of men with a prior history of breast cancer.

3. Aetna considers diagnostic mammography medically necessary for members with signs or symptoms of breast disease or history of breast cancer.

   Note: Diagnostic mammography is covered regardless of whether the member has preventive services benefits

4. Aetna considers digital mammography an acceptable alternative to film mammography.

5. Aetna considers computer-aided detection (CAD) a medically necessary adjunct to mammography.

6. Aetna considers xeroradiography for breast imaging experimental and investigational because this method of radiography is obsolete.

7. Aetna considers breast tomosynthesis imaging experimental and investigational because of insufficient evidence of its effectiveness.

8. Aetna considers contrast-enhanced spectral mammography experimental and investigational because of insufficient evidence of its effectiveness.

See also CPB 0105 - Magnetic Resonance Imaging (MRI) of the Breast; CPB 0269 - Breast Biopsy Procedures and CPB 0386 - Breast Transillumination and Electrical Impedance Scanning (EIS), and Elastography.

Background

This policy is based on the recommendations of the American Cancer Society, American College of Radiology, and the American College of Obstetricians and Gynecologists.

A mammogram is an x-ray of the breast. A screening mammography is one of several tools that are used for early detection of breast cancer in asymptomatic women. Other screening tools include the clinical breast examination and breast self-examination. Diagnostic mammography is used to diagnose breast cancer in women who have signs or symptoms of breast disease.

Screening mammography aims to reduce morbidity and mortality from breast cancer by early detection and treatment of occult malignancies. There is extensive evidence from a variety of well-conducted, randomized controlled studies that annual or biennial mammography is effective in reducing breast cancer mortality by 30 % in women aged 50 to 69 years. Data on women under age 50 are less clear. Results from the Canadian National Breast Screening Study

(CNBSS) suggest that the contribution of mammography over good physical examinations to breast cancer mortality reduction may be less than has been assumed. This observation re-emphasizes a truism of screening -- that it is not necessary to detect cancers as early as possible to obtain a benefit -- it is only necessary to detect them early enough. What is early enough in any individual case is uncertain because there are insufficient outcomes data. This has made it difficult for professional societies to develop specific mammography screening recommendations for high-risk women.

The U.S. Preventive Services Task Force (USPSTF) revised their recommendations for mammography screening in 2009. Whereas they had formerly recommended routine screening every 1 to 2 years starting at age 40, they now recommend against routine screening for women aged 40 to 49 and biennial rather than annual screening for women aged 50 to 74. In their 2009 recommendations, the USPSTF recommend that women aged 40 to 49 consider their personal risk for developing breast cancer before deciding to participate in regular, biennial screening. The USPSTF concluded that the current evidence is insufficient to assess the additional benefits and harms of (i) screening mammography in women aged 75 years or older, (ii) clinical breast examination (CBE) beyond screening mammography in women aged 40 years or older, and (iii) either digital mammography or magnetic resonance imaging instead of film mammography as screening modalities for breast cancer. In addition, the USPSTF recommended against clinicians teaching women how to perform breast self-examination.

The American Medical Association (AMA), the Society of Breast Imaging (SBI), the American College of Radiology (ACR), and the American Cancer Society (ACS), all support screening with mammography and CBE beginning at age 40. Recent recommendations from the SBI and the ACR (2010) released after the 2009 USPSTF recommendations, which recommended that average-risk women wait until age 50 to undergo screening mammography, continue to support yearly screening mammography beginning at age 40 for women at average-risk for breast cancer. The American College of Obstetricians and Gynecologists (ACOG, 2000) supports screening with mammography beginning at age 40 and CBE beginning at age 19. The Canadian Task Force on Preventive Health Care (CTFPHC), the American Academy of Family Physicians (AAFP), and the American College of Preventive Medicine (ACPM) recommend beginning mammography for average-risk women at age 50. The AAFP and ACPM recommend that mammography in high-risk women begin at age 40, and AAFP recommends that all women aged 40 to 49 be counseled about the risks and benefits of mammography before making decisions about screening.

A 1997 Consensus Development Panel convened by the National Institutes of Health concluded that the evidence was insufficient to determine the benefits of mammography among women aged 40 to 49. This panel recommended that women aged 40 to 49 should be counseled about potential benefits and harms before making decisions about mammography. In 2001, the CTFPHC concluded there was insufficient evidence to recommend for or against mammography in women aged 40 to 49.

Organizations differ on their recommendations for the appropriate interval for mammography. Annual mammography is recommended by AMA, ACR, and ACS. Mammography every 1 to 2 years is recommended by AAFP, ACPM, and the CTFPHC. ACOG recommends mammography every 1 to 2 years for women aged 40 to 49 and annually for women aged 50 and older.

Berg et al (2008) compared the diagnostic yield, defined as the proportion of women with positive screen test results and positive reference standard, and performance of screening with ultrasound plus mammography versus mammography alone in women at elevated risk of breast cancer. A total of 2,809 women, with at least heterogeneously dense breast tissue in at least 1 quadrant, were recruited from 21 sites to undergo mammographic and physician-performed ultrasonographic examinations in randomized order by a radiologist masked to the other examination results. Reference standard was defined as a combination of pathology and 12-month follow-up and was available for 2,637 (96.8 %) of the 2,725 eligible participants. Main outcome measures included diagnostic yield, sensitivity, specificity, and diagnostic accuracy (assessed by the area under the receiver operating characteristic curve) of combined mammography plus ultrasound versus mammography alone and the positive predictive value of biopsy recommendations for mammography plus ultrasound versus mammography alone. A total of 40 participants (41 breasts) were diagnosed with cancer: 8 suspicious on both ultrasound and mammography, 12 on ultrasound alone, 12 on mammography alone, and 8 participants (9 breasts) on neither. The diagnostic yield for mammography was 7.6 per 1,000 women screened (20 of 2,637) and increased to 11.8 per 1,000 (31 of 2,637) for combined mammography plus ultrasound; the supplemental yield was 4.2 per 1,000 women screened (95 % confidence interval [CI]: 1.1 to 7.2 per 1,000; p = 0.003 that supplemental yield is 0). The diagnostic accuracy for mammography was 0.78 (95 % CI: 0.67 to 0.87) and increased to 0.91 (95 % CI: 0.84 to 0.96) for mammography plus ultrasound (p = 0.003 that difference is 0). Of 12 supplemental cancers detected by ultrasound alone, 11 (92 %) were invasive with a median size of 10 mm (range of 5 to 40 mm; mean [SE], 12.6 [3.0] mm) and 8 of the 9 lesions (89 %) reported had negative nodes. The positive predictive value of biopsy recommendation after full diagnostic workup was 19 of 84 for mammography (22.6 %; 95 % CI: 14.2 % to 33 %), 21 of 235 for ultrasound (8.9 %, 95 % CI: 5.6 % to 13.3 %), and 31 of 276 for combined mammography plus ultrasound (11.2 %; 95 % CI: 7.8 % to 15.6 %). The authors concluded that adding a single screening ultrasound to mammography will yield an additional 1.1 to 7.2 cancers per 1,000 high-risk women, but it will also substantially increase the number of false positives.

Although digital mammography has not shown greater accuracy than film mammography, it has become standard of

care. The Food and Drug Administration (FDA)'s initial market approval of digital mammography technology was based, in part, on studies that demonstrated its effectiveness in patients referred for further testing after an initial suspicious mammogram (FDA, 2004). In that setting, the FDA found the accuracy of digital technology was similar to that of screen film. Studies are currently underway to evaluate the effectiveness of digital mammography in screening of the general population.

Hendrick and colleagues (2008) retrospectively compared the accuracy for cancer diagnosis of digital mammography with soft-copy interpretation with that of screen-film mammography for each digital equipment manufacturer, by using results of biopsy and follow-up as the reference standard. The American College of Radiology Imaging Network Digital Mammographic Imaging Screening Trial (DMIST) collected screening mammography studies performed by using both digital and screen-film mammography in 49,528 women (mean age of 54.6 years; range of 19 to 92 years). Digital mammography systems from 4 manufacturers (Fischer, Fuji, GE, and Hologic) were used. For each digital manufacturer, a cancer-enriched reader set of women screened with both digital and screen-film mammography in DMIST was constructed. Each reader set contained all cancer-containing studies known for each digital manufacturer at the time of reader set selection, together with a subset of negative and benign studies. For each reader set, 6 or 12 experienced radiologists attended 2 randomly ordered reading sessions 6 weeks apart. Each radiologist identified suspicious findings and rated suspicion of breast cancer in identified lesions by using a 7-point scale. Results were analyzed according to digital manufacturer by using areas under the receiver operating characteristic curve (AUCs), sensitivity, and specificity for soft-copy digital and screen-film mammography. Results for Hologic digital are not presented owing to the fact that few cancer cases were available. The implemented design provided 80 % power to detect average AUC differences of 0.09, 0.08, and 0.06 for Fischer, Fuji, and GE, respectively. No significant difference in AUC, sensitivity, or specificity was found between Fischer, Fuji, and GE soft-copy digital and screen-film mammography. Large reader variations occurred with each modality. The authors concluded that no statistically significant differences were found between soft-copy digital and screen-film mammography for Fischer, Fuji, and GE digital mammography equipment.

Pisano and associates (2008) retrospectively compared the accuracy of digital versus film mammography in population subgroups of the DMIST defined by combinations of age, menopausal status, and breast density, by using either biopsy results or follow-up information as the reference standard. For analysis, AUCs for each modality were compared within each subgroup evaluated (age less than 50 versus 50 to 64 versus greater than or equal to 65 years; dense versus non-dense breasts at mammography; and pre- or peri-menopausal versus post-menopausal status for the 2 younger age cohorts) while controlling for multiple comparisons ($p < 0.002$ indicated a significant difference). All DMIST cancers were evaluated with respect to mammographic detection method (digital versus film versus both versus neither), mammographic lesion type (mass, calcifications, or other), digital machine type, mammographic and pathologic size and diagnosis, existence of prior mammographic study at time of interpretation, months since prior mammographic study, and compressed breast thickness. A total of 33 centers enrolled 49,528 women. Breast cancer status was determined for 42,760 women, the group included in this study. Pre- or peri-menopausal women younger than 50 years who had dense breasts at film mammography comprised the only subgroup for which digital mammography was significantly better than film (AUCs, 0.79 versus 0.54; $p = 0.0015$). Breast Imaging Reporting and Data System-based sensitivity in this subgroup was 0.59 for digital and 0.27 for film mammography. AUCs were not significantly different in any of the other subgroups. For women aged 65 years or older with fatty breasts, the AUC showed a non-significant tendency toward film being better than digital mammography (AUCs, 0.88 versus 0.70; $p = 0.0025$). The authors concluded that digital mammography performed significantly better than film for pre- and peri-menopausal women younger than 50 years with dense breasts, but film tended non-significantly to perform better for women aged 65 years or older with fatty breasts.

Computer-aided detection and diagnosis (CAD) systems consist of computer programs that are designed to recognize patterns in images. CAD may be applied to digital mammograms or to plain film mammograms that have been digitized. CAD has been used for 2 different purposes: (i) to improve radiologists' ability to identify suspicious areas that may otherwise be overlooked on screening mammograms (detection), and (ii) to distinguish between benign and malignant lesions (diagnosis). The radiologist remains the reader and interpreter of the mammogram. CAD assists the radiologist by identifying areas warranting further review.

The American Cancer Society breast cancer screening guidelines (Smith et al, 2003) indicate that for digital mammography and CAD there is “some clinical evidence for effectiveness or equivalence to screen-film mammography for screening” (Evidence Level B).

Bennett et al (2006) assessed current evidence to ascertain if the accuracy of single reading with CAD compares with that of double reading. These researchers performed a literature review to identify studies where both protocols had been investigated and compared. They identified 8 studies that compared single reading with CAD against double reading, of which 6 reported on comparisons of both sensitivity and specificity. Of the 6 studies identified, 3 showed no differences in either sensitivity or specificity; 1 showed single reading with CAD had a higher sensitivity at the same specificity; 1 showed that single reading with CAD had a higher specificity at the same sensitivity. However, 1 study, in a real-life setting, showed that single reading with CAD had a higher sensitivity but a lower specificity. The authors concluded that as the majority of the studies were not in a real-life setting, used test sets, lacked sufficient training in the use of CAD and simulated double reading (using a protocol of recall if one suggests), current evidence

is therefore limited as to the accuracy, in terms of sensitivity and specificity, of single reading with CAD in comparison with the most common practice in the United Kingdom of double reading using a protocol of consensus or arbitration.

Fenton and colleagues (2007) stated that CAD identifies suspicious findings on mammograms to assist radiologists. Since the FDA approved the technology in 1998, it has been disseminated into practice, but its effect on the accuracy of interpretation is unclear. These investigators determined the association between the use of CAD at mammography facilities and the performance of screening mammography from 1998 through 2002 at 43 facilities in 3 states. They had complete data for 222,135 women (a total of 429,345 mammograms), including 2,351 women who received a diagnosis of breast cancer within 1 year after screening. They calculated the specificity, sensitivity, and positive predictive value of screening mammography with and without CAD, as well as the rates of biopsy and breast-cancer detection and the overall accuracy, measured as the area under the receiver-operating-characteristic (ROC) curve. A total of 7 facilities (16 %) implemented CAD during the study period. Specificity decreased from 90.2 % before implementation to 87.2 % following implementation ($p < 0.001$), the positive predictive value decreased from 4.1 % to 3.2 % ($p = 0.01$), and the rate of biopsy increased by 19.7 % ($p < 0.001$). The increase in sensitivity from 80.4 % before implementation of CAD to 84.0 % after implementation was insignificant ($p = 0.32$). The change in the cancer-detection rate (including invasive breast cancers and ductal carcinomas in situ) was insignificant (4.15 cases per 1,000 screening mammograms before implementation and 4.20 cases after implementation, $p = 0.90$). Analyses of data from all 43 facilities showed that the use of CAD was associated with significantly lower overall accuracy than was non-use (area under the ROC curve, 0.871 versus 0.919; $p = 0.005$). The authors concluded that the use of CAD is associated with reduced accuracy of interpretation of screening mammograms. The increased rate of biopsy with the use of CAD is not clearly associated with improved detection of invasive breast cancer. This is in agreement with Bazzocchi et al (2007) who noted that there is still considerable variation among different studies in the level of benefit deriving from CAD. Thus, the role of these systems in clinical practice is still debated, and their real contribution to the overall management of the diagnostic process is still unclear.

An assessment of CAD in mammography screening by the Swedish Council on Health Technology Assessment (SBU, 2011) concluded that the scientific evidence is insufficient to determine whether CAD plus single reading by one breast radiologist would yield results that are at least equivalent to those obtained in standard practice, i.e. double reading where two breast radiologists independently read the x-ray images.

Screening film mammography has been shown to reduce the mortality rate from breast cancer; however, conventional mammography does not detect all breast cancers. A significant factor contributing to the limitations of mammography is the structure overlap that results on a 2-dimensional mammogram. Structure overlap not only obscures lesions, but can mimic abnormalities, thus contributing to reductions in both the sensitivity and specificity of mammography. A number of new imaging techniques and enhancements for digital mammography have recently become available or are likely to become available in the near future.

Breast tomosynthesis is a 3-dimensional imaging technique that involves acquiring images of a stationary compressed breast at multiple angles during a short scan. The individual images are then reconstructed into a series of thin, high-resolution slices that can be displayed individually or in a dynamic cine mode. While holding the breast stationary, images are acquired at a number of different x-ray source angles. Objects at different heights in the breast project differently for each angle. The data are then reconstructed to generate images that enhance objects from a given height by appropriate shifting of the projections relative to one another. Three important areas in tomosynthesis system requirements are: (i) detector efficiency and dose, (ii) field of view, and (iii) equipment geometry. While holding the breast stationary, an x-ray tube is rotated over a limited angular range and a series of low-dose exposures are made every few degrees, creating a series of digital images. The x-ray tube is rotated about +/-15 degrees, and 11 exposures are made every 3 degrees during a total scan of a few seconds. These individual images are then reconstructed into slices. There are 2 basic tomosynthesis system designs that differ in the motion of the detector during acquisition. One method moves the detector in concert with the x-ray tube so as to maintain the shadow of the breast on the detector. An altenate method is to keep the detector stationary relative to the breast platform. The tomosynthesis reconstruction process consists of computing high-resolution images whose planes are parallel to the breast support plates. These images are reconstructed with slice separation of 1 mm; thus, a 5-cm compressed breast tomosynthesis study will have 50 reconstructed slices. The reconstructed tomosynthesis slices can be displayed similarly to computed tomography reconstructed slices. Proponents of breast tomosynthesis hope it will resolve many of the tissue overlap reading problems that are a major source of recalls and additional imaging in 2-D mammography examinations (Smith, 2005). Tomosynthesis is currently in clinical trials to evaluate its effectiveness. Initial pilot studies suggested that breast tomosynthesis has comparable or superior image quality to that of film-screen mammography and has the potential to decrease the recall rate when used adjunctively with digital screening mammography (Good et al, 2008; Chen et al, 2007; Poplack et al, 2007); however, further studies are needed to evaluate its effectiveness in clinical practice.

Helvie (2010) discussed recent developments in advanced derivative technologies associated with digital mammography. Digital breast tomosynthesis, its principles, development, and early clinical trials, were reviewed. Contrast-enhanced digital mammography and combined imaging systems with digital mammography and ultrasound

were also discussed. Although all these methods are currently research programs, they hold promise for improving cancer detection and characterization if early results are confirmed by clinical trials.

Spangler and colleagues (2011) compared the ability of digital breast tomosynthesis and full field digital mammography (FFDM) to detect and characterize calcifications. A total of 100 paired examinations were performed utilizing FFDM and digital breast tomosynthesis: 20 biopsy-proven cancers, 40 biopsy-proven benign calcifications, and 40 randomly selected negative screening studies were retrospectively reviewed by 5 radiologists in a crossed multi-reader multi-modal observer performance study. Data collected included the presence of calcifications and forced Breast Imaging, Reporting and Data System (BI-RADS) scores. Receiver operator curve analysis using BI-RADS was performed. Overall calcification detection sensitivity was higher for FFDM (84 % [95 % CI: 79 % to 88 %]) than for digital breast tomosynthesis (75 % [95 % CI: 70 % to 80 %]). In the cancer cohort, 75 (76 %) of 99 interpretations identified calcification in both modes. Of those, a BI-RADS score less than or equal to 2 was rendered in 3 (4 %) and 9 (12 %) cases with FFDM and digital breast tomosynthesis, respectively. In the benign cohort, 123 (62 %) of 200 interpretations identified calcifications in both modes. Of those, a BI-RADS score greater than or equal to 3 was assigned in 105 (85 %) and 93 (76 %) cases with FFDM and digital breast tomosynthesis, respectively. There was no significant difference in the non-parametric computed AUC using the BI-RADS scores (FFDM, AUC = 0.76 and SD = 0.03; digital breast tomosynthesis, AUC = 0.72 and SD = 0.04 [p = 0.1277]). The authors concluded that in this small data set, FFDM appears to be slightly more sensitive than digital breast tomosynthesis for the detection of calcification. However, diagnostic performance as measured by AUC using BI-RADS was not significantly different. With improvements in processing algorithms and display, digital breast tomosynthesis could potentially be improved for this purpose.

On February 2011, the FDA approved the Selenia Dimensions 3D System (Digital Breast Tomosynthesis), a mammography device that provides digital 2D and 3D images for the screening and diagnosis of breast cancer.

Guidelines on breast cancer screening from the American College of Obstetricians and Gynecologists (ACOG, 2011) considered, but did not recommend, breast tomosynthesis. The guidelines concluded that "[c]olor Doppler ultrasonography, computer-aided detection, positron emission tomography, scintimammography, and digital breast tomosynthesis have shown promise in selected clinical situations or as adjuncts to mammography for breast cancer diagnosis. However, these technologies are not considered alternatives to routine mammography."

The ongoing TOMMY trial: (A comparison of TOMosynthesis with digital MammographY in the UK NHS Breast Screening Programme) will compare the accuracy of digital breast tomosynthesis (DBT) with standard digital full field mammography(FFDM) in the diagnosis of breast cancer. The aim of the trial is to assess whether DBT could improve upon digital mammography as a screening tool, particularly in certain groups of women, e.g. those with a family history of breast cancer, or women with dense breasts. The diagnostic accuracy of DBT, FFDM and DBT+FFDM will be evaluated in an independent retrospective reading study and compared to the final clinical outcome for each case.

Sahiner et al (2012) designed a computer-aided detection (CADe) system for clustered micro-calcifications in reconstructed DBT volumes and performed a preliminary evaluation of the CADe system. Institutional review board approval and informed consent were obtained in this study. A data set of 2-view DBT of 72 breasts containing micro-calcification clusters was collected from 72 subjects who were scheduled to undergo breast biopsy. Based on tissue sampling results, 17 cases had breast cancer and 55 were benign. A separate data set of 2-view DBT of 38 breasts free of clustered micro-calcifications from 38 subjects was collected to independently estimate the number of false-positives (FPs) generated by the CADe system. A radiologist experienced in breast imaging marked the biopsied cluster of micro-calcifications with a 3D bounding box using all available clinical and imaging information. A CADe system was designed to detect micro-calcification clusters in the reconstructed volume. The system consisted of pre-screening, clustering, and FP reduction stages. In the pre-screening stage, the conspicuity of micro-calcification-like objects was increased by an enhancement-modulated 3D calcification response function. An iterative thresholding and 3D object growing method was used to detect cluster seed objects, which were used as potential centers of micro-calcification clusters. In the cluster detection stage, micro-calcification candidates were identified using a 2nd iterative thresholding procedure, which was applied to the signal-to-noise ratio (SNR) enhanced image voxels with a positive calcification response. Starting with each cluster seed object as the initial cluster center, a dynamic clustering algorithm formed a cluster candidate by including micro-calcification candidates within a 3D neighborhood of the cluster seed object that satisfied the clustering criteria. The number, size, and SNR of the micro-calcifications in a cluster candidate and the cluster shape were used to reduce the number of FPs. The pre-screening stage detected a cluster seed object in 94 % of the biopsied micro-calcification clusters at a threshold of 100 cluster seed objects per DBT volume. After clustering, the detection sensitivity was 90 % at 15 marks per DBT volume. After FP reduction, at 85 % sensitivity, the average number of FPs estimated using the data set containing micro-calcification clusters was 3.8 per DBT volume, and that estimated using the data set free of micro-calcification clusters was 3.4. The detection performance for malignant micro-calcification clusters was superior to that for benign clusters. The authors concluded that these findings indicated the feasibility of the 3D approach to the detection of clustered micro-calcifications in DBT and that the newly designed enhancement-modulated 3D calcification response function is promising for pre-screening. They stated that further work is needed to assess the generalizability of this approach and to improve its performance.

Skaane and colleagues (2012) noted that DBT is a promising new technology. Some experimental clinical studies have shown positive results, but the future role and indications of this new technique, whether in a screening or clinical setting, need to be evaluated. These investigators compared DM and DBT in a side-by-side feature analysis for cancer conspicuity, and examined if there is a potential additional value of DBT to standard state-of-the-art conventional imaging work-up with respect to detection of additional malignancies. The study had ethics committee approval. A total of 129 women underwent 2D DM including supplementary cone-down and magnification views and breast ultrasonography if indicated, as well as DBT. The indication for conventional imaging in the clinical setting included a palpable lump in 30 (23 %), abnormal mammographic screening findings in 54 (42 %), and surveillance in 45 (35 %) of the women. The women were examined according to present guidelines, including spot-magnification views, ultrasonography, and needle biopsies, if indicated. The DBT examinations were interpreted several weeks after the conventional imaging without knowledge of the conventional imaging findings. In a later session, 3 radiologists performed a side-by-side feature analysis for cancer conspicuity in a sample of 50 cases. State-of-the-art conventional imaging resulted in needle biopsy of 45 breasts, of which 20 lesions were benign and a total of 25 cancers were diagnosed. The remaining 84 women were dismissed with a normal/definitely benign finding and without indication for needle biopsy. The subsequent DBT interpretation found suspicious findings in 4 of these 84 women, and these 4 women had to be called back for repeated work-up with knowledge of the tomosynthesis findings. These delayed work-ups resulted in 2 cancers (increasing the cancer detection by 8 %) and 2 FP findings. The side-by-side feature analysis showed higher conspicuity scores for tomosynthesis compared to conventional 2D for cancers presenting as spiculated masses and distortions. The authors concluded that DBT is a promising new technique. The authors' preliminary clinical experience showed that there is a potential for increasing the sensitivity using this new technique, especially for cancers manifesting as spiculated masses and distortions.

Also, an UpToDate review on "Breast imaging: Mammography and ultrasonography" (Venkataraman, 2012) states that "Tomosynthesis is a modification of digital mammography and uses a moving x-ray source and digital detector. A three dimensional volume of data is acquired and reconstructed using computer algorithms to generate thin sections of images …. The examination has a slightly longer exposure time of 10 seconds per acquisition compared to standard digital mammography, which could increase the radiation dose per acquisition and increase the risk of motion artifacts. This technique shows promise in screening women with dense breast tissue and with high risk for breast cancer".

Bernardi et al (2012a) stated that there is limited evidence on the role of 3D mammography with tomosynthesis in breast screening, although early studies suggested that it may improve specificity. These researchers prospectively evaluated the effect of integrating 3D mammography as a triage to assessment in 158 consecutive recalls to assessment (recalled in standard 2D-mammographic screening) in asymptomatic subjects. Radiologists provided 3D mammography-based opinion as to whether recall/assessment was warranted or unnecessary, and all subjects proceeded to assessment. 3D triage was positive (confirmed the need for assessment) in all 21 subjects with breast cancer (there were no false-negatives), and would have avoided recall in 102 of 137 (74.4 %) subjects with a negative/benign final outcome in whom 3D triage did not recommend recall. Proportion of true negative 3D triage (as a proxy for potential reduction in recalls) was slightly higher in dense than non-dense breasts, did not differ across age-groups, but was significantly associated with the type of lesion seen on imaging (being highest for distortions, asymmetric densities, and lesions with ill-defined margins). While the simulation design may have over-estimated the potential for 3D mammography triage to reduce recalls, this study clearly demonstrates its capability to improve breast screening specificity and to reduce recall rates. The authors stated that future studies of 3D mammography should further assess its role as a recall-reducing strategy in screening practice and should include formal cost-analysis.

Bernardi et al (2012b) supplemented the paucity of information available on logistical aspects of the application of 3D mammography in breast screening. These investigators prospectively examined the effect on radiographers' and radiologists' workload of implementing 3D mammography in screening by comparing image acquisition time and screen-reading time for 2D mammography with that of combined 2D+3D mammography. Radiologists' accuracy was also calculated. Average acquisition time (measured from start of first-view breast positioning to compression release at completion of last view) for 7 radiographers, based on 20 screening examinations, was longer for 2D+3D (4 min 3 s; range of 3 min 53 s to 4 min 18 s) than 2D mammography (3 min 13 s; range of 3 min 0 s to 3 min 26 s; $p < 0.01$). Average radiologists' reading time per screening examination (3 radiologists reading case-mix of 100 screens: 10 cancers, 90 controls) was longer for 2D+3D (77 s; range of 60 to 90 s) than for 2D mammography (33 s; range of 25 to 46 s; $p < 0.01$). 2D+3D screen-reading was associated with detection of more cancers and with substantially fewer recalls than 2D mammography alone. The authors concluded that relative to standard 2D mammography, combined 2D+3D mammography prolongs image acquisition time and screen-reading time (at initial implementation), and appears to be associated with improved screening accuracy. They stated that these findings provided relevant information to guide larger trials of integrated 3D mammography (2D+3D) and its potential implementation into screening practice.

Zhao et al (2012) noted that mammography is the primary imaging tool for screening and diagnosis of human breast cancers, but approximately 10 to 20 % of palpable tumors are not detectable on mammograms and only about 40 % of biopsied lesions are malignant. These researchers reported a high-resolution, low-dose phase contrast X-ray

tomographic method for 3D diagnosis of human breast cancers. By combining phase contrast X-ray imaging with an image reconstruction method known as equally sloped tomography, these investigators imaged a human breast in 3D and identified a malignant cancer with a pixel size of 92 μm and a radiation dose less than that of dual-view mammography. According to a blind evaluation by 5 independent radiologists, this method can reduce the radiation dose and acquisition time by approximately 74 % relative to conventional phase contrast X-ray tomography, while maintaining high image resolution and image contrast. The authors concluded that these results demonstrated that high-resolution 3D diagnostic imaging of human breast cancers can, in principle, be performed at clinical compatible doses.

Houssami and Skaane (2013) stated that DBT, a 3D derivative of DM, reduces the effect of tissue superimposition and may improve mammographic interpretation. These investigators examined the evidence on the accuracy of DBT in clinical studies. Published studies of DBT were relatively small studies, mostly test-set observer (reader) studies or clinical series that included symptomatic and screen-recalled cases, and were generally enriched with cancers. With these limitations in mind, the evidence showed some consistent findings, summarized as follows: 2-view DBT has at least equal or better accuracy than standard 2-view DM, whereas 1-view DBT does not have better accuracy than standard DM; the addition of DBT to standard mammography (for mammographic interpretation or for assessment or triage of screen-recalled abnormalities) increases accuracy; improved accuracy from using DBT (relative to, or added to, DM) may be due to increased cancer detection or due to reduced false positive recalls, or both; and subjective interpretation of cancer conspicuity consistently found that cancers were equally or more conspicuous on DBT relative to DM. Preliminary data from population screening trials suggested that the integration of DBT with conventional DM (screen-reading using combined 2D + 3D mammography) may substantially improve breast cancer detection, although final results are not yet available, and many logistical issues need further evaluation to determine the potential implications and cost of combined 2D + 3D mammographic screening. At present, there is insufficient evidence to justify a change from standard DM to DBT however the available data strongly support investment in new large-scale population screening trials. These trials need to avoid the 'double' acquisitions required for 2D + 3D mammograms, and should therefore focus on evaluating integrated 2Dsynthetic + 3D mammography (where 2D-images are reconstructed from the DBT acquisition), and should consider using a randomized design.

Alakhras et al (2013) reviewed the major limitations in current mammography and described how these may be addressed by DBT. Digital breast tomosynthesis is a novel imaging technology in which an x-ray fan beam sweeps in an arc across the breast, producing tomographic images and enabling the production of volumetric, 3D data. It can reduce tissue overlap encountered in conventional 2D mammography, and thus has the potential to improve detection of breast cancer, reduce the suspicious presentations of normal tissues, and facilitate accurate differentiation of lesion types.

Also, an ACOG technology assessment on “Digital breast tomosynthesis” (2013) stated that this strategy of 2D plus 3D mammography would need to be compared to other potential strategies for comparative cost-effectiveness, such as the use of computer aided detection. Additional studies will be needed to confirm whether digital mammography with tomosynthesis is a cost-effective approach capable of replacing digital mammography alone as the first-line screening modality of choice for breast cancer screening. Furthermore, the ACOG technology assessment also noted the concern with the high-dose of radiation with breast tomosynthesis.

Poller et al (2012) stated that annual ultrasound screening may detect small, node-negative breast cancers that are not seen on mammography; and magnetic resonance imaging (MRI) may reveal additional breast cancers missed by both mammography and ultrasound screening. These researchers examined supplemental cancer detection yield of ultrasound and MRI in women at elevated risk for breast cancer. From April 2004 to February 2006, a total of 2,809 women at 21 sites with elevated cancer risk and dense breasts consented to 3 annual independent screens with mammography and ultrasound in randomized order. After 3 rounds of both screenings, 612 of 703 women who chose to undergo an MRI had complete data. The reference standard was defined as a combination of pathology (biopsy results that showed in-situ or infiltrating ductal carcinoma or infiltrating lobular carcinoma in the breast or axillary lymph nodes) and 12-month follow-up. Main outcome measures included cancer detection rate (yield), sensitivity, specificity, positive-predictive value (PPV3) of biopsies performed and interval cancer rate. A total of 2,662 women underwent 7,473 mammogram and ultrasound screenings, 110 of whom had 111 breast cancer events: 33 detected by mammography only, 32 by ultrasound only, 26 by both, and 9 by MRI after mammography plus ultrasound; 11 were not detected by any imaging screen. Among 4,814 incidence screens in the 2nd and 3rd years combined, 75 women were diagnosed with cancer. Supplemental incidence-screening ultrasound identified 3.7 cancers per 1,000 screens (95 % CI: 2.1 to 5.8; $p < 0.001$). Sensitivity for mammography plus ultrasound was 0.76 (95 % CI: 0.65 to 0.85); specificity, 0.84 (95 % CI: 0.83 to 0.85); and PPV3, 0.16 (95 % CI: 0.12 to 0.21). For mammography alone, sensitivity was 0.52 (95 % CI: 0.40 to 0.64); specificity, 0.91 (95 % CI: 0.90 to 0.92); and PPV3, 0.38 (95 % CI: 0.28 to 0.49; $p < 0.001$ all comparisons). Of the MRI participants, 16 women (2.6 %) had breast cancer diagnosed. The supplemental yield of MRI was 14.7 per 1,000 (95 % CI: 3.5 to 25.9; $p = 0.004$). Sensitivity for MRI and mammography plus ultrasound was 1.00 (95 % CI: 0.79 to 1.00); specificity, 0.65 (95 % CI: 0.61 to 0.69); and PPV3, 0.19 (95 % CI: 0.11 to 0.29). For mammography and ultrasound, sensitivity was 0.44 (95 % CI: 0.20 to 0.70, $p = 0.004$); specificity 0.84 (95 % CI: 0.81 to 0.87; $p < 0.001$); and PPV3, 0.18 (95 % CI: 0.08 to 0.34; $p = 0.98$). The number of screens needed to detect 1 cancer was 127 (95 % CI: 99 to 167) for mammography;

234 (95 % CI: 173 to 345) for supplemental ultrasound; and 68 (95 % CI: 39 to 286) for MRI after negative mammography and ultrasound results. The authors concluded that the addition of screening ultrasound or MRI to mammography in women at increased risk of breast cancer resulted in not only a higher cancer detection yield but also an increase in false-positive findings.

Currently, there are no recommendations to screen men at risk for hereditary breast cancer with mammography. Robson (2002) explained "There are no established guidelines for screening of men at risk for hereditary breast cancer. It is reasonable to suggest periodic self-examination and evaluation by a provider experienced in clinical breast examination. The utility of screening mammography in men is unknown, although it is technically possible in at least some individuals." However, diagnostic mammography may be indicated in men with a breast mass on clinical examination (American Cancer Society, 2003).

Xeroradiography (Xerox Corporation, Stamford, CT) is an outmoded X-ray imaging method that had been used especially in mammographic screening for breast cancer. With Xeroradiography, X-rays pass through the body to an X-ray-sensitive metal plate. The plate is then processed through a unique photocopying-type machine, and the X-ray image transferred to paper rather than X-ray film. Unlike photographically recorded X-ray images, Xeroradiographs produce a "positive" image in which the denser elements appear darker. Unlike X-ray films, the Xeroradiographic image is a mirror image of the object. The primary advantage of Xeroradiography over conventional plain film mammography is that the former produces instant radiographs. However, Xeroradiography has become outmoded because the radiation exposure required is much higher than with conventional radiographs, and the Xeroradiographic image has a number of defects, such as excessive contrast and edge enhancement.

Fredenberg and colleagues (2010) noted that spectral imaging is a method in medical x-ray imaging to extract information about the object constituents by the material-specific energy dependence of x-ray attenuation. The authors have investigated a photon-counting spectral imaging system with 2 energy bins for contrast-enhanced mammography. System optimization and the potential benefit compared to conventional non-energy-resolved absorption imaging were studied. A framework for system characterization was set up that included quantum and anatomical noise and a theoretical model of the system was benchmarked to phantom measurements. Optimal combination of the energy-resolved images corresponded approximately to minimization of the anatomical noise, which is commonly referred to as energy subtraction. In that case, an ideal-observer detectability index could be improved close to 50% compared to absorption imaging in the phantom study. Optimization with respect to the signal-to-quantum-noise ratio, commonly referred to as energy weighting, yielded only a minute improvement. In a simulation of a clinically more realistic case, spectral imaging was predicted to perform approximately 30 % better than absorption imaging for an average glandularity breast with an average level of anatomical noise. For dense breast tissue and a high level of anatomical noise, however, a rise in detectability by a factor of 6 was predicted. Another approximately 70 to 90 % improvement was found to be within reach for an optimized system. The authors concluded that contrast-enhanced spectral mammography is feasible and beneficial with the current system, and there is room for additional improvements. Inclusion of anatomical noise is essential for optimizing spectral imaging systems.

Schmitzberger et al (2011) demonstrated the feasibility of low-dose photon-counting tomosynthesis in combination with a contrast agent (contrast material-enhanced tomographic mammography) for the differentiation of breast cancer. All studies were approved by the institutional review board, and all patients provided written informed consent. A phantom model with wells of iodinated contrast material (3 mg of iodine per milliliter) 1, 2, 5, 10, and 15 mm in diameter was assessed. A total of 9 patients with malignant lesions and 1 with a high-risk lesion (atypical papilloma) were included (all women; mean age of 60.7 years). A multi-slit photon-counting tomosynthesis system was utilized (spectral imaging) to produce both low- and high-energy tomographic data (below and above the k edge of iodine, respectively) in a single scan, which allowed for dual-energy visualization of iodine. Images were obtained prior to contrast material administration and 120 and 480 seconds after contrast material administration. Four readers independently assessed the images along with conventional mammograms, ultrasonographic images, and magnetic resonance images. Glandular dose was estimated. Contrast agent was visible in the phantom model with simulated spherical tumor diameters as small as 5 mm. The average glandular dose was measured as 0.42 mGy per complete spectral imaging tomosynthesis scan of one breast. Because there were 3 time-points (prior to contrast medium administration and 120 and 480 seconds after contrast medium administration), this resulted in a total dose of 1.26 mGy for the whole procedure in the breast with the abnormality. Seven of 10 cases were categorized as Breast Imaging Reporting and Data System score of 4 or higher by all four readers when reviewing spectral images in combination with mammograms. One lesion near the chest wall was not captured on the spectral image because of a positioning problem. The authors concluded that the use of contrast-enhanced tomographic mammography has been demonstrated successfully in patients with promising diagnostic benefit. They stated that further studies are necessary to fully assess diagnostic sensitivity and specificity.

An American College of Obstetrician and Gynecologist technology assessment on "Digital breast tomosynthesis" (ACOG, 2013) noted that "Clinical data suggest that digital mammography with tomosynthesis produces a better image, improved accuracy, and lower recall rates compared with digital mammography alone. Further study will be necessary to confirm whether digital mammography with tomosynthesis is a cost-effective approach, capable of replacing digital mammography alone as the first-line screening modality of choice for breast cancer screening".

An UpToDate review on “Breast imaging: Mammography and ultrasonography” (Venkataraman and Slanetz, 2014) states that “At present, tomosynthesis is approved only to be performed in conjunction with a conventional mammogram. Hence, when performed in the screening setting, the patient is exposed to approximately twice the usual radiation dose, which sometimes is greater if the patient had dense or thick breasts. This technique shows promise in screening women with dense breast tissue and with high risk for breast cancer, although there are no prospective large studies to justify its routine use at the present time”.

A BlueCross BlueShield Association TEC Assessment, “Use of digital breast tomosynthesis with mammography for breast cancer screening or diagnosis” (BCNSA, 2014), concluded that “Improved health outcomes following the addition of breast tomosynthesis to screening or diagnostic mammography [has] not been demonstrated in the investigational setting. Based on the above, the addition of digital breast tomosynthesis to screening or diagnostic mammography does not meet the TEC criteria”.

National Cancer Institute’s Factsheet on “Mammograms” (NCI, 2014) states that “Three-dimensional (3D) mammography, also known as breast tomosynthesis, is a type of digital mammography in which x-ray machines are used to take pictures of thin slices of the breast from different angles and computer software is used to reconstruct an image. This process is similar to how a computed tomography (CT) scanner produces images of structures inside of the body. 3D mammography uses very low dose x-rays, but, because it is generally performed at the same time as standard two-dimensional (2D) digital mammography, the radiation dose is slightly higher than that of standard mammography. The accuracy of 3D mammography has not been compared with that of 2D mammography in randomized studies. Therefore, researchers do not know whether 3D mammography is better or worse than standard mammography at avoiding false-positive results and identifying early cancers”.

NCCN guidelines on breast cancer screening (NCCN, 2014) conclude: "Early studies show promise for tomosynthesis mammography. Two large trials showing a combined use of digital mammography and tomosynthesis showed improved cancer detection and decreased call back rates; of note, this is double the dose of radiation and is a factor in recommending this modality. Definitive studies are still pending."

An assessment by the Standing Committee on Screening of the Screening Section of the Australian Department of Health and Ageing (2013) stated that: "Whilst there is some evidence that DBT is at least as sensitive and specific as DM, it is not clear whether the use of DBT in large populations outside of a research setting would deliver the same results or whether the additional cost is justified. The issue of radiation dose also needs to be considered in terms of safety for women being screened.'

Allec et al (2011) noted that the accumulation of injected contrast agents allows the image enhancement of lesions through the use of contrast-enhanced mammography. In this technique, the combination of 2 acquired images is used to create an enhanced image. There exist several methods to acquire the images to be combined, which include dual energy subtraction using a single detection layer that suffers from motion artifacts due to patient motion between image acquisition. To mitigate motion artifacts, a detector composed of 2 layers may be used to simultaneously acquire the low and high energy images. In this work, these researchers evaluated both of these methods using amorphous selenium as the detection material to find the system parameters (tube voltage, filtration, photoconductor thickness and relative intensity ratio) leading to the optimal performance. They then compared the performance of the 2 detectors under the variation of contrast agent concentration, tumor size and dose. The detectability was found to be most comparable at the lower end of the evaluated factors. The single-layer detector not only led to better contrast, due to its greater spectral separation capabilities, but also had lower quantum noise. The single-layer detector was found to have a greater detectability by a factor of 2.4 for a 2.5 mm radius tumor having a contrast agent concentration of 1.5 mg ml(-1) in a 4.5 cm thick 50 % glandular breast. The authors stated that the inclusion of motion artifacts in the comparison is part of ongoing research efforts.

Furthermore, an UpToDate review on "MRI of the breast and emerging technologies" (Slanetz, 2013) states that "Emerging Imaging Technology for Breasr Cancer Detection -- Recognition of the limitations of mammography, ultrasound, and breast MRI has led to investigation of other breast imaging techniques including contrast-enhanced dual energy digital mammography, high-field strength MRI, magnetic resonance spectroscopy, diffusion weighted imaging, breast specific gamma imaging, and positron emission mammography". Contrast enhanced spectral mammography is not mentioned in this review.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes covered if selection criteria are met:

+ 77051

+ 77052

77055

77056

77057

HCPCS codes covered if selection criteria are met:

G0202	Screening mammography, producing direct digital image, bilateral, all views
G0204	Diagnostic mammography, producing direct digital image, bilateral, all views
G0206	Diagnostic mammography, producing direct digital image, unilateral, all views

ICD-9 codes covered if selection criteria are met:

174.0 - 174.9	Malignant neoplasm of female breast
198.81	Secondary malignant neoplasm of breast
217	Benign neoplasm of breast
233.0	Carcinoma in situ of breast
238.3	Neoplasm of uncertain behavior of breast
610.0 - 611.9	Disorders of breast
V10.3	Personal history of malignant neoplasm of breast
V16.3	Family history of malignant neoplasm of breast
V76.11	Screening mammogram for high-risk patient
V76.12	Other screening mammogram

The above policy is based on the following references:

1. Centers for Disease Control and Prevention. Update: National Breast and Cervical Cancer Early Detection Program -- July 1991-September 1995. MMWR Morb Mortal Wkly Rep. 1996;45(23):484-487.
2. Esserman L, Kerlikowske K. Should we recommend screening mammography for women aged 40 to 49? Oncology (Huntingt). 1996;10(3):357-364, 370-376.
3. Feig SA, Hendrick RE. Radiation risk from screening mammography of women aged 40-49 years. J Natl Cancer Inst Monogr. 1997;(22):119-124.
4. Feig SA. Ductal carcinoma in situ. Implications for screening mammography. Radiol Clin North Am. 2000;38(4):653-668, vii.
5. Ferrini R, Mannino E, Ramsdell E, et al. Screening mammography for breast cancer: American College of Preventive Medicine practice policy statement. Am J Prev Med. 1996;12(5):340-341.
6. Harris KM, Vogel VG. Breast cancer screening. Cancer Metastasis Rev. 1997;16(3-4):231-262.
7. Kerlikowske K, Barclay J. Outcomes of modern screening mammography. J Natl Cancer Inst Monogr. 1997; (22):105-111.
8. Kerlikowske K, Grady D, Barclay J, et al. Effect of age, breast density, and family history on the sensitivity of first screening mammography. JAMA. 1996;276(1):33-38.
9. Kerlikowske K, Grady D, Barclay J, et al. Likelihood ratios for modern screening mammography. Risk of breast cancer based on age and mammographic interpretation. JAMA. 1996;276(1):39-43.
10. Kopans DB. Updated results of the trials of screening mammography. Surg Oncol Clin N Am. 1997;6(2):233-263.
11. Legorreta AP, Brooks RJ, Leibowitz AN, et al. Cost of breast cancer treatment. A 4-year longitudinal study. Arch Intern Med. 1996;156(19):2197-2201.
12. Miller AB, Baines CJ, To T, Wall C. Canadian National Breast Screening Study: 1. Breast cancer detection and death rates among women aged 40 to 49 years. CMAJ. 1992;147(10):1459-1476.
13. Miller AB, To T, Baines CJ, et al. Canadian National Breast Screening Study-2: 13-year results of a randomized trial in women aged 50-59 years. J Natl Cancer Inst. 2000;92(18):1490-1499.
14. Miller AB. Screening and detection. In: The Breast: Comprehensive Management of Benign and Malignant Diseases. 2nd ed. KI Bland, EM Copeland III, eds. Philadelphia, PA: W.B. Saunders Company;1998:625-633.
15. Rajkumar SV, Hartmann LC. Screening mammography in women aged 40-49 years. Medicine (Baltimore). 1999;78(6):410-416.
16. Reichle. JL. Benefits of screening mammography: A review for the primary care physician. South Med J. 1998;91(6):510-517.
17. Ringash J. Preventive health care, 2001 update: Screening mammography among women aged 40-49 years at average risk of breast cancer. CMAJ. 2001;164(4):469-476.

18. Roetzheim R, Fox SA, Leake B, et al. The influence of risk factors on breast carcinoma screening of Medicare-insured older women. National Cancer Institute Breast Cancer Screening Consortium. Cancer. 1996;78(12):2526-2534.
19. American College of Obstetricians and Gynecologists (ACOG). Routine Cancer Screening. ACOG Committee Opinion Number 247. Washington, DC: ACOG; December 2000.
20. Tierney LM, McPhee SJ, Papadakis MA, eds. Current Medical Diagnosis & Treatment 2001. 40th ed. New York, NY: McGraw-Hill; 2001:711-712.
21. National Institutes of Health (NIH). Breast cancer screening in women ages 40-49. NIH Consensus Statement 103. Bethesda, MD: NIH; 1997. Available at: http://text.nlm.nih.gov/nih/cdc/www/103txt.html. Accessed August 28, 2001.
22. Wyatt SW, Long DM, Lee NC, et al. State legislation related to breast cancer: 1980-1994. J Public Health Manag Pract. 1996;2(2):64-69.
23. Canadian Task Force on Preventive Health Care (CTFPHC). Preventive health care, 2001 update: Screening mammography among women aged 40-49 years at average risk of breast cancer. London, ON; CTFPHC; 2001. Available at: http://www.ctfphc.org. Accessed July 2, 2002.
24. Baxter N; Canadian Task Force on Preventive Health Care. Preventive health care, 2001 update: Should women be routinely taught breast self-examination to screen for breast cancer? CMAJ. 2001;164 (13):1837-1846.
25. Royal New Zealand College of General Practitioners. Early detection of breast cancer. Wellington, New Zealand: Royal New Zealand College of General Practitioners; 1999.
26. New Zealand Health Technology Assessment (NZHTA). The early detection and diagnosis of breast cancer: A literature review - an update. Christchurch, New Zealand: NZHTA; 1999.
27. American College of Obstetricians and Gynecologists (ACOG). Primary and preventive care: Periodic assessments. ACOG Committee Opinion 246. Washington, DC: ACOG; 2000.
28. Empire Medicare Services. Xeroradiography. Medicare Part B Medical Policy. Policy No. Rad. #37. New York, NY: Empire Medicare Services; November 17, 1978. Available at: http://www.empiremedicare.com/Newypolicy/policy/RAD37.htm. Accessed July 2, 2002.
29. Robson ME. Clinical considerations in the management of individuals at risk for hereditary breast and ovarian cancer. Cancer Control. 2002;9(6):457-465.
30. U.S. Preventive Services Task Force. Screening for breast cancer: Recommendations and rationale. Ann Intern Med. 2002;137(5 Part 1):344-346.
31. American College of Obstetricians and Gynecologists (ACOG). ACOG practice bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 42, April 2003. Breast cancer screening. Obstet Gynecol. 2003;101(4):821-831.
32. Smith RA, Saslow D, Sawyer KA, et al. American Cancer Society guidelines for breast cancer screening: Update 2003. CA Cancer J Clin. 2003;53(3):141-169.
33. American Cancer Society (ACS). Breast Cancer (Men) Detailed Guide. Atlanta, GA: ACS: revised January 7, 2003. Available at: http://www.cancer.org/docroot/CRI/content/CRI_2_4_7x_CRC_Male_Breast_CancerPDF.asp. Accessed October 24, 2003.
34. American Academy of Family Physicians. Summary of policy recommendations for periodic health examinations. Leawood, KS: American Academy of Family Physicians; August 2003.
35. Levine C, Armstrong K, Chopra S, et al. Diagnosis and Management of Specific Breast Abnormalities. Summary, Evidence Report/Technology Assessment: Number 33. AHRQ Publication No. 01-E045. Rockville, MD: Agency for Healthcare Research and Quality; April 2001.
36. Humphrey L, Chan BKS, Detlefsen S, Helfand M. Screening for breast cancer. Systematic Evidence Review. Prepared for the Agency for Healthcare Research and Quality (AHRQ) by the Oregon Health Sciences University Evidence-based Practice Center. Contract No. 290-97-0018, Task Order No. 2. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); 2002.
37. Humphrey LL, Helfand M, Chan BKS. Breast cancer screening with mammography. A summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med. 2002;137(5):347-367
38. Sauven P; Association of Breast Surgery Family History Guidelines Panel. Guidelines for the management of women at increased familial risk of breast cancer. Eur J Cancer. 2004;40(5):653-665.
39. U.S. Food and Drug Administration (FDA). Digital mammography for breast cancer screening: An innovative approach to establishing efficacy. In: The Critical Path to New Medical Products. Rockville, MD: FDA; 2004. Available at: http://www.fda.gov/oc/initiatives/criticalpath/digitalmamm.html. Accessed February 15, 2005.
40. Freer TW, Ulissey MJ. Screening mammography with computer-aided detection: Prospective study of 12,860 patients in a community breast center. Radiology. 2001;220:781-786.
41. Irwig L, Houssami N, van Vliet C. New technologies in screening for breast cancer: A systematic review. Br J Cancer. 2004; April 27:1-5.
42. Gur D, Sumkin JH, Rockette HE, et al. Changes in breast cancer detection and mammography recall rates after the introduction of a computer-aided detection system. J Natl Cancer Inst. 2004;96(3):185-190.
43. Elmore JG, Carney PA. Computer-aided detection of breast cancer: Has promise outstripped performance? J Natl Cancer Inst. 2004;96(3):162-163.
44. Astley SM, Gilbert FJ. Computer-aided detection in mammography. Clin Radiol. 2004;59(5):390-399.
45. Taylor P, Champness J, Given-Wilson R, et al. Impact of computer-aided detection prompts on the sensitivity and specificity of screening mammography. Health Technol Assess. 2005;9(6):iii, 1-58.

46. Astley SM. Computer-aided detection for screening mammography. Acad Radiol. 2004;11(10):1139-1143.
47. Helvie MA, Hadjiiski L, Makariou E, et al. Sensitivity of noncommercial computer-aided detection system for mammographic breast cancer detection: Pilot clinical trial. Radiology. 2004;231:208-214.
48. Baum F, Fischer U, Obenauer S, Grabbe E. Computer-aided detection in direct digital full-field mammography: Initial results. Eur Radiol. 2002;12:3015-3017.
49. Chan HP, Sahiner B, Helvie MA, et al. Improvement of radiologists' characterization of mammographic masses by using computer-aided diagnosis: An ROC study. Radiology. 1999;212(3):817-827.
50. Chuong H, Hailey D, Warburton R, et al. Digital mammography versus film-screen mammography: Technical, clinical and economic assessments. Technology Report No. 30. Ottawa, ON: Canadian Coordinating Office for Health Technology Assessment; October 2002.
51. Comite d'Evaluation et de Diffusion des Innovations Technologiques (CEDIT). Digital mammography - systematic review, expert panel. Paris, France: CEDIT; 2001.
52. Alberta Heritage Foundation for Medical Research (AHFMR). 'Soft copy' digital mammography. Techscan. Edmonton, AB: AHFMR; November 2000.
53. Department of Health and Human Services, Centers for Medicare and Medicaid Services (CMS). Diagnostic and screening mammography. Change Request 1837. Trans. No. 1724. Baltimore, MD: CMS: September 27, 2001.
54. National Academy of Sciences, Institute of Medicine, National Cancer Policy Board, Committee on the Early Detection of Breast Cancer. Mammography and Beyond: Developing Technologies for the Early Detection of Breast Cancer. Washington, DC: National Academy Press; 2001.
55. National Academy of Sciences, Institute of Medicine, Board on Science, Technology and Economic Policy. Saving Women's Lives. Strategies for Improving Breast Cancer Detection and Diagnosis, Washington, DC: National Academy Press; 2004.
56. Elmore JG, Armstrong K, Lehman CD, Fletcher SW. Screening for breast cancer. JAMA. 2005;293(10):1245-1256.
57. California Technology Assessment Forum (CTAF). Full field digital mammography. Technology Assessment. San Francisco, CA: CTAF; February 15, 2006. Available at:http://ctaf.org/ass/viewfull.ctaf?id=65198186100. Accessed June 6, 2006.
58. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Computer-aided detection with full-field digital mammography. Technology Assessment. Chicago, IL: BCBSA; May 2006;21(3). Available at: http://www.bcbs.com/tec/vol21/21_03.html. Accessed June 6, 2006.
59. Fenton JJ, Taplin SH, Carney PA, et al. Influence of computer-aided detection on performance of screening mammography. N Engl J Med. 2007;356(14):1399-1409.
60. Hall FM. Breast imaging and computer-aided detection. N Engl J Med. 2007; 356:1464-1466.
61. Bazzocchi M, Mazzarella F, Del Frate C, et al. CAD systems for mammography: A real opportunity? A review of the literature. Radiol Med (Torino). 2007;112(3):329-353.
62. Qaseem A, Snow V, Sherif K, et al; Clinical Efficacy Assessment Subcommittee of the American College of Physicians. Screening mammography for women 40 to 49 years of age: A clinical practice guideline from the American College of Physicians. Ann Intern Med. 2007;146(7):511-515.
63. Ontario Ministry of Health and Long-Term Care, Medical Advisory Secretariat (MAS). Breast cancer screening and optimum imaging breast technologies for women 40-49 years of age - mammography. Health Technology Policy Assessment. Toronto, ON: MAS; January 2007.
64. Tosteson AN, Stout NK, Fryback DG, et al. Cost-effectiveness of digital mammography breast cancer screening. Ann Intern Med. 2008;148:1-10
65. Miller D, Livingstone V, Herbison P. Interventions for relieving the pain and discomfort of screening mammography. Cochrane Database Syst Rev. 2008;(1):CD002942.
66. Berg WA, Blume JD, Cormack JB, et al; ACRIN 6666 Investigators. Combined screening with ultrasound and mammography vs mammography alone in women at elevated risk of breast cancer. JAMA. 2008;299(18):2151-2163.
67. Hendrick RE, Cole EB, Pisano ED, et al. Accuracy of soft-copy digital mammography versus that of screen-film mammography according to digital manufacturer: ACRIN DMIST retrospective multireader study. Radiology. 2008;247(1):38-48.
68. Pisano ED, Hendrick RE, Yaffe MJ, et al; DMIST Investigators Group. Diagnostic accuracy of digital versus film mammography: Exploratory analysis of selected population subgroups in DMIST. Radiology. 2008;246(2):376-383.
69. Niklason LT, Christian BT, Niklason LE, et al. Digital tomosynthesis in breast imaging. Radiology. 1997;205:399-406.
70. Simonetti G, Cossu E, Montanaro M, et al. What's new in mammography. Eur J Radiol. 1998;27 Suppl 2:S234-241.
71. Poplack SP, Tosteson AT, Grove MR, et al. Mammography in 53,803 women from the New Hampshire Mammography Network. Radiology. 2000;217:832-840.
72. Smith AP, Hall PA, Marcello DM. Emerging technologies in breast cancer detection. Radiol Manage. 2004;26(4):16-24.
73. Smith A. Full-field breast tomosynthesis. Radiol Manage. 2005;27:25-31.
74. Gong X, Glick SJ, Liu B, et al. A computer simulation study comparing lesion detection accuracy with digital

mammography, breast tomosynthesis, and cone-beam CT breast imaging. Med Phys. 2006;33(4):1041-1052.
75. Schulz-Wendtland R, Wenkel E, Lell M, et al. Experimental phantom lesion detectability study using a digital breast tomosynthesis prototype system. Rofo. 2006;178(12):1219-1223.
76. Park JM, Franken EA Jr, Garg M, et al. Breast tomosynthesis: Present considerations and future applications. Radiographics. 2007;27 Suppl 1:S231-240.
77. Poplack SP, Tosteson TD, Kogel CA, Nagy HM. Digital breast tomosynthesis: Initial experience in 98 women with abnormal digital screening mammography. AJR Am J Roentgenol. 2007;189(3):616-623.
78. Sinha SP, Roubidoux MA, Helvie MA, et al. Multi-modality 3D breast imaging with x-ray tomosynthesis and automated ultrasound. Conf Proc IEEE Eng Med Biol Soc. 2007;2007:1335-1338.
79. Diekmann F, Bick U. Tomosynthesis and contrast-enhanced digital mammography: Recent advances in digital mammography. Eur Radiol. 2007;17(12):3086-3092.
80. Chen SC, Carton AK, Albert M, et al. Initial clinical experience with contrast-enhanced digital breast tomosynthesis. Acad Radiol. 2007;14(2):229-238.
81. Suryanarayanan S, Karellas A, Vedantham S, et al. Comparison of tomosynthesis methods used with digital mammography. Acad Radiol. 2000;7(12):1085-1097.
82. National Comprehensive Cancer Network (NCCN). Breast cancer screening and diagnosis guidelines. NCCN Clinical Practice Guidelines in Oncology V.1.2008. Fort Washington, PA: NCCN; April 2008. Available at: http://www.nccn.org/professionals/physician_gls/PDF/breast-screening.pdf. Accessed September 4, 2008.
83. Taylor P, Potts HW. Computer aids and human second reading as interventions in screening mammography: Two systematic reviews to compare effects on cancer detection and recall rate. Eur J Cancer. 2008;44(6):798-807.
84. Gromet M. Comparison of computer-aided detection to double reading of screening mammograms: Review of 231,221 mammograms. AJR Am J Roentgenol. 2008;190(4):854-859.
85. Noble M, Bruening W, Uhl S, Schoelles K. Computer-aided detection mammography for breast cancer screening: Systematic review and meta-analysis. Arch Gynecol Obstet. 2009;279(6):881-890.
86. U.S. Preventive Services Task Force. Screening for breast cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2009;151:716.
87. Purins A, Mundy L, Hiller JE. Breast tomosynthesis: A breast cancer screening tool. Horizon Scanning Prioritising Summary. Adelaide, SA: Adelaide Health Technology Assessment (AHTA); 2009;25.
88. Moulton K, Wright MD. Digital tomosynthesis of the breast: Clinical effectiveness, cost-effectiveness, and guidelines. Health Technology Assessment. Health Technology Inquiry Service (HTIS). Ottawa, ON: Canadian Agency for Drugs and Technologies in Health (CADTH); December 7, 2009.
89. Kelly KM, Dean J, Comulada WS, Lee SJ. Breast cancer detection using automated whole breast ultrasound and mammography in radiographically dense breasts. Eur Radiol. 2010;20(3):734-742.
90. Lee CH, Dershaw DD, Kopans D, et al. Breast cancer screening with imaging: Recommendations from the Society of Breast Imaging and the ACR on the use of mammography, breast MRI, breast ultrasound, and other technologies for the detection of clinically occult breast cancer. J Am Coll Radiol. 2010;7(1):18-27.
91. Helvie MA. Digital mammography imaging: Breast tomosynthesis and advanced applications. Radiol Clin North Am. 2010;48(5):917-929.
92. Spangler ML, Zuley ML, Sumkin JH, et al. Detection and classification of calcifications on digital breast tomosynthesis and 2D digital mammography: A comparison. AJR Am J Roentgenol. 2011;196(2):320-324.
93. Swedish Council on Health Technology Assessment (SBU). Computer-Aided Detection (CAD) in mammography screening. Summary and Conclusions. SBU Alert 2011-05. Stockholm, Sweden: SBU; 2011.
94. Canadian Task Force on Preventive Health Care, Tonelli M, Gorber SC, Joffres M, et al. Recommendations on screening for breast cancer in average-risk women aged 40-74 years. CMAJ. 2011;183(17):1991-2001.
95. Kerlikowske K, Hubbard RA, Miglioretti DL, et al; Breast Cancer Surveillance Consortium. Comparative effectiveness of digital versus film-screen mammography in community practice in the United States: A cohort study. Ann Intern Med. 2011;155(8):493-502.
96. Robertson C, Ragupathy SK, Boachie C, et al; Mammographic Surveillance Health Technology Assessment Group. Surveillance mammography for detecting ipsilateral breast tumour recurrence and metachronous contralateral breast cancer: A systematic review. Eur Radiol. 2011;21(12):2484-2491.
97. Gotzsche PC, Nielsen M. Screening for breast cancer with mammography. Cochrane Database Syst Rev. 2011; (1):CD001877.
98. American College of Obstetricians and Gynecologists (ACOG). Breast cancer screening. Washington, DC: American College of Obstetricians and Gynecologists (ACOG); August 2011. Availabe at: http://www.guideline.gov/content.aspx?id=34275&search=Breast+tomosynthesis+. Accessed October 29, 2012.
99. van Ravesteyn NT, Miglioretti DL, Stout NK, et al. Tipping the balance of benefits and harms to favor screening mammography starting at age 40 years: A comparative modeling study of risk. Ann Intern Med. 2012;156(9):609-617.
100. Berg WA, Zhang Z, Lehrer D, et al; ACRIN 6666 Investigators. Detection of breast cancer with addition of annual screening ultrasound or a single screening MRI to mammography in women with elevated breast cancer risk. JAMA. 2012;307(13):1394-1404.
101. Food and Drug Administration. Selenia Dimensions 3D System - P080003. February 11, 2011. FDA: Silver Spring, MD. Available at:

http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm246400.htm. Accessed October 29, 2012.
102. Sahiner B, Chan HP, Hadjiiski LM, et al. Computer-aided detection of clustered microcalcifications in digital breast tomosynthesis: A 3D approach. Med Phys. 2012;39(1):28-39.
103. Skaane P, Gullien R, Bjorndal H, et al. Digital breast tomosynthesis (DBT): Initial experience in a clinical setting. Acta Radiol. 2012;53(5):524-529.
104. Venkataraman S. Breast imaging: Mammography and ultrasonography. UpToDate [serial online]. Waltham, MA: UpToDate; reviewed September 2012.
105. Fredenberg E, Hemmendorff M, Cederström B, et al. Contrast-enhanced spectral mammography with a photon-counting detector. Med Phys. 2010;37(5):2017-2029.
106. Schmitzberger FF, Fallenberg EM, Lawaczeck R, et al. Development of low-dose photon-counting contrast-enhanced tomosynthesis with spectral imaging. Radiology. 2011;259(2):558-564.
107. Allec N, Abbaszadeh S, Karim KS. Single-layer and dual-layer contrast-enhanced mammography using amorphous selenium flat panel detectors. Phys Med Biol. 2011;56(18):5903-5923.
108. Bernardi D, Ciatto S, Pellegrini M, et al. Prospective study of breast tomosynthesis as a triage to assessment in screening. Breast Cancer Res Treat. 2012a;133(1):267-271.
109. Bernardi D, Ciatto S, Pellegrini M, et al. Application of breast tomosynthesis in screening: Incremental effect on mammography acquisition and reading time. Br J Radiol. 2012b;85(1020):e1174-e1178.
110. Zhao Y, Brun E, Coan P, et al. High-resolution, low-dose phase contrast X-ray tomography for 3D diagnosis of human breast cancers. Proc Natl Acad Sci USA. 2012;109(45):18290-18294.
111. Houssami N, Skaane P. Overview of the evidence on digital breast tomosynthesis in breast cancer detection. Breast. 2013;22(2):101-108.
112. Alakhras M, Bourne R, Rickard M, et al. Digital tomosynthesis: A new future for breast imaging? Clin Radiol. 2013;68(5):e225-e236.
113. Slanetz PJ. MRI of the breast and emerging technologies. UpToDate [serial online]. Waltham, MA: UpToDate; reviewed April 2013.
114. Zuley ML, Bandos AI, Ganott MA, et al. Digital breast tomosynthesis versus supplemental diagnostic mammographic views for evaluation of noncalcified breast lesions. Radiology. 2013;266(1):89-95.
115. Rafferty EA, Park JM, Philpotts LE, et al. Assessing radiologist performance using combined digital mammography and breast tomosynthesis compared with digital mammography alone: Results of a multicenter, multireader trial. Radiology. 2013;266(1):104-113.
116. Skaane P, Bandos AI, Gullien R, et al. Comparison of digital mammography alone and digital mammography plus tomosynthesis in a population-based screening program. Radiology. 2013;267(1):47-56.
117. American College of Obstetricians and Gynecologists (ACOG). Digital breast tomosynthesis. Technology Assessment No. 9. Obstet Gynecol. 2013;121(6):1415-1417.
118. Canadian Agency for Drugs and Technologies in Health (CADTH). Digital tomosynthesis for the screening and diagnosis of breast cancer: A review of the diagnostic accuracy. Rapid Response Report: Summary with Critical Appraisal. Ottawa, ON: CADTH; September 26, 2013.
119. Venkataraman S, Slanetz PJ. Breast imaging: Mammography and ultrasonography. UpToDate [serial online]. Waltham, MA: UpToDate; reviewed May 2014.
120. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Use of digital breast tomosynthesis with mammography for breast cancer screening or diagnosis. Technology Assessment. Chicago, IL: BCBSA; February 2014. Available at: http://www.bcbs.com/blueresources/tec/vols/28/use-of-digital-breast.html. Accessed May 7, 2014.
121. National Comprehensive Cancer Network (NCCN). Breast cancer screening and diagnosis. NCCN Clinical Practice Guidelines in Oncology. v.1.2014. Fort Washington, PA: NCCN; 2014.
122. Standing Committee on Screening by the Screening Section, Department of Health and Ageing. Digital Breast Tomosynthesis. Overview of the evidence and issues for its use in screening for breast cancer. Canberra, ACT: Department of Health; April 2013.

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CME Editor: Ayalew Tefferi, MD
CME Author: Elihu H. Estey, MD

CME Information: Acute myeloid leukemia: 2013 update on risk-stratification and management

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Upon completion of this educational activity, participants will be better able to:

1. Assess the relative importance of "treatment related mortality" and "resistance to therapy" as causes of therapeutic failure in AML
2. Be familiar with the need to obtain information about NPM and CEBPA mutations and FLT3 internal tandem duplications
3. Know several means to measure minimal residual disease (MRD) and the prognostic significance of MRD
4. Understand what results of randomized trials using chemotherapy +/− gemtuzumab ozogamicin tell us about the heterogeneity of AML

Activity Disclosures

No commercial support has been accepted related to the development or publication of this activity.

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Annual Clinical Updates in Hematological Malignancies:
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Acute myeloid leukemia: 2013 update on risk-stratification and management

Elihu H. Estey[1,2]*

Disease overview: Acute myeloid leukemia (AML) results from accumulation of abnormal blasts in the marrow. These cells interfere with normal hematopoiesis, can escape into the peripheral blood, and infiltrate CSF and lung. It is likely that many different mutations, epigenetic aberrations, or abnormalities in micro RNA expression can produce the same morphologic disease with these differences responsible for the very variable response to therapy, which is AMLs principal feature.

Diagnosis: This rests on demonstration that the marrow or blood has > 20% blasts of myeloid lineage. Blast lineage is assessed by multiparameter flow cytometry, with CD33 and CD13 being surface markers typically expressed by myeloid blasts. It should be realized that clinical/prognostic considerations, not the blast % per se, should be the main factor determining how a patient is treated.

Risk Stratification: Two features determine risk: the probability of treatment-related mortality (TRM) and, more important, even in patients aged >75 with Zubrod performance status 1, the probability of resistance to standard therapy despite not incurring TRM. The chief predictor of resistance is cytogenetics, with a monosomal karyotype (MK) denoting the disease is essentially incurable with standard therapy even if followed by a standard allogeneic transplant (HCT). The most common cytogentic finding is a normal karyotype(NK) and those of such patients with an NPM1 mutation but no FLT3 internal tandem duplication (ITD), or with a CEBPA mutation, have a prognosis similar to that of patients with the most favorable cytogenetics (inv 16 or t[8;21]) (60–70% cure rate). In contrast, NK patients with a FLT3 ITD have only a 30–40% chance of cure even after HCT. Accordingly analyses of NPM1, FLT3, and CEBPA should be part of routine evaluation, much as is cytogenetics. Risk is best assessed considering several variables simultaneously rather than, for example, only age. Increasing evidence indicates that other mutations and abnormalities in microRNA (miRNA) expression also affect resistance as do post treatment factors, in particular the presence of minimal residual disease. These newer mutations and MRD are discussed in this update.

Risk-adapted therapy: Patients with inv (16) or t(8;21) or who are NPM1+/FLT3ITD—can receive standard therapy (daunorubicin + cytarabine) and should not receive HCT in first CR. It seems likely that use of a daily daunorubicin dose of 90 mg/m^2 will further improve outcome in these patients. There appears no reason to use doses of cytarabine > 1 g/m^2 (for example bid X 6 days), as opposed to the more commonly used 3 g/m^2. Patients with an unfavorable karyotype (particularly MK) are unlikely to benefit from standard therapy (even with dose escalation) and are thus prime candidates for clinical trials of new drugs or new approaches to HCT; the latter should be done in first CR. Patients with intermediate prognoses (for example NK and NPM and FLT3ITD negative) should also receive HCT in first CR and can plausibly receive either investigational or standard induction therapy, with the same prognostic information about standard therapy leading one patient to choose the standard and another an investigational option. This update discusses results with newer agents: quizartinib and crenolanib, gemtuzumab ozogamicin, clofarabine and cladribine, azacitidine and decitabine, volasertib, and means to prevent relapse after allogeneic transplant.

The diagnosis of AML essentially is made as it was in 2012.Thus this review will emphasize new developments in risk stratification and treatment using as references many papers published in 2012. Am. J. Hematol. 88:318–327, 2013. © **2013 Wiley Periodicals, Inc.**

Risk Stratification

TRM vs. resistance

It is important to re-emphasize that the major cause of therapeutic failure in AML is resistance to treatment rather than treatment-related mortality (TRM) [1,2]. Although various criteria for TRM exist, Walter et al.[3] using data from 2,238 MD Anderson Cancer Center (MDA) and 1,127 Southwest Oncology Group (SWOG) patients given 3 days of an anthracycline + either standard doses of ara-C (100 mg/m^2 daily X 7, i.e. "3+7") or higher doses noted a sharp decline in weekly mortality rate once 4 weeks had elapsed from start of induction therapy suggesting that patients who died in these 4 weeks were a distinct group. Using 4-weeks as a criterion they developed (by bootstrapping) a model that predicts TRM. Of note covariates such as performance status, creatinine, albumin, platelet count, WBC and blast count and de novo vs. secondary AML can replace age in

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REVIEW ARTICLE

Prognostic significance of FLT3 internal tandem duplication, nucleophosmin 1, and CEBPA gene mutations for acute myeloid leukemia patients with normal karyotype and younger than 60 years: a systematic review and meta-analysis

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Abstract Diagnosis and classification of acute myeloid leukemia (AML) are based on morphology and genetics. An increasing number of gene mutations have been found, and some are used for risk classification in AML patients with normal karyotype (cytogenetically normal (CN)-AML). In this systematic review and meta-analysis, we examined three frequent mutations in CN-AML: mutations of *fms*-related tyrosine kinase 3 (*FLT3-ITD*), mutated nucleophosmin (*NPM1*), and mutations of the CCAAT enhancer-binding protein alpha (*CEBPA*) gene. A systematic literature search of publications listed in the electronic databases (Embase, Pubmed, Healthstar, BIOSIS, ISI Web of Knowledge and Cochrane) from 2000 up to March 2012 was performed (Fig. 1). Nineteen studies were included and qualitatively analyzed. Two to four studies entered the quantitative meta-analysis incorporating 1,378 to 1,942 patients with CN-AML. Meta-analysis for overall survival (OS) and relapse-free survival (RFS) showed *FLT3*-ITD to predict an unfavorable prognosis, with hazard ratios (HR) of 1.86 and 1.75, respectively. In contrast, meta-analysis of the impact of *NPM1* and *CEBPA* mutations on OS yielded an HR of 0.56 for each mutation, while analysis of impact on RFS produced HRs of 0.37 and 0.42, respectively. This systematic review and meta-analysis aimed to evaluate the prognostic value of mutations in the *NPM1*, *CEBPA*, and *FLT3* genes. *FLT3*-ITD was associated with worse prognosis, whereas mutations in *NPM1* and *CEBPA* genes were associated with a favorable prognosis.

Keywords Acute myeloid leukemia · Normal karyotype · Mutations of fms-related tyrosine kinase 3 · Mutated nucleophosmin · Mutations of the CEBPA gene · Meta-analysis

Introduction

Acute myeloid leukemia (AML) is a clonal disease of hematopoietic progenitor cells that accumulate a variety of mutations and epigenetic events leading to increased proliferation, disruption of differentiation, lack of apoptosis, and suppression of normal hematopoietic progenitor cells [1]. The natural history of disease is thus characterized by the lack of mature blood cells like granulocytes, lymphocytes, erythrocytes, or platelets. Without therapy, the outcome is typically fatal within weeks or months due to severe infection, bleeding, or wasting. Acquired genetic abnormalities determine the pathogenesis and the course of the disease. Until the recent developments in molecular genetics, diagnosis and classification were based on cytological criteria and cytogenetic alterations. The different karyotypes have enabled physicians to classify AML patients as low, intermediate, and high risk. Typically, AML patients are treated with chemotherapy. In adult patients younger than 60 years, therapy includes an induction

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Comparative Effectiveness Review: Prostate Cancer Antigen 3 Testing for the Diagnosis and Management of Prostate Cancer

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Purpose: We compared the effectiveness of PCA3 (prostate cancer antigen 3) and select comparators for improving initial or repeat biopsy decision making in men at risk for prostate cancer, or treatment choices in men with prostate cancer.

Materials and Methods: MEDLINE®, EMBASE®, Cochrane Database and gray literature were searched from January 1990 through May 2012. Included studies were matched, and measured PCA3 and comparator(s) within a cohort. No matched analyses were possible. Differences in independent performance estimates between PCA3 and comparators were computed within studies. Studies were assessed for quality using QUADAS (Quality Assessment of Diagnostic Accuracy Studies) and for strength of evidence using GRADE (Grading of Recommendations Assessment, Development and Evaluation) criteria.

Results: Among 1,556 publications identified, 34 observational studies were analyzed (24 addressed diagnostic accuracy and 13 addressed treatment decisions). Most studies were conducted in opportunistic cohorts of men referred for procedures and were not designed to answer key questions. Two study biases (partial verification and sampling) were addressed by analyses, allowing some conclusions to be drawn. PCA3 was more discriminatory than total prostate specific antigen increases (eg at an observed 50% specificity, summary sensitivities were 77% and 57%, respectively). Analyses indicated that this finding holds for initial and repeat biopsies, and that the markers were independent predictors. For all other biopsy decision making comparisons and associated health outcomes, strength of evidence was insufficient. For treatment decision making, strength of evidence was insufficient for all outcomes and comparators.

Conclusions: PCA3 had a higher diagnostic accuracy than total prostate specific antigen increases, but strength of evidence was low (limited confidence in effect estimates). Strength of evidence was insufficient to conclude that PCA3 testing leads to improved health outcomes. For all other outcomes and comparators, strength of evidence was insufficient.

Key Words: prostatic neoplasms; prostate cancer antigen 3, human; biopsy; predictive value of tests; comparative effectiveness research

Abbreviations and Acronyms

CER = comparative effectiveness review
cPSA = complexed prostate specific antigen
EVN = externally validated nomogram
FPR = false-positive rate
fPSA = free prostate specific antigen
KQ = key question
PCA3 = prostate cancer antigen 3
PSA = prostate specific antigen
PSAD = prostate specific antigen density
SOE = strength of evidence
TEP = Technical Expert Panel
tPSA = total prostate specific antigen

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Editor's Note: This article is the first of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 818 and 819.

PROSTATE cancer is the second leading cause of cancer death in the United States.[1] The rationale for tPSA screening was to reduce the prevalence of advanced prostate cancer and related mortality through early detection and treatment. While a decrease in prostate cancer related mortality has been observed,[1,2] studies in screened populations have raised concerns about the test's low specificity and the risks of treatment in men with early stage disease that may never progress to symptoms.[3] The balance of

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Platinum Priority – Review – Prostate Cancer

Editorial by Zoran Culig on pp. 588–589 of this issue

The Role of Genetic Markers in the Management of Prostate Cancer

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Abstract

Context: Despite widespread screening for prostate cancer (PCa) and major advances in the treatment of metastatic disease, PCa remains the second most common cause of cancer death for men in the Western world. In addition, the use of prostate-specific antigen testing has led to the diagnosis of many potentially indolent cancers, and aggressive treatment of these cancers has caused significant morbidity without clinical benefit in many cases. The recent discoveries of inherited and acquired genetic markers associated with PCa initiation and progression provide an opportunity to apply these findings to guide clinical decision making.

Objective: In this review, we discuss the potential use of genetic markers to better define groups of men at high risk of developing PCa, to improve screening techniques, to discriminate indolent versus aggressive disease, and to improve therapeutic strategies in patients with advanced disease.

Evidence acquisition: PubMed-based literature searches and abstracts through January 2012 provided the basis for this literature review. We also examined secondary sources from reference lists of retrieved articles and data presented at recent congresses. Cited review articles are only from the years 2007–2012, favoring more recent discussions because of the rapidly changing field. Original research articles were curated based on favoring large sample sizes, independent validation, frequent citations, and basic science directly related to potentially clinically relevant prognostic or predictive markers. In addition, all authors on the manuscript evaluated and interpreted the data acquired.

Evidence synthesis: We address the use of inherited genetic variants to assess risk of PCa development, risk of advanced disease, and duration of response to hormonal therapies. The potential for using urine measurements such as prostate cancer antigen 3 (*PCA3*) RNA and the transmembrane protease, serine 2 v-ets erythroblastosis virus E26 oncogene homolog (avian) (*TMPRSS2-ERG*) gene fusion to aid screening is discussed. Multiple groups have developed gene expression signatures from primary prostate tumors correlating with poor prognosis, and attempts to improve and standardize these signatures as diagnostic tests are presented. Massive sequencing efforts are underway to define important somatic genetic alterations (amplifications, deletions, point mutations, translocations) in PCa, and these alterations hold great promise as prognostic markers and for predicting response to therapy. We provide a rationale for assessing genetic markers in metastatic disease for guiding choice of therapy and for stratifying patients in clinical trials, and discuss challenges in clinical trial design incorporating the use of these markers.

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European Association of Urology

Collaborative Review – Prostate Cancer

Contemporary Role of Prostate Cancer Antigen 3 in the Management of Prostate Cancer

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Abstract

Context: Newly discovered biomarkers ideally should prove clinical usefulness, provide additional detection, staging, and prognosis information to improve individual risk assessment, and potentially permit targeted cancer therapy.
Objective: To review, display, and evaluate the current evidence regarding the biologic and analytic approach of urinary prostate cancer gene 3 (PCA3) in prostate cancer (PCa) detection, staging, and prognosis, and its therapeutic potential.
Evidence acquisition: A systematic and comprehensive Medline search was performed using the Medical Subject Headings search terms *PCA3, DD3, UPM3, prostate cancer, cell-lines, prostate tissue, prostate biopsy, detection, diagnosis, radical prostatectomy, staging, grading, progression,* and *gene therapy*. Results were restricted to English-language papers published within the period 1999–2011.
Evidence synthesis: The *PCA3* gene is highly overexpressed in specific PCa cell lines and prostatic tumours. In 2006, a simple and robust urine test (Progensa) became commercially available. Despite its costs, prostate cancer antigen 3 (PCA3) is superior to prostate-specific antigen (PSA) and percent free PSA in the early detection of PCa. PCA3 improves the diagnostic accuracy of externally validated nomograms among men with an elevated PSA undergoing biopsy. PCA3 independently predicts low-volume disease and pathologically insignificant PCa but is not associated with locally advanced disease and is limited in the prediction of aggressive cancer. Preliminary data demonstrate that combining PCA3 with other new biomarkers further improves diagnostic and prognostic accuracy. Finally, findings of the first PCA3-Gene-ViroTherapy study suggest therapeutic potential by exploiting PCA3 overexpression.
Conclusions: PCA3, integrated in novel biopsy nomograms or risk stratification tools, can be used to counsel or confirm biopsy indications. If confirmed in further studies, using PCA3 together with established staging risk factors could assist clinicians in specific pretreatment decision making. So far no evidence for the usefulness of PCA3 in active surveillance programs has been presented.
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Next Review: 06/11/2015
Review History
Definitions
Additional Information
Clinical Policy Bulletin Notes

Policy

Aetna considers prostate-specific antigen (PSA) screening a medically necessary preventive service for men aged 40 years and older, and for men under 40 years of age who are at high-risk for prostate cancer. Risk groups include African-American men and men with a family history of prostate cancer.

When used for routine screening, annual PSA screening is considered medically necessary, but additional PSA tests may be considered medically necessary in men with previously elevated PSAs or signs or symptoms of disease.

Aetna considers diagnostic PSA testing medically necessary for men of all ages with signs or symptoms of prostate cancer, and for follow-up of men with prostate cancer.

Aetna considers annual digital rectal examination (DRE) a medically necessary preventive service.

Aetna considers alpha-methylacyl coenzyme A racemase (AMACR), early prostate cancer antigen, endoglin, E twenty-six (ETS) gene fusions, human glandular kallikrein 2 (hK2) (also known as kallikrein-related peptidase 2 [KLK2]), interleukin-6, prostate cancer gene 3 (PCA3), TMPRSS2:ERG gene fusion, and transforming growth factor-beta 1 experimental and investigational for prostate cancer screening because they have no proven value for this indication.

Aetna considers measurement of selenium in the blood or in tissues (such as toenail clippings) experimental and investigational to assess the risk of developing prostate cancer because it has no proven value for this indication.

Aetna considers the analysis of prostatic fluid electrolyte composition (e.g., citrate, zinc; not an all inclusive list) experimental and investigational for prostate cancer screening because it has no proven value for this indication.

See also CPB 0352 - Tumor Markers.

Note: Some plans exclude coverage of preventive services. Please check benefit plan descriptions for details. Medically necessary diagnostic PSA testing is covered regardless of whether the member has preventive service benefits.

Background

The decision to perform routine prostate cancer screening with digital rectal examination (DRE) or prostate-specific antigen (PSA) is left to the discretion of the clinician. Patients who request screening should be given objective information about the potential benefits and harms of early detection and treatment.

The American Cancer Society (ACS) recommends PSA screening for all men over age 50 and at age 45 for men at higher risk (e.g., men with a family history of prostate cancer and African-American men). Similar recommendations have been issued by the American Urological Association (AUA) and the American College of Radiology. The ACS, however, acknowledges that currently there is no clinical trial evidence that screening for prostate cancer is associated with a reduction in mortality.

The updated ACS guideline for the early detection of prostate cancer (Wolf et al, 2010) recommends both the PSA blood test and DRE should be offered annually, beginning at age 50, to men who have at least a 10-year life expectancy. Men at high-risk (African-American men and men with a strong family of 1 or more first-degree relatives (father, brothers) diagnosed at an early age) should begin testing at age 45. Men at even higher risk, due to multiple first-degree relatives affected at an early age, could begin testing at age 40. Depending on the results of this initial test, no further testing might be needed until age 45. The ACS states that information should be provided to all men about what is known and what is uncertain about the benefits and limitations of early detection and treatment of prostate cancer so that they can make an informed decision about testing. Men who ask their doctor to make the decision on their behalf should be tested. The ACS states that discouraging testing is inappropriate. Furthermore, not offering testing is also inappropriate.

In a review on prostate cancer screening, Ilic and colleagues (2011) concluded that prostate cancer screening did not significantly decrease all-cause or prostate cancer-specific mortality in a combined meta-analysis of 5 randomized controlled trials. Any benefits from prostate cancer screening may take greater than 10 years to accrue; therefore, men who have a life expectancy of less than 10 to 15 years should be informed that screening for prostate cancer is not beneficial and has harms.

The AUA recommends that prostate cancer screening be offered to men over age 50, and at age 40 for men at high-risk, with the physician explaining the uncertain benefits and risks of such screening.

The National Comprehensive Cancer Network (2005) recommends baseline screening beginning at age 40, especially for men in high-risk groups. Risk groups include African-American men and men with a family history of prostate cancer.

Most professional societies do not recommend routine screening for prostate cancer with DRE or serum tumor markers (e.g., PSA). These include the American Academy of Family Physicians, the U.S. Preventive Services Task Force (USPSTF), the Institute for Clinical Systems Improvement, the Canadian Task Force on the Periodic Health Examination, the American College of Preventive Medicine, the U.S. Office of Technology Assessment, the American Society for Internal Medicine and American College of Physicians, the National Cancer Institute, the Centers for Disease Control and Prevention, and the technology assessment agencies of Canada, England, Sweden, and Australia.

The USPSTF evaluated randomized, controlled trials of the benefits of prostate cancer screening, cohort and cross-sectional studies of the psychological harms of false-positive PSA test results, and evidence on the natural history of PSA-detected prostate cancer and concluded that in men younger than age 75 years, the current evidence is insufficient to determine whether treatment for prostate cancer detected by screening improves health outcomes compared with treatment after clinical detection. In men aged 75 years or older, the USPSTF found no direct evidence of benefits of prostate cancer screening and recommends against screening for prostate cancer in men aged 75 years or older.

If screening is to be performed, the generally accepted approach is to screen with DRE and PSA and to limit screening to men with a life expectancy of greater than 10 years. There is currently insufficient evidence to determine the need and optimal interval for repeat screening or whether PSA thresholds must be adjusted for density, velocity, or age.

Schenk-Braat and Bangma (2006) noted that PSA is currently the most important biochemical marker for the diagnosis of prostate cancer. Because of the limited specificity of PSA, clinically irrelevant tumors and benign abnormalities are also detected that can potentially lead to over-treatment and the associated physical as well as emotional burden for the patient. Furthermore, PSA is used as an indicator of progression or clinical response following prostate cancer therapy, but the prognostic value of this marker is limited. Ongoing research is examining several alternative markers (e.g., osteoprotegerin, human kallikrein 2, and the gene DD3(PCA3)) that may improve the specificity of current PSA-based diagnostics and the prognostic value of PSA.

Prostate-specific antigen velocity, the yearly rate of increase of PSA, has not been proven to improve the test characteristics of PSA, Schroder and colleagues (2006) stated that PSA-driven screening has been applied to a large part of the male population in many countries. An elevated PSA in secondary screens may indicate benign enlargement of the prostate rather than prostate cancer. In such cases the yearly rate of increase of PSA (PSA velocity [PSAV]) may improve the test characteristics of PSA. These investigators examined if PSAV predict prostate cancer in pre-screened populations. Data from the European Randomized Study of Screening for Prostate Cancer Rotterdam were used to study the issue. Relative sensitivity, relative specificity, and positive predictive value (PPV) were calculated. Logistic regression analysis was used to compare odds ratios for positive biopsies. The relationship between PSAV and parameters of tumor aggressiveness was investigated. A total of 588 consecutive participants were identified who presented at their first screening with PSA values less than 4.0 and who progressed to PSA values greater than 4.0 ng/ml 4 years later were included in this study. None was biopsied in round-1, all were biopsied in round-2. Relative sensitivity and specificity depend strongly on PSAV cut-offs of 0.25 to 1.0 ng/ml/year. The use of PSAV cut-offs did not improve the PPV of the PSA cut-off of 4.0 ng/ml, nor did any of the PSAV cut-offs improve the odds ratio (OR) for identifying prostate cancer with respect to the cut-off value of 4.0 ng/ml. The rate of aggressive cancers seems to increase with increasing PSAV. The authors concluded that PSAV did not improve the detection characteristics of a PSA cut-off of 4.0 ng/ml in secondary screening after 4 years.

Wolters et al (2009) evaluated the value of PSAV in screening for prostate cancer. Specifically, the role of PSAV in lowering the number of unnecessary biopsies and reducing the detection rate of indolent prostate cancer was evaluated. All men included in the study cohort were subjects in the European Randomized Study of Screening for Prostate Cancer (ERSPC), Rotterdam section. During the first and second screening round, a PSA test was performed on 2,217 men, and all underwent a biopsy during the second screening round 4 years later. Prostate specific antigen velocity was calculated and biopsy outcome was classified as benign, possibly indolent prostate cancer, or clinically significant prostate cancer. A total of 441 cases of prostate cancer were detected, 333 were classified as clinically significant and 108 as possibly indolent. The use of PSAV cut-offs reduced the number of biopsies but led to important numbers of missed (indolent and significant) prostate cancer; PSAV was predictive for prostate ancer (OR: 1.28, $p < 0.001$) and specifically for significant prostate cancer (OR: 1.46, $p < 0.001$) in uni-variate analyses. However, multi-variate analyses using age, PSA, prostate volume, DRE and transrectal ultrasonography outcome, and previous biopsy (yes/no) showed that PSAV was not an independent predictor of prostate cancer (OR: 1.01, $p = 0.91$) or significant prostate cancer (OR: 0.87, $p = 0.30$). The authors concluded that the use of PSAV as a biopsy indicator would miss a large number of clinically significant cases of prostate cancer with increasing PSAV cut-offs. In this study, PSAV was not an independent predictor of a positive biopsy in general or significant prostate cancer on biopsy. Thus, PSAV does not improve the ERSPC screening algorithm.

The role of selenium in cancer prevention has been the subject of recent study and debate.

Population studies suggest that people with cancer are more likely to have low selenium levels (measured in the blood or in tissues such as toenail clippings) than healthy matched individuals. However, in most cases it is not clear if low selenium levels are a cause or merely a consequence of disease. Initial evidence from the Nutritional Prevention of Cancer (NPC) trial suggests that selenium supplementation reduces the risk of prostate cancer among men with normal baseline PSA levels and low selenium blood levels. The ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT) aims to definitively address the role of selenium in prostate cancer prevention. The study, which spans from 2001 to 2013, will include 32,400 men. Currently, it is unclear if selenium is beneficial in the treatment of prostate cancer or any type of cancer. Measurement of body selenium (e.g., in serum, toenail clippings) has no proven value in the prevention of prostate cancer.

Costello and Franklin (2009) proposed that changes in prostatic fluid composition could provide accurate and reliable biomarkers for the screening of prostate cancer. Most notable is the consistent and significant decrease in citrate and zinc that is associated with the development and progression of prostate cancer. These researchers provided the clinical and physiological basis and the evidence in support of the utility of prostatic fluid analysis as an effective approach for screening/detection of prostate cancer, especially early stage and "at-risk" subjects. The problem of interference from benign prostatic hypertrophy that hampers PSA testing is eliminated in the potential prostatic fluid biomarkers. The potential development of rapid, simple, direct, accurate clinical tests would provide additional advantageous conditions. The authors stated that further exploration and development of citrate, zinc and other electrolytes as prostatic fluid biomarkers are needed to address this critical prostate cancer issue.

A long-term randomized controlled clinical trial found prostate cancer screening had no effect on mortality (Andriole et al, 2009). From 1993 through 2001, investigators randomly assigned 76,693 men at 10 U.S. study centers to receive either annual screening (38,343 subjects) or usual care as the control (38,350 subjects). Men in the screening group were offered annual PSA testing for 6 years and DRE for 4 years. The subjects and health care providers received the results and decided on the type of follow-up evaluation. Usual care sometimes included screening, as some organizations have recommended. The numbers of all cancers and deaths and causes of death were ascertained. In the screening group, rates of compliance were 85 % for PSA testing and 86 % for DRE. Rates of screening in the control group increased from 40 % in the first year to 52 % in the sixth year for PSA testing and ranged from 41 to 46 % for DRE. After 7 years of follow-up, the incidence of prostate cancer per 10,000 person-years was 116 (2,820 cancers) in the screening group and 95 (2,322 cancers) in the control group (rate ratio, 1.22; 95 % confidence interval [CI]: 1.16 to 1.29). The incidence of death per 10,000 person-years was 2.0 (50 deaths) in the screening group and 1.7 (44 deaths) in the control group (rate ratio, 1.13; 95 % CI: 0.75 to 1.70). The data at 10 years were 67 % complete and consistent with these overall findings. An important limitation of this study is that subjects in the control group underwent considerable screening outside of the clinical trial. An accompanying editorial (Barry, 2009) commented that serial PSA screening has at best a modest effect on prostate cancer mortality during the first decade of follow-up, and that this benefit comes at the cost of substantial over-diagnosis and over-treatment.

Available evidence shows that the majority of men with low-risk prostate tumors receive aggressive treatment, despite the risk of complications. Shao and colleagues (2010) used the Surveillance, Epidemiology and End Results (SEER) database to study the records of 123,934 men over the age of 25 who had newly diagnosed prostate cancer from 2004 to 2006. About 14 % of the men had PSA values lower than 4, generally younger men. In that group, 54 % had low-risk disease that could be safely monitored for progression with little risk. Nonetheless, 75 % of them received aggressive treatment, including a radical prostatectomy and radiation therapy. Among men in that group over the age of 65, in which "watchful waiting" is generally advised for low-risk disease, 66 % had aggressive therapy. In both cases, the percentages were similar to those in the group with PSA levels between 4 and 20.

Mazzola et al (2011) stated that the introduction and widespread adoption of PSA has revolutionized the way prostate cancer is diagnosed and treated. However, the use of PSA has also

led to over-diagnosis and over-treatment of prostate cancer resulting in controversy about its use for screening. Prostate specific antigen also has limited predictive accuracy for predicting outcomes after treatment and for making clinical decisions about adjuvant and salvage therapies. Thus, there is an urgent need for novel biomarkers to supplement PSA for detection and management of prostate cancer. A plethora of promising blood- and urine-based biomarkers have shown promise in early studies and are at various stages of development (human kallikrein 2, early prostate cancer antigen, transforming growth factor-beta 1, interleukin-6, endoglin, prostate cancer gene 3 (PCA3), alpha-methylacyl coenzyme A racemase (AMACR) and E twenty-six (ETS) gene fusions).

Pettersson et al (2012) stated that whether the genomic re-arrangement trans-membrane protease, serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (ERG) has prognostic value in prostate cancer is unclear. Among men with prostate cancer in the prospective Physicians' Health and Health Professionals Follow-Up Studies, these researchers identified re-arrangement status by immunohistochemical assessment of ERG protein expression. They used Cox models to examine associations of ERG over-expression with biochemical recurrence and lethal disease (distant metastases or cancer-specific mortality). In a meta-analysis including 47 additional studies, these investigators used random-effects models to estimate associations between re-arrangement status and outcomes. The cohort consisted of 1,180 men treated with radical prostatectomy between 1983 and 2005. During a median follow-up of 12.6 years, 266 men experienced recurrence and 85 men developed lethal disease. These researchers found no significant association between ERG over-expression and biochemical recurrence [hazard ratio (HR), 0.99; 95 % CI: 0.78 to 1.26] or lethal disease (HR, 0.93; 95 % CI: 0.61 to 1.43). The meta-analysis of prostatectomy series included 5,074 men followed for biochemical recurrence (1,623 events), and 2,049 men followed for lethal disease (131 events). TMPRSS2:ERG was associated with stage at diagnosis [risk ratio (RR)(≥T3 vs. T2), 1.23; 95% CI, 1.16-1.30) but not with biochemical recurrence (RR, 1.00; 95 % CI: 0.86 to 1.17) or lethal disease (RR, 0.99; 95 % CI: 0.47 to 2.09). The authors concluded that the findings of this meta-analysis suggested that TMPRSS2:ERG, or ERG over-expression, is associated with tumor stage but does not strongly predict recurrence or mortality among men treated with radical prostatectomy.

Salagierski et al (2012) stated that widespread PSA screening together with the increase in the number of biopsy cores has led to increased prostate cancer incidence. Standard diagnostic tools still cannot unequivocally predict prostate cancer progression, which often results in a significant over-treatment rate. These investigators presented recent findings on PCA3 and TMPRSS:ERG fusion, and described their clinical implications and performance. The PubMed® database was searched for reports on PCA3 (130 articles), TMPRSS:ERG and ETS fusion (180 publications) since 1999. In recent years advances in genetics and biotechnology have stimulated the development of non-invasive tests to detect prostate cancer. Serum and urine molecular biomarkers have been identified, of which PCA3 has already been introduced clinically. The identification of prostate cancer specific genomic aberrations, i.e., TMPRSS2:ERG gene fusion, might improve diagnosis and affect prostate cancer treatment. The authors concluded that although several recently developed markers are promising, often showing increased specificity for prostate cancer detection compared to that of PSA, their clinical application is limited.

Choudhury et al (2012) noted that despite widespread screening for prostate cancer and major advances in the treatment of metastatic disease, prostate cancer remains the second most common cause of cancer death for men in the Western world. In addition, the use of PSA testing has led to the diagnosis of many potentially indolent cancers, and aggressive treatment of these cancers has caused significant morbidity without clinical benefit in many cases. The recent discoveries of inherited and acquired genetic markers associated with prostate cancer initiation and progression provide an opportunity to apply these findings to guide clinical decision-making. In this review, these investigators discussed the potential use of genetic markers to better define groups of men at high risk of developing prostate cancer, to improve screening techniques, to discriminate indolent versus aggressive disease, and to improve therapeutic strategies in patients with advanced disease. PubMed-based literature searches and abstracts through January 2012 provided the basis for this literature review. These researchers also examined secondary sources from reference lists of

retrieved articles and data presented at recent congresses. Cited review articles were only from the years 2007 to 2012, favoring more recent discussions because of the rapidly changing field. Original research articles were curated based on favoring large sample sizes, independent validation, frequent citations, and basic science directly related to potentially clinically relevant prognostic or predictive markers. In addition, all authors on the manuscript evaluated and interpreted the data acquired. These investigators addressed the use of inherited genetic variants to assess risk of prostate cancer development, risk of advanced disease, and duration of response to hormonal therapies. The potential for using urine measurements such as PCA3 RNA and TMPRSS2-ERG gene fusion to aid screening was discussed. Multiple groups have developed gene expression signatures from primary prostate tumors correlating with poor prognosis, and attempts to improve and standardize these signatures as diagnostic tests were presented. Massive sequencing efforts are underway to define important somatic genetic alterations (amplifications, deletions, point mutations, translocations) in prostate cancer, and these alterations hold great promise as prognostic markers and for predicting response to therapy. These researchers provided a rationale for assessing genetic markers in metastatic disease for guiding choice of therapy and for stratifying patients in clinical trials, and discussed challenges in clinical trial design incorporating the use of these markers. The authors concluded that the use of genetic markers has the potential to aid disease screening, improve prognostic discrimination, and prediction of response to treatment. However, most markers have not been prospectively validated for providing useful prognostic or predictive information or improvement upon clinicopathologic parameters already in use. They stated that significant efforts are underway to develop these research findings into clinically useful diagnostic tests in order to improve clinical decision making.

Furthermore, an UpToDate review on "Screening for prostate cancer" (Hoffman, 2013) does not mention the use of human kallikrein 2 and TMPRSS2:ERG gene fusion as screening tools for prostate cancer.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes covered if selection criteria are met:

84152

84153

84154

CPT codes not covered for indications listed in the CPB:

PCA3:

84255 Chemistry, Selenium

TMPRSS2:ERG gene fusion:

No specific code

Other CPT codes related to the CPB:

88271

88272

88273

88274

88275

88291

Modifier 0Z

HCPCS codes covered if selection criteria are met:

G0102	Prostate cancer screening; digital rectal examination
G0103	Prostate cancer screening; prostate specific antigen test (PSA)

HCPCS codes not covered for indications listed in the CPB:

S3721	Prostate cancer antigen 3 (PCA3) testing

ICD-9 codes covered if selection criteria are met:

185	Malignant neoplasm of prostate
222.2	Benign neoplasm of prostate
233.4	Carcinoma in situ of prostate
236.5	Neoplasm of uncertain behavior of prostate
600.00 - 600.91	Hypertrophy of prostate
602.3	Dysplasia of prostate
790.93	Elevated prostate specific antigen (PSA)
V10.46	Personal history of malignant neoplasm of prostate
V16.42	Family history of malignant neoplasm of prostate
V76.44	Special screening for malignant neoplasm of prostate
V84.03	Genetic susceptibility to malignant neoplasm of prostate

Other ICD-9 codes related to the CPB:

788.20 - 788.29	Retention of urine
788.41 - 788.43	Frequency of urination and polyuria
788.61 - 788.65	Other abnormality of urination

The above policy is based on the following references:

1. U.S. Preventive Services Task Force. Screening for prostate cancer: Recommendations and rationale. Ann Intern Med. 2002;137(11):915-916.
2. American College of Radiology (ACR). Resolution No. 36. Reston, VA: ACR; October 1991.
3. Canadian Task Force on the Periodic Health Examination. Canadian Guide to Clinical Preventive Health Care. Ottawa, ON: Canada Communications Group; 1994.
4. U.S. Congress, Office of Technology Assessment (OTA). Costs and effectiveness of prostate cancer screening in elderly men. Pub. No. OTA-PB-H-145. Washington, DC: U.S. Government Printing Office; 1995.
5. Coley CM, Barry MJ, Fleming C, et al. Early detection of prostate cancer. Part I: Prior probability and effectiveness of tests. Ann Intern Med. 1997;126(5):394-406.
6. Coley CM, Barry MJ, Fleming C, et al. Early detection of prostate cancer. Part II: Estimating the risks, benefits, and costs. American College of Physicians. Ann Intern Med. 1997;126(6):468-469.
7. Middleton RG. Prostate cancer: Are we screening and treating too much? Ann Intern Med. 1997;126(6):465-467.
8. Ferrini R, Woolf SH. Screening for prostate cancer in American men. American College of Preventive Medicine Practice Policy Statement. Am J Prev Med. 1998;15(1):81-84.
9. von Eschenbach A, Ho R, Murphy GP, et al. American Cancer Society guideline for early

detection of prostate cancer: Update 1997. CA Cancer J Clin. 1997;47(5):261-264.
10. Centers for Disease Control and Prevention (CDC). Prostate cancer: Can we reduce deaths and preserve quality of life? At-a-Glance 2000. Atlanta, GA: CDC; 2000. Available at: http://www.cdc.gov/cancer/prostate/prospdf/proaag00.pdf. Accessed July 28, 2000.
11. National Cancer Institute (NCI). Screening for prostate cancer (PDQ). Screening/detection -- Health professionals. Bethesda, MD: NCI; May 2000. Available at: http://cancernet.nci.nih.gov/cancertopics/pdq/screening/prostate/healthprofessional. Accessed July 28, 2000.
12. American Academy of Family Physicians. Summary of policy recommendations for periodic health examinations. Leawood, KS: American Academy of Family Physicians; August 2003.
13. Institute for Clinical Systems Improvement (ICSI). Preventive services for adults. ICSI Health Care Guidelines. Bloomington, MN: ICSI; September 2004.
14. Peters S, Jovell AJ, Garcia-Altes A, Serra-Prat M. Screening and clinical management of prostate cancer: A cross-national comparison. Int J Technol Assess Health Care. 2001;17:215-221.
15. Harris R, Lohr KN. Screening for prostate cancer: An update of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med. 2002;137(11):917-929.
16. Brawer MK. Clinical usefulness of assays for complexed prostate-specific antigen. Urol Clin North Am. 2002;29(1):193-203, xi.
17. Small EJ, Roach M 3rd. Prostate-specific antigen in prostate cancer: A case study in the development of a tumor marker to monitor recurrence and assess response. Semin Onco. 2002;29(3):264-273.
18. Slaughter PM, Pinfold SP, Laupacis A. Prostate-specific antigen (PSA) screening in asymptomatic men. Toronto, ON: Institute for Clinical Evaluative Sciences (ICES); 2002.
19. So A, Goldenberg L, Gleave ME. Prostate specific antigen: An updated review. Can J Urol. 2003;10(6):2040-2050.
20. Denmeade SR, Isaacs JT. The role of prostate-specific antigen in the clinical evaluation of prostatic disease. BJU Int. 2004;93 Suppl 1:10-15.
21. Han M, Gann PH, Catalona WJ. Prostate-specific antigen and screening for prostate cancer. Med Clin North Am. 2004;88(2):245-265, ix.
22. National Comprehensive Cancer Network (NCCN). Prostate cancer early detection. Clinical Practice Guidelines in Oncology. Version 1.2004. Jenkintown, PA: NCCN; March 3, 2004.
23. American Cancer Society (ACS). Recommendations from the American Cancer Society Workshop on Early Prostate Cancer Detection, May 4-6, 2000 and ACS guideline on testing for early prostate cancer detection: Update 2001. CA Cancer J Clin. 2001;51(1):39-44.
24. Smith RA, Cokkinides V, Eyre HJ. American Cancer Society guidelines for the early detection of cancer, 2003. CA Cancer J Clin. 2003;53(1):27-43.
25. American Urological Association. Prostate-specific antigen (PSA) best practice policy. Oncology. 2000;14(2):267-286.
26. Carroll P, Coley C, McLeod D, et al. Prostate-specific antigen best practice policy--part I: Early detection and diagnosis of prostate cancer. Urology. 2001;57(2):217-224.
27. Carroll P, Coley C, McLeod D, et al. Prostate-specific antigen best practice policy--part II: Prostate cancer staging and post-treatment follow-up. Urology. 2001;57(2):225-229.
28. University of Michigan Health System. Adult preventive health care: Cancer screening. Ann Arbor, MI: University of Michigan Health System; May 2004.
29. Medical Services Advisory Committee (MSAC). Prostate specific antigen (PSA) near patient testing for diagnosis and management of prostate cancer. MSAC Application 1068. Canberra, ACT: MSAC; 2005.
30. Ilic D, O'Connor D, Green S, Wilt T. Screening for prostate cancer. Cochrane Database Syst Rev. 2006:(3):CD004720.
31. Mambourg F, Van den Bruel A, Devriese S, et al. Health technology assessment: The use of prostate specific antigen (PSA) in prostate cancer screening. KCE Reports Vol. 31B. Brussels, Belguim: Belgian Health Care Knowledge Centre (KCE); 2006.
32. Schenk-Braat EA, Bangma CH. The search for better markers for prostate cancer than prostate-specific antigen. Ned Tijdschr Geneeskd. 2006;150(23):1286-1290.
33. Ilic D, O'Connor D, Green S, Wilt T. Screening for prostate cancer: A Cochrane systematic review. Cancer Causes Control. 2007;18(3):279-285.

34. Bryant RJ, Hamdy FC. Screening for prostate cancer: An update. Eur Urol. 2008;53(1):37-44.
35. Lim LS, Sherin K; ACPM Prevention Practice Committee. Screening for prostate cancer in U.S. men ACPM position statement on preventive practice. Am J Prev Med. 2008;34(2):164-170.
36. Lin K, Lipsitz R, Miller T, Janakiraman S. Benefits and harms of prostate-specific cancer screening: An evidence update for the U.S. Preventive Services Task Force. Evidence Synthesis No. 63. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); 2008.
37. U.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2008;149:185-191.
38. Satia JA, King IB, Morris JS, Stratton K, White E. Toenail and plasma levels as biomarkers of selenium exposure. Ann Epidemiol. 2006;16(1):53-58.
39. van den Brandt PA, Zeegers MP, Bode P, Goldbohmm A. Toenail selenium levels and the subsequent risk of prostate cancer: A prospective Cohort study. Cancer Epidemiology, Biomarkers & Prevention. 2003;12:866-871. Available at: http://cebp.aacrjournals.org/cgi/reprint/12/9/866.pdf. Accessed August 14, 2008.
40. Reid ME, Duffield-Lillico AJ, Slate E, et al. The nutritional prevention of cancer: 400 mcg per day selenium treatment. Nutr Cancer. 2008;60(2):155-163.
41. Costello LC, Franklin RB. Prostatic fluid electrolyte composition for the screening of prostate cancer: A potential solution to a major problem. Prostate Cancer Prostatic Dis. 2009;12(1):17-24.
42. Schröder FH, Roobol MJ, van der Kwast TH, et al. Does PSA velocity predict prostate cancer in pre-screened populations? Eur Urol. 2006;49(3):460-465; discussion 465.
43. Wolters T, Roobol MJ, Bangma CH, Schröder FH. Is prostate-specific antigen velocity selective for clinically significant prostate cancer in screening? European Randomized Study of Screening for Prostate Cancer (Rotterdam). Eur Urol. 2009;55(2):385-392.
44. Schröder FH, Hugosson J, Roobol MJ, et al; ERSPC Investigators. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med. 2009;360(13):1320-1328.
45. Shao YH, Albertsen PC, Roberts CB, et al. Risk profiles and treatment patterns among men diagnosed as having prostate cancer and a prostate-specific antigen level below 4.0 ng/mL. Arch Intern Med. 2010;170(14):1256-1261.
46. Lu-Yao GL, Albertsen PC, Moore DF, et al. Outcomes of localized prostate cancer following conservative management. JAMA. 2009;302(11):1202-1209.
47. Shappley WV 3rd, Kenfield SA, Kasperzyk JL, et al. Prospective study of determinants and outcomes of deferred treatment or watchful waiting among men with prostate cancer in a nationwide cohort. J Clin Oncol. 2009;27(30):4980-4985.
48. Zietman A. Evidence-based medicine, conscience-based medicine, and the management of low-risk prostate cancer. J Clin Oncol. 2009;27(30):4935-4936.
49. Esserman L, Shieh Y, Thompson I. Rethinking screening for breast cancer and prostate cancer. JAMA. 2009;302(15):1685-1692.
50. Andriole GL, Crawford ED, Grubb RL 3rd, et al; PLCO Project Team. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med. 2009;360(13):1310-1319.
51. Barry MJ. Screening for prostate cancer -- the controversy that refuses to die. N Engl J Med. 2009;360(13):1351-1354.
52. Greene KL, Albertsen PC, Babaian RJ, et al. Prostate specific antigen best practice statement: 2009 update. J Urol. 2009;182(5):2232-2241.
53. Shao YH, Albertsen PC, Roberts CB, et al. Risk profiles and treatment patterns among men diagnosed as having prostate cancer and a prostate-specific antigen level below 4.0 ng/ml. Arch Intern Med. 2010;170(14):1256-1261.
54. Wolf AM, Wender RC, Etzioni RB, et al; American Cancer Society Prostate Cancer Advisory Committee. American Cancer Society guideline for the early detection of prostate cancer: Update 2010. CA Cancer J Clin. 2010;60(2):70-98.
55. Mazzola CR, Ghoneim T, Shariat SF. Emerging biomarkers for the diagnosis, staging and prognosis of prostate cancer. Prog Urol. 2011;21(1):1-10.
56. Ilic D, O'Connor D, Green S, Wilt TJ. Screening for prostate cancer: An updated Cochrane systematic review. BJU Int. 2011;107(6):882-891.

57. Djulbegovic M, Beyth RJ, Neuberger MM, et al. Screening for prostate cancer: Systematic review and meta-analysis of randomised controlled trials. BMJ. 2010;341:c4543.
58. Chou R, Croswell JM, Dana T, et al. Screening for prostate cancer: A review of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med. 2011;155(11):762-771.
59. U.S. Preventive Services Task Force. Screening for prostate cancer: Current recommendation. Rockville, MD: Agency for Healthcare Research and Quality; 2012. Available at: http://www.uspreventiveservicestaskforce.org/prostatecancerscreening.htm. Accessed: June 5, 2012.
60. National Comprehensive Cancer Network (NCCN). Prostate cancer early detection. NCCN Clinical Practice Guidelines in Oncology version 2.2012. Fort Washington, PA: NCCN; 2012.
61. Pettersson A, Graff RE, Bauer SR, et al. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: A cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2012;21(9):1497-1509.
62. Salagierski M, Schalken JA. Molecular diagnosis of prostate cancer: PCA3 and TMPRSS2:ERG gene fusion. J Urol. 2012;187(3):795-801.
63. Choudhury AD, Eeles R, Freedland SJ, et al. The role of genetic markers in the management of prostate cancer. Eur Urol. 2012;62(4):577-587.
64. Hoffman RM. Screening for prostate cancer. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed April 2013.
65. Qaseem A, Barry MJ, Denberg TD, et al. Screening for prostate cancer: A guidance statement from the Clinical Guidelines Committee of the American College of Physicians. Ann Intern Med. 2013;158(10):761-769.

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Technology Assessment

Technology Assessment Program

Technology Assessment of Molecular Pathology Testing for the Estimation of Prognosis for Common Cancers

Final
May 29, 2014

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RTI International-University of North Carolina at Chapel Hill Evidence Based Practice Center" (RTI-UNC EPC)

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Peer Reviewers

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Structured Abstract

Structured Abstract

Objectives: To conduct a systematic review and meta-analysis assessing the prognostic value and test performance (analytic validity, clinical validity, clinical utility, and harms) associated with 11 prognostic molecular pathology tests. Many of these tests are indicated for prediction of therapeutic responses, but this review focuses on their potential use as prognostic factors. Our overarching question was whether there is direct evidence that the addition of these molecular pathology tests changed physician decisionmaking and improved outcomes for adult patients.

We evaluated the following tests: microsatellite instability assessment by polymerase chain reaction (PCR) for colorectal cancer (CRC), MLH1 promoter methylation for CRC, *KRAS* mutation testing for CRC, *BRAF* mutation testing for CRC, Oncotype DX Colon mRNA expression for CRC, Oncotype DX Breast mRNA expression for breast cancer, MammaPrint mRNA expression for breast cancer, *ALK* cytogenetics for lung cancer, *EGFR* mutation testing for lung cancer, *KRAS* mutation testing for lung cancer, and UroVysion cytogenetics for urinary bladder cancer.

Data Sources: PubMed®, the Cochrane Library, and EMBASE® (through November 2013); reference lists of pertinent review articles and included studies, test developers' Web sites, ClinicalTrials.gov, the Food and Drug Administration (FDA) Web site, Health Services Research Projects in Progress, the European Union Clinical Trials Register, the College of American Pathologists (CAP), and data submitted by companies that developed the tests.

Review Methods: Two investigators independently selected, extracted data from, and rated risk of bias of each study. For clinical validity, we conducted meta-analyses to estimate weighted summary hazard ratios when three or more studies reported an eligible outcome. For the other review questions, we did not find sufficient data to conduct meta-analyses. Therefore, we synthesized the information qualitatively. We graded strength of evidence based on established guidance.

Results: Evidence from multiple studies supports associations between test results and prognosis, with added value beyond known independent prognostic factors, for MammaPrint, Oncotype DX Breast, *KRAS* mutation testing for lung cancer, *BRAF* and *KRAS* mutation testing for CRC, and microsatellite instability for CRC for at least one of our included outcomes (i.e., risk of recurrence, cancer-specific survival, or overall survival). Although UroVysion is marketed as a diagnostic rather than a prognostic test, limited evidence from two small studies (total N=168) rated as low or medium risk of bias supported associations between test result and prognosis for risk of recurrence. We found no studies that directly assessed the impact of a test of interest on both physician decision-making and downstream health outcomes to establish clinical utility. We attempted to construct an indirect chain of evidence to answer the overarching question, but we were unable to do so. Even in the cases where the tests seemed to add value in determining prognosis (i.e., evidence of clinical validity), we found no evidence that using the test was related to improved outcomes for patients. However, for impact of test use on treatment decisions, we found moderate strength of evidence that Oncotype DX Breast leads to changes in treatment decisions. Although the decision changes were observed in both directions for

individual patients, studies consistently showed an overall shift to less-intensive treatment recommendations as a result of using Oncotype DX Breast, with fewer recommendations for chemotherapy (and less exposure to potential harms of chemotherapy).

Limitations: We were not able to directly address the prognostic value of these tests for the Medicare population. There were several studies that included patients from the Medicare age group and although the impact of these tests were not analyzed with specific reference to this population, we did not come across any evidence that suggested that the prognostic value of these tests would be different for the Medicare population.

Conclusions: Modest evidence supports added prognostic value (i.e., clinical validity) for over half of the tests evaluated, and that Oncotype DX Breast leads to changes in treatment decisions, but we found no evidence to determine whether using the tests to estimate prognosis leads to improved outcomes for patients.

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Executive Summary

Background

Molecular pathology tests that identify pathogenic mutations and cytogenetic translocations help us define the molecular subtypes of common cancers. Because several of these acquired mutations/translocations may predict response to specific therapies, screening tests for "targetable" mutations are now commonly available in clinical laboratory improvement amendments (CLIA)-approved clinical labs. It is unclear whether these test results can also serve as independent prognostic factors. This review aims to clarify the value of certain molecular pathology tests for improving our estimates of prognosis for common cancers (breast, lung, colon, urinary bladder) affecting Medicare beneficiaries. The main purpose of this review is to determine whether these tests improve estimation of prognosis (for recurrence), affect physician decision making, and/or improve clinical outcomes when compared with traditional assessment of prognosis of recurrence. These genetic tests are used in two different contexts. In one, the tests are used in a specific context of a diagnostic/therapy combination, where the diagnostic test is being used to predict response to a very specific treatment. In the second context, the genetic tests are used to estimate the patient's prognosis, and physicians use this prognostic information to choose from a variety of different treatment options. CMS requested this report to evaluate the second context. Therefore, studies that evaluate specific diagnostic/therapy combinations are excluded from this report

The following tests are under consideration for this assessment and apply to all Key Questions (KQs): microsatellite instability (MSI) for colorectal cancer (CRC), MLH1 promoter methylation for CRC, *KRAS* mutations for CRC, *BRAF* mutations for CRC, Oncotype DX Colon mRNA expression for CRC, Oncotype DX Breast mRNA expression for breast cancer, MammaPrint mRNA expression for breast cancer, *ALK* cytogenetics for lung cancer, *EGFR* mutations for lung cancer, *KRAS* mutations for lung cancer, and UroVysion cytogenetics for urinary bladder cancer.

Our overarching question was whether there is direct evidence that the addition of the results of these molecular pathology tests to traditional prognostic factors changed physician decisionmaking and improved clinical outcomes for adult patients. We also examined analytic validity, clinical validity and utility, and any harms to patients associated with these tests. In this review, we address the following KQs:

KQ 1. Overarching Question: Is there direct evidence that the addition of the specified molecular pathology tests used alone or in combination with traditional prognostic factors changes physician decisionmaking and improves outcomes for adult patients with CRC, breast, lung, or bladder cancer compared with the use of traditional factors to predict risk of recurrence (RR) for adults with these cancers?

KQ 2. Analytic Validity: Does existing evidence establish the technical accuracy and reliability of these tests for detecting the relevant molecular analytes?

KQ 3. Clinical Validity: Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4. Clinical Utility: Does existing evidence support clinical utility of the molecular pathology tests?

KQ 4a. What is the evidence that the prognostic information provided by the molecular pathology tests modifies physician decisions regarding use of adjuvant antineoplastic chemo- and/or radiotherapy, enhanced diagnostic testing for recurrence, and/or surgery among adult patients with malignant tumors?

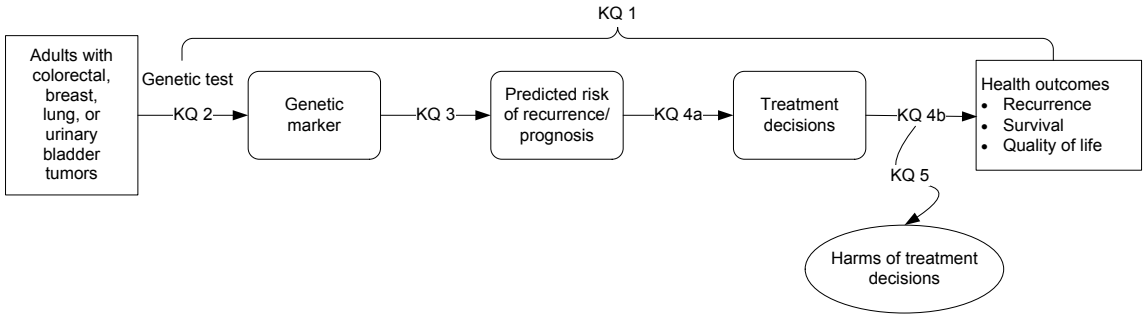
KQ 4b. What is the evidence that modified decisions lead to improved outcomes, including patient-centered outcomes such as improved quality of life, reduced disease recurrence, increased overall survival (OS) or disease-free survival (DFS), or reduced therapeutic side effects?

KQ 5. What are the harms associated with treatment decisions that are informed by the molecular pathology tests?

Analytic Framework

We developed an analytic framework to guide the systematic review process (Figure A).

Figure A. Analytic framework



Abbreviation: KQ = Key Question.

Methods

Literature Search Strategy

We searched PubMed®, the Cochrane Library, and EMBASE® for English-language, adult, and human-only studies published from inception through November 2013. Searches were run by an experienced Evidence-based Practice Center (EPC) librarian and were peer reviewed by another EPC librarian. We manually searched reference lists of pertinent review articles and studies meeting our inclusion criteria to look for any relevant citations that our searches might have missed. We searched for unpublished studies relevant to this review using test developers' Web sites, ClinicalTrials.gov, the Food and Drug Administration Web site, Health Services Research Projects in Progress, and the European Union Clinical Trials Register. In addition, we requested information from the College of American Pathologists (CAP) and from relevant companies, asking for data that they believe should be considered for the review.

Eligibility Criteria

We developed eligibility criteria for Populations, Interventions (i.e., tests for this report), Comparators, Outcomes, Timing, Settings (PICOTS), and study designs. Briefly, we included studies of adult patients with one of the cancer types of interest that evaluated an eligible molecular pathology test (listed in the Background). For KQs 1, 4, and 5, we included studies that compare at least 1 of the 11 molecular pathology tests plus standard prognostic factors with the standard prognostic factors alone to determine whether the molecular pathology test adds independent prognostic value (benefit) or introduces additional harms (KQ 5). Studies that compare an eligible molecular pathology test alone with standard prognostic factors were also eligible for inclusion. For KQ 2 (analytic validity), we included studies of test performance, including intra/interlab reproducibility. For KQ 3 (clinical validity), we included studies comparing patients with different test results (e.g., those with a mutation versus those who are wild-type) to establish prognostic value, with a multivariate analysis to adjust for known factors. To inform whether these molecular pathology tests *add value* above standard prognostic factors, we required that results were either adjusted for known prognostic factors or were specifically addressed in other ways, such as through inclusion/exclusion criteria of the study or stratification.

We required studies to report one of the following outcomes: for KQ 1, direct evidence of the impact of the test on physician decisionmaking and also on health outcomes (OS, DFS, time to recurrence, or quality of life); for KQ 2, tissue sample acceptance/rejection criteria, test performance, intra- and interlab reproducibility for the same specimen, lower limit of detection in admixtures of carcinoma and normal cells, and quality controls; for KQ 3, assessment of added prognostic value (i.e., by assessing discrimination, calibration, or association of test results with recurrence) beyond known independent prognostic factors for recurrence, cancer-specific survival (CSS), and overall survival (OS); for KQ 4a, modification of physician decisions about treatment; for KQ 4b, health outcomes (OS, DFS, time to recurrence, quality of life); and for KQ 5, adverse effects of tests, adverse effects of adjuvant therapy, and decreased quality of life.

We did not include studies focused on patients with advanced/metastatic cancer or studies focused on predicting response to treatments (e.g., studies focused on response to gefitinib for people with *EGFR*-mutant advanced lung adenocarcinomas). Progression-free survival was not an eligible outcome. Additional details of the eligibility criteria, including eligible study designs, are provided in the full technical report.

Study Selection

Two members of the research team independently reviewed each title and abstract identified through searches for eligibility. Studies marked for possible inclusion by either reviewer and those that lacked adequate information to determine eligibility underwent a full-text review. Two members of the research team independently reviewed each full-text article to determine eligibility. If the reviewers disagreed, they resolved conflicts by discussion and consensus or by consulting a third member of the review team.

Data Extraction

We designed and used structured data extraction forms to gather pertinent information from each article; this included characteristics of study populations, settings, interventions, comparators, study designs, methods, and results. Trained reviewers extracted the relevant data

from each included article. All data extractions were reviewed for completeness and accuracy by a second member of the team.

Risk-of-Bias Assessment of Individual Studies

We assessed the risk of bias (RoB) (internal validity) using an approach supported by the *Methods Guide for Medical Test Reviews*.[1] For studies of analytic validity, we considered the potential for bias due to flaws in the sample selection, testing protocol, reference standards, verification procedures, interpretation, and analysis. We used the questions relevant for prognostic tests from the QUADAS-2[2,3] (which was developed for diagnostic tests) for assessing risk of relevant biases. For studies of clinical validity and clinical utility, we assessed the potential for selection bias, confounding, performance bias, attrition bias, and detection bias. We assessed these biases using relevant questions and predefined criteria based on guidance from the AHRQ *Methods Guide for Effectiveness and Comparative Effectiveness Reviews* and the RTI Question Bank.[4] We rated RoB for each study as low, medium, high, or unclear. Two independent reviewers assessed the RoB for each study. Disagreements between the two reviewers were resolved by discussion and consensus or by a third member of the team.

Data Synthesis

For clinical validity (KQ 3), we conducted meta-analyses using techniques described in Hedges and Vevea.[4] We estimated summary hazard ratios (HRs) for outcomes (for any given test-cancer pair) with three or more independent adjusted HR estimates. We tested the null hypothesis of homogeneity of effect sizes across the studies[4,5] for each of the outcomes. If the null hypothesis was rejected at a significance level of 0.05, the summary HR was estimated using a random effects model; if not, it was estimated using a fixed effects model. In both cases, the inverse of the variance was used to estimate a weighted summary HR.

For the other KQs, we did not find sufficient data to conduct meta-analyses. Therefore, we synthesized the information qualitatively, in tabular and narrative format.

We did not include studies with a high or unclear RoB in our main analyses/main data syntheses.

Strength of the Body of Evidence

We graded the strength of evidence (SOE) as high, moderate, low, or insufficient based on the guidance established for the EPC Program.[6,7] Developed to grade the overall strength of a body of evidence, this approach incorporates four key domains: RoB (includes study design and aggregate quality), consistency, directness, and precision of the evidence. It also considers other optional domains. For each test being evaluated in this review, two reviewers assessed each domain for each key outcome and determined an overall SOE grade based on domain ratings. In the event of disagreements on the domain or overall grade, they resolved differences by consensus discussion or by consulting with a third investigator.

We attempted to find studies that directly address the overarching question (KQ 1). If we did not find adequate direct evidence addressing the overarching question, we attempted to construct an indirect chain of evidence (using studies identified for the other KQs). We graded the SOE separately for analytic validity and for evidence on our overarching KQ. For analytic validity, we graded the SOE for the following: sensitivity and specificity, positive and negative predictive

value, and intra/interlab reproducibility. For evidence on the overarching KQ, we graded the SOE for the following outcomes: RR, CSS, OS, and decisions about treatment.

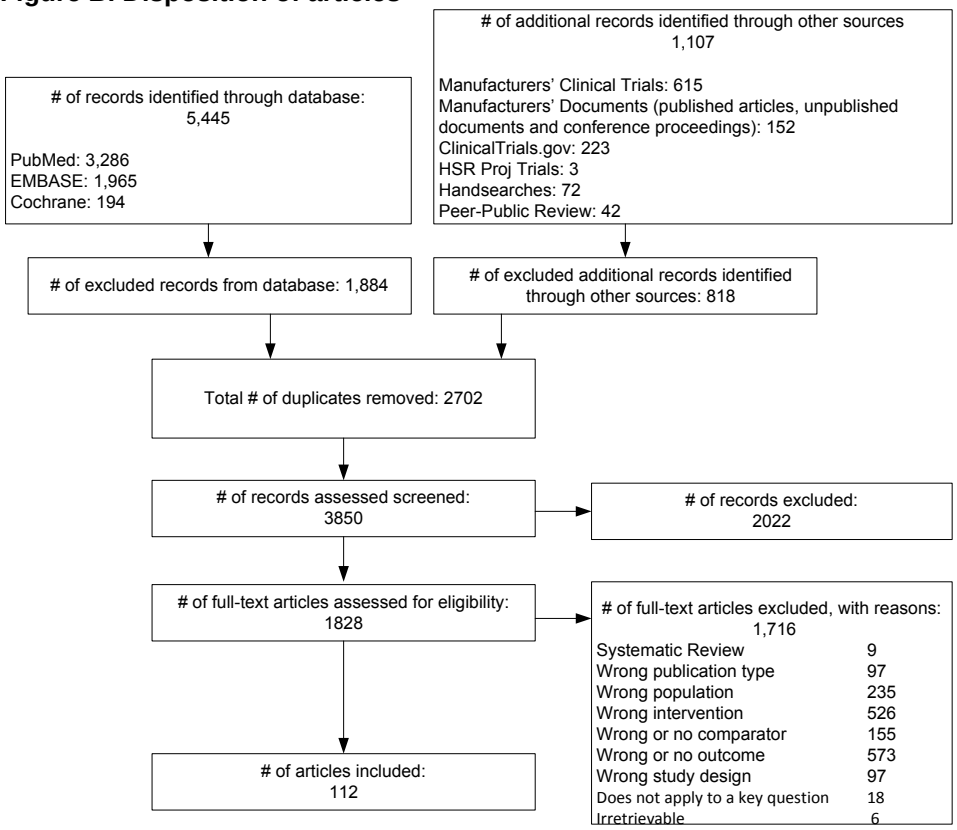
Applicability

We assessed applicability of the evidence following guidance from the *Methods Guide for Comparative Effectiveness Reviews*.[5,8] We used the PICOTS framework to explore factors that affect applicability.

Results

We included 112 publications reporting on tests of interest for the 4 cancers (Figure B).

Figure B. Disposition of articles



We found no studies that directly addressed our overarching questions (KQ 1) (i.e., no study directly assessed the impact of a molecular pathology test result of interest on both physician decisionmaking and downstream health outcomes). We attempted to construct an indirect chain of evidence to answer the overarching question, but no studies addressed whether modified decisions lead to improved health outcomes (KQ 4b, clinical utility), so we were unable to do so. The furthest downstream evidence that we found addressed whether prognostic information provided by the molecular pathology tests modifies treatment decisions (KQ 4a).

The majority of studies meeting inclusion criteria for our review focused on clinical validity (KQ 3), and they evaluated associations between prognostic tests and outcomes. For two tests, MammaPrint and Oncotype DX Breast, we found studies rated as low or medium RoB

addressing whether prognostic information provided by their mRNA abundance risk stratification tests modified treatment decisions (KQ 4a).

Analytic Validity

Included studies provide some evidence regarding analytic validity (KQ 2) for all of the included tests. Data from included studies was supplemented with proficiency tests results provided by the College of American Pathologists (CAP) for five tests for which this data was available.

Data on intra- and interlab reproducibility is available in the primary literature and through national organization proficiency testing programs. Beau-Faller[9] found high interlab reproducibility for EGFR and KRAS mutation testing. Cronin's[10] group at Genomic Health (Clin Chem 2007) found high intralab reproducibility for Oncotype quantitative RT-PCR mRNA abundance measurements relevant to the Oncotype DX tests for breast and colon carcinoma. The College of American Pathologists sends proficiency test unknowns to CLIA-approved US clinical laboratories or International clinical laboratories, an excellent mechanism for assessing nationwide interlab reproducibility. The three most recent surveys for each of these analytes showed average accuracy rates of 95 percent for EGFR (413/438), 98 percent for *KRAS* (602/612), 99 percent for *BRAF* (528/533), 99 percent for MSI (288/291), and 99 percent for UroVysion (552/555) (data presented are percentage of labs with correct results; and number with correct results/total number of labs that tested in parentheses).[11-15]

Clinical Validity

Included studies provided some evidence on clinical validity for 9 of the included tests, adjusted for known prognostic factors (Table A). Evidence from multiple studies supports clinical validity, with added value beyond traditional prognostic factors, for MammaPrint, Oncotype DX Breast, *KRAS* mutation testing for lung cancer, *BRAF* mutation testing for CRC, *KRAS* mutation testing for CRC, and MSI for CRC for at least one of our included outcomes (i.e., RR, CSS, or OS). For UroVysion, limited evidence from 2 small studies (total N=168) rated as low or medium RoB supported prognostic value for RR. *EGFR* lung cancer did not add prognostic value to the traditional factors used to determine prognosis. For CRC, evidence did not adequately support added prognostic value for Oncotype DX Colon. The metric used to assess the clinical validity of the test for recurrence, CSS, or OS in all of these studies was the HR. HRs in this report range from 0.57 to 3.93. If the test is noninformative, we expect that the probability of experiencing the end point would be the same for either group, with an HR of 1. If the HR is greater than 1, the probability of the endpoint is higher in the group with the higher hazard. If the HR is lower than 1, the probability of experiencing the endpoint is lower in the group with the lower hazard. For example, an HR of 2 for CSS indicates that one group (e.g., those with high risk results for Oncotype DX Breast) has twice the rate per unit of time as the comparison group (e.g., those with low-risk test results).

Table A. Summary of findings on clinical validity

Test: Cancer	Outcome	N studies; N subjects	Results Effect Size (95% CI)	Evidence from Multiple Studies Supports Association between Test Result and Prognosis?
MammaPrint: Breast	RR	6; 1,913	HR: 2.84 (2.11 to 3.89) for poor prognosis vs. good prognosis	Yes
	CSS	5; 1,615	HR: 3.3 (2.22 to 4.9) for poor prognosis vs. good prognosis	Yes
	OS	1; 144	HR: 1.67 (0.73 to 3.82) for poor prognosis vs. good prognosis	No
Oncotype DX Breast	RR	6; 3,222	HR: 2.97 (2.19 to 4.02) for high risk vs. low risk	Yes
	CSS	2; 1,234	HR: 2.02 (1.35 to 3.00) for high risk vs. low risk	Yes
	OS	1; 668	HR: 1.65 (1.24 to 2.19) for high risk vs. low risk	No, single study
EGFR: Lung	RR	6; 1,870	HR: 0.87 (0.65 to 1.15); No association	No
	CSS	0; 0	NA	No
	OS	6; 1,820	HR: 0.76 (0.50 to 1.19); No association	No
KRAS: Lung	RR	4; 611	2.84 (1.14 to 7.1) *KRAS* mutation associated with greater RR	Yes
	CSS	0; 0	NA	No
	OS	2; 253	2.69 (1.91 to 3.8); 3.33 (1.03 to 10.82)	Yes[a]
BRAF: CRC	RR	5; 4,106	HR 1.07 (0.76 to 1.52) for wild-type vs. mutation	No
	CSS	7; 5,409	HR 1.50 (1.26 to 1.77) for wild-type vs. mutation	Yes
	OS	11; 7,610	HR 1.45 (1.29 to 1.62) for wild-type vs. mutation	Yes
KRAS: CRC	RR	5; 4,085	HR 1.02 (0.91 to 1.14) for wild-type vs. mutation	No
	CSS	2; 1,174	HR 1.30 (1.02 to 1.66) for wild-type vs. mutation	Yes
	OS	10; 5,328	HR 1.22 (0.93 to 1.60) for wild-type vs. mutation	No
MSI: CRC	RR	10; 7,130	HR 0.60 (0.50 to 0.72) for MSI-H vs. MSS	Yes
	CSS	6; 3,439	HR 0.65 (0.51 to 0.82) for MSI-H vs. MSS	Yes
	OS	12; 8,839	HR 0.57 (0.43 to 0.77) for MSI-H vs. MSS	Yes
Oncotype DX: CRC	RR	1; 690	HR 1.68 (1.18 to 2.38)	No, single study
	CSS	0; 0	NA	No
	OS	0; 0	NA	No
UroVysion: Bladder	RR	2; 168	Association between mutation and RR in 2 small studies	Yes, but limited to 168 subjects
	CSS	0; 0	NA	No
	OS	0; 0	NA	No

[a] Meta-analyses were conducted only when we had 3 or more HRs to combine

Notes: Table includes results of our main analyses that were based on studies rated as low or medium RoB. HRs reported are results of our meta-analyses of studies rated as low or medium RoB reporting adjusted associations for clinical validity (KQ 3).

Abbreviations: *BRAF* = gene name; CI = confidence interval; CRC = colorectal cancer; CSS = cancer-specific survival; *EGFR* = gene name; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; MSI-H = microsatellite instability high; MSS = microsatellite stability: N = number; NA = not applicable; OS = overall survival; RR = risk of recurrence; vs. = versus.

Clinical Utility and the Overarching Question

Table B summarizes the evidence on the overarching question, clinical utility, and the impact of test use on treatment decisions. Ultimately, we found insufficient SOE to answer the overarching question for most tests. Even in the cases where the tests seemed to add value in determining prognosis (i.e., evidence of clinical validity), we found no evidence that using the test was related to improved outcomes for patients. For a few tests, we found low SOE, suggesting that using the test would not improve outcomes for patients—for these tests we found

evidence that did not support clinical validity (because with evidence suggesting lack of clinical validity, it is unlikely that the tests will be found to have clinical utility).

For impact on treatment decisions, we found moderate SOE that one test, Oncotype DX Breast, leads to changes in decisions. Although the decision changes were observed in both directions for individual patients, studies consistently showed an overall shift to less-intensive treatment recommendations as a result of using Oncotype DX Breast, with fewer recommendations for chemotherapy (and therefore less exposure to potential harms of chemotherapy; but studies did not follow patients to actually report on harms or to assess the overall balance of clinical benefits and harms). We found just one study of low or medium RoB for the impact of MammaPrint on treatment decisions, and we concluded that evidence was insufficient to determine the impact of MammaPrint on treatment decisions, primarily because of unknown consistency and imprecision.

Table B. Summary of findings and strength of evidence for impact on treatment decisions, clinical utility, and our overarching question

Test: Cancer	Outcome	N studies; N subjects	Conclusions	Strength of Evidence
MammaPrint: Breast	RR	6; 1,913	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	5; 1,615	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	1; 144	Study assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	1; 427	Adjuvant therapy was used less if the prognosis signature is used	Insufficient
Oncotype DX Breast	RR	6; 3,222	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	2; 1,234	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	1; 668	All studies assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	16; 2,251	~30% of treatment decisions changed by the test	Moderate
EGFR: Lung	RR	6; 1,870	All studies assessed clinical validity and found no prognostic value; test is unlikely to improve outcomes	Low
	CSS	0; 0	NA	Insufficient
	OS	6;1,820	No prognostic value; none of the reported HRs were statistically significant; test unlikely to improve mortality	Low
	Decisions about Rx	0; 0	NA	Insufficient
KRAS: Lung	RR	4; 611	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	0; 0	NA	Insufficient
	OS	2; 253	Studies assessed clinical validity; no prognostic value; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient

Table B. Summary of findings and strength of evidence for impact on treatment decisions, clinical utility, and our overarching question (continued)

Test: Cancer	Outcome	N studies; N subjects	Conclusions	Strength of Evidence
BRAF: CRC	RR	5; 4,106	All studies assessed clinical validity; no prognostic value; test is unlikely to improve outcomes	Low
	CSS	7; 5,409	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	10; 7,610	All studies assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient
KRAS: CRC	RR	5; 4,085	All studies assessed clinical validity; no prognostic value; test is unlikely to improve outcomes	Low
	CSS	2; 1,174	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	OS	10; 5,328	All studies assessed clinical validity; no prognostic value; test is unlikely to improve mortality	Low
	Decisions about Rx	0; 0	NA	Insufficient
MSI: CRC	RR	10; 7,130	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	6; 3,439	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	12; 8,839	All studies assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient
Oncotype DX: CRC	RR	1; 690	Study assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	0; 0	NA	Insufficient
	OS	0; 0	NA	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient
UroVysion: Bladder	RR	2; 168	Both studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	0; 0	NA	Insufficient
	OS	0; 0	NA	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient

Abbreviations: *BRAF* = gene name; CRC = colorectal cancer; CSS = cancer-specific survival; *EGFR* = gene name; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; N = number; NA = not applicable; OS = overall survival; RR = risk of recurrence; Rx = treatment.

Discussion

Our review demonstrated that the weight of published research to date in the area of molecular pathology tests for improving estimates of prognosis has focused on the clinical validity of the tests of interest in giving information about prognosis and little emphasis on how these tests can be integrated into the overall care of cancer patients in terms of measuring changes in management decisions or the effect of those altered decisions on downstream outcomes of value to patients. Such changes in management may be occurring and may be of benefit, or possibly of harm, to patients, but they have not been measured and studied, with the notable exception of the Oncotype DX assay in breast cancer, which does have a sizeable body of evidence to suggest an effect on treatment decisions (resulting in fewer recommendations for chemotherapy), though not yet a clear effect on downstream outcomes.

At this point, physicians can, in many cases, rely on the prognostic value of molecular pathology tests, share test results with patients, and discuss whether the test result indicates a better- or worse-than-average prognosis. However, in most cases, physicians cannot be sure whether using this information will improve any clinical outcomes. Although having accurate information about prognosis may be valued by patients independent of its effects on survival and recurrence outcomes, this has yet to be demonstrated for the tests of interest.

Limitations

Many of the included studies had methodological limitations, introducing some RoB. For example, most of them were observational studies assessing associations between test results and outcomes, and are susceptible to potential confounding. To limit such bias, we only included studies for KQ 3 that adjusted for most or all standard prognostic factors. Also, we assessed potential selection bias and confounding in our RoB assessments—limiting our main data syntheses to studies with low or medium RoB.

We were also not able to address the prognostic value of the tests for the Medicare population due to a lack of data that was specific to that age group. However, several studies included subjects from the Medicare agegroup and although there wre no analyses done specific to the agegroup, there was no evidence that suggested that the prognostic value of the tests would be different for this group.

Future Research

We found no direct evidence of the impact of the information provided by these tests on downstream health outcomes such as patients' quality of life or survival. Thus, future research should focus on quality of life, survival, and other health outcomes.

There is no information on the differential effects of the test by race or cancer subtype (e.g., ductal versus lobular in breast cancer) or location (e.g., proximal versus distal in CRC). As described in the results, the subpopulations that were represented in the studies varied in terms of stage, tumor type, and location in the case of CRC. Race and location have both been shown to be important predictors of the prognostic value of genetic markers in CRC.[16,17] Similar differences in prognostic value by subpopulations could be a factor in terms of *EGFR* and *KRAS* in the lung. There is thus a need to create an evidence base that replicates results in the same subpopulations, particularly in CRC and lung cancer.

As in many studies in the oncology field, the published literature uses a variety of specific definitions for outcomes such as recurrence, distant recurrence, DFS, CSS, and OS, making comparison of effects across studies more difficult. Future research should take into account careful selection of the most appropriate endpoints, both in the context of the existing body of literature and the endpoints of most clinical relevance to doctors and patients.

Conclusions

We found modest evidence supporting added prognostic value (i.e., clinical validity), beyond traditional prognostic factors, for MammaPrint; Oncotype DX Breast; *KRAS* mutation testing for lung; *BRAF* mutation testing for CRC; *KRAS* mutation testing for CRC; and MSI for CRC for RR, CSS, and/or OS. For UroVysion, which is marketed as a diagnostic (not prognostic) test, limited evidence supported an association between test result and prognosis for RR. For impact

of test use on treatment decisions, we found moderate SOE that Oncotype DX Breast leads to changes in treatment decisions, resulting in fewer recommendations for chemotherapy.

We found no studies that directly assessed the impact of test use on downstream health outcomes to establish clinical utility. Even for the tests with good evidence supporting clinical validity, we found no evidence that using the test was related to improved outcomes for patients.

Many of the included tests are currently used to predict response to specific treatments, an aspect that was not evaluated in this report. Determining whether the tests have clinical utility for predicting therapeutic response is beyond the scope of this review.

References

1. Rector TS, Taylor BC, Wilt TJ. Systematic review of prognostic tests. Chapter 12 of Methods Guide for Medical Test Reviews. AHRQ Publication No. 12-EHC017. Rockville, MD: Agency for Healthcare Research and Quality; June 2012. www.effectivehealthcare.ahrq.gov/reports/final.cfm. Also published in a special supplement to the Journal of General Internal Medicine, July 2012.
2. Whiting PF, Rutjes AW, Sterne J, et al. A quality assessment tool for diagnostic accuracy studies. Bristol, UK: University of Bristol http://www.bris.ac.uk/quadas/resources/quadas2.pdf. Accessed September 12, 2013.
3. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011 Oct 18;155(8):529-36. PMID: 22007046.
4. Hedges LV, Vevea JL. Fixed- and random-effects models in meta-analysis. Psychological Methods. 1998;3(4):486-504.
5. Atkins D, Chang SM, Gartlehner G, et al. Assessing applicability when comparing medical interventions: AHRQ and the Effective Health Care Program. J Clin Epidemiol. 2011 Nov;64(11):1198-207. PMID: 21463926.
6. Norris S, Atkins D, Bruening W, et al. Selecting Observational Studies for Comparing Medical Interventions. Methods Guide for Effectiveness and Comparative Effectiveness Reviews. Rockville, MD: Agency for Healthcare Research and Quality; 2010.
7. Owens DK, Lohr KN, Atkins D, et al. AHRQ series paper 5: Grading the strength of a body of evidence when comparing medical interventions—Agency for Healthcare Research and Quality and the Effective Health-Care Program. J Clin Epidemiol. 2010 May;63(5):513-23. PMID: 19595577.
8. Atkins D, Chang S, Gartlehner G, et al. Assessing the Applicability of Studies When Comparing Medical Interventions. Agency for Healthcare Research and Quality, Methods Guide for Comparative Effectiveness Reviews. AHRQ Publication No. 11-EHC019-EF. Rockville, MD: January 2011. http://effectivehealthcare.ahrq.gov/.
9. Beau-Faller M, Degeorges A, Rolland E, et al. Cross-validation study for epidermal growth factor receptor and KRAS mutation detection in 74 blinded non-small cell lung carcinoma samples: a total of 5550 exons sequenced by 15 molecular French laboratories (evaluation of the EGFR mutation status for the administration of EGFR-TKIs in non-small cell lung carcinoma [ERMETIC] project--part 1). J Thorac Oncol. 2011 Jun;6(6):1006-15. PMID: 21532509.
10. Cronin M, Sangli C, Liu ML, et al. Analytical validation of the Oncotype DX genomic diagnostic test for recurrence prognosis and therapeutic response prediction in node-negative, estrogen receptor-positive breast cancer. Clin Chem. 2007 Jun;53(6):1084-91. PMID: 17463177.
11. CAP Molecular Oncology Committee. EGFR Educational Challenge Participant Summary Report. Surveys 2012 EGFR-B, 2013 EGFR-A, 2013 EGFR-B. . Northfield, IL: College of American Pathologists; 2012, 2013.
12. CAP Molecular Oncology Committee. KRAS Educational Challenge Participant Summary Report. Surveys 2012 KRAS-B, 2013 KRAS-A, 2013 KRAS-B. . Northfield, IL: College of American Pathologists; 2012, 2013.
13. CAP Molecular Oncology Committee. BRAF Educational Challenge Participant Summary Report. Surveys 2012 BRAF-B, 2013 BRAF-A, 2013 BRAF-B. Northfield, IL: College of American Pathologists; 2012, 2013.
14. CAP Molecular Oncology Committee. MSI Educational Challenge Participant Summary Report. Surveys 2012 MSI-B, 2013 MSI-A, 2013 MSI-B. Northfield, IL: College of American Pathologists; 2012, 2013.

15. CAP/ACMG Cytogenetics Resource Committee. FISH for Urothelial Carcinoma Educational Challenge Participant Summary Report. Surveys 2013 CYI-A, CYI-B, CYI-C. . Northfield, IL: College of American Pathologists; 2013.

16. Katkoori VR, Jia X, Shanmugam C, et al. Prognostic significance of p53 codon 72 polymorphism differs with race in colorectal adenocarcinoma. Clin Cancer Res. 2009 Apr 1;15(7):2406-16. PMID: 19339276.

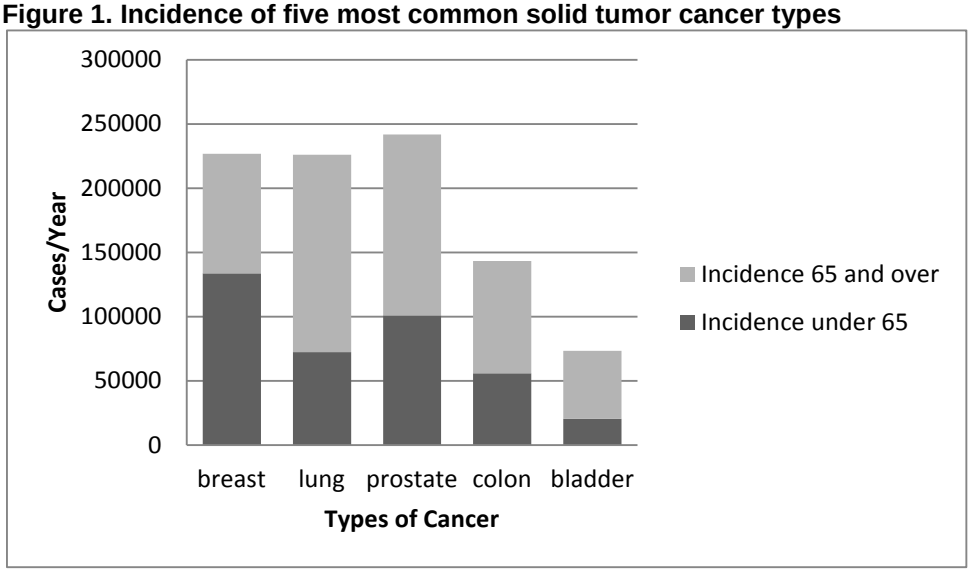
17. Manne U, Weiss HL, Myers RB, et al. Nuclear accumulation of p53 in colorectal adenocarcinoma: prognostic importance differs with race and location of the tumor. Cancer. 1998 Dec 15;83(12):2456-67. PMID: 9874449.

Background and Objectives

Burden of Cancer in the Medicare Population

Cancer is a leading cause of death in the United States. More than 1.6 million new cases of cancer are expected to be diagnosed in the United States in 2012.[1] Because rates of cancer incidence increase with age, almost half of these new cases will occur in the Medicare beneficiary population. As illustrated in Figure 1, adults ages 65 or older carry a disproportionate burden of new cancer diagnoses for the five most common solid tumor cancer types. For newly diagnosed Medicare beneficiaries with cancer and their care providers, estimating the prognosis of their cancer is a key step in understanding their diagnosis and forming a treatment plan. As our understanding of cancer has evolved to include the DNA mutational diversity and mRNA expression differences in the neoplastic clones, molecular pathology tests are playing an increasingly important role in determining cancer prognosis. This review aims to clarify the value of certain molecular pathology tests in determining prognosis for common cancers (breast, lung, colon, bladder) affecting Medicare beneficiaries.

Figure 1. Incidence of five most common solid tumor cancer types



Source: Data drawn from National Cancer Institute, Surveillance, Epidemiology, and End Results (SEER) Program, http://seer.cancer.gov/.[1] This figure represents an estimate of new cancer cases in the 65 or older population for the year 2012 based on overall incidence estimates for 2012 and historical distribution of new cases by age between 2005 and 2009 provided in the SEER Cancer Statistics Review.

What Is a Prognostic Test?

Prognosis is "a forecasting of the probable course and termination of an illness."[2] A prognostic test is used to predict a patient's probability of developing a disease or experiencing a medical event.[3] Determining prognosis is one of the most important steps for a newly diagnosed cancer patient and his or her health care providers. For people with cancer, a prognostic test is used to provide estimates for the likelihood of recurrence after curative-intent treatment, death from cancer, or death due to any cause after a diagnosis of cancer. In cancer research studies, these estimates are frequently referred to as recurrence-free survival, cancer-specific survival,

and overall survival. Recurrence of the cancer, cancer-specific deaths, and all-cause death are outcomes of great interest for the physician and patient as well as for researchers.

Prognostic estimates are important to cancer patients for several reasons. First, the patients and caregivers are naturally interested in finding out what to expect after the cancer is diagnosed and treated, for emotional and psychological reasons as well as for planning purposes. Second, prognostic information is often used to assist in treatment decisions, and to weigh the potential harms of treatment against the risk of disease recurrence. For example, if a patient were told that his cancer has a 10 percent chance of recurrence after initial surgical treatment, he might decide not to undergo additional chemotherapy with the potential for significant harms, whereas a patient given a 60 percent chance of recurrence might be more likely to decide to have chemotherapy. Pieces of information that distinguish patients who have different likelihoods of specific cancer outcomes are often referred to as *prognostic factors*.

Standard Prognostic Factors in Solid Tumor Cancer

The stage of a patient's cancer is one of the most powerful factors used to assess a patient's prognosis. The TNM staging system is based on the local extent of invasion by the tumor (T), whether cancer cells have spread to nearby (regional) lymph nodes (N), and whether distant (to other parts of the body) metastasis (M) has occurred.[4,5]

In addition, the grade of the tumor; other pathologic features; and other patient characteristics such as age, sex, and race are all factors that can affect prognosis depending on the primary site of the cancer. Table 1 lists the most widely accepted and validated prognostic factors for the cancer types of interest for this review.

Molecular Pathology Tests and Cancer Prognosis

As the science of cancer pathology has evolved over the past several decades, scientists have increasingly appreciated that there is biological diversity within cancers occurring in the same anatomic location and that patients with anatomically similar cancers may, in fact, have a variety of outcomes driven by molecular subtypes. The search for biomarkers to identify patients with better or worse prognoses within similar stage cancers began with the identification of distinct histologic cell types (e.g., squamous cell versus adenocarcinoma as two kinds of nonsmall cell lung cancer). These distinctions have been enriched by immunohistochemical assays, which can identify altered expression of cellular proteins. Such markers may or may not be associated with differences in prognosis. A key example includes the expression of the human epidermal growth factor 2 (HER2) receptor in breast cancer in which HER overexpression is associated with poor prognosis[6], but also serves as a predictor of response to anti-HER2 therapy. Such biomarkers have become a part of routine clinical care, and help clinicians offer more personalized prognoses and therapies to patients.

Table 1. Key standard prognostic factors in nonmetastatic solid tumors

Cancer Type	Prognostic Factor	Relationship
Colorectal	• Depth of invasion of bowel wall[a] • Invasion of adjacent structures[a] • Lymph node involvement[a] • Grade • Special cellular subtypes (e.g., mucinous, signet ring) • Lymphovascular invasion • Elevated carcinoembryonic antigen (CEA) level • Location (rectum vs. colon) • Sex • Age	Compton et al., 2000[7] Sun et al., 2009[8]
Breast	• Tumor size[a] • Tumor grade • Lymph node involvement[a] • Hormone (estrogen and progesterone) receptor status • HER2 receptor status • Inflammatory disease[a] • Age • Special cellular subtypes (e.g., mucinous, tubular)	Fitzgibbons et al., 2000[9] Rakha et al., 2008[10]
Lung	• Tumor size[a] • Invasion of adjacent tissues[a] • Spread to more than one lobe[a] • Lymph node involvement[a] • Cell type • Performance status • Weight loss • Sex	Groome et al., 2007[11] Sculier et al., 2008[12]
Bladder	• Depth of invasion of bladder wall[a] • Invasion of adjacent structures[a] • Lymph node involvement[a] • Lymphatic or vascular invasion	International Bladder Cancer Nomogram Consortium et al., 2006[13] Shariat et al., 2006[14]

[a] Factor included in American Joint Committee on Cancer staging criteria.

Abbreviations: CEA = carcinoembryonic antigen; HER2 = Human Epidermal Growth Factor Receptor 2, also known as Neu..

The search for better markers to estimate prognosis has been further enhanced with the advances in our understanding of the human genome. Genetic diversity between tumors of the same cancer type may or may not be associated with differences in prognosis. In some cases, tests for DNA mutations, DNA translocations, or mRNA expression differences (hereafter referred to as "molecular pathology tests") may offer a more accurate estimate of prognosis as an alternative or complement to traditional prognostic factors. To the extent that the results of a molecular pathology test add distinct information about a patient's likelihood of a certain outcome, such as recurrence, they can be valuable in patient education and decisionmaking.

Although molecular pathology tests are potentially valuable new tools, all such tests for common cancers are not created equal. Molecular pathology tests may vary in the extent to which they have been validated in groups of patients for whom long-term outcome data are available, and in the extent to which the patient population used in validation represents the average patient with the cancer of interest. Molecular pathology tests may also vary in their performance characteristics, including reproducibility, sensitivity and specificity, and lower limit of detection. In addition, some molecular pathology tests may change prognostic estimates by large increments when used in addition to traditional predictors, whereas others refine prognostic

estimates only slightly or not at all. All of these characteristics influence the degree to which a molecular pathology test is useful in clinical oncology practice.

How Is Prognosis Estimated?

Researchers developing prognostic models generally start with information about patient and cancer characteristics for a group of patients whose cancer outcomes (such as survival or cancer recurrence) are already known. These data are used to figure out mathematical relationships between the potential prognostic factors and the outcome of interest. Researchers must consider which outcome they wish to predict, identify the population for which they wish to make an estimate, and consider all the potential prognostic factors to include in their model based on prior literature and clinical observation.[15] Multivariable prognostic models are statistical tools that enable physicians and patients to estimate prognosis based on a combination of clinical and laboratory factors. For most cancers, the following outcomes are of greatest interest: all-cause survival (measured as time from diagnosis to death due to any cause), disease-specific survival (time from diagnosis to death due to cancer), and recurrence (time from initial surgical resection and treatment to recurrence). Thus, the mathematical models that estimate prognosis will assess the ability of the prognostic markers to predict one of these three outcomes. After comparing potential models to determine which one works best, the model's performance should be tested by using statistical methods and applying the model to different groups of patients to see if it performs as well as it did in the initial patient group. In many cases, these models can be simplified into nomograms or risk calculators that can be used in the office setting to estimate prognosis for individual patients. The usefulness of these tools depends on the validity of the predictive model that underlies them.

All prognostic estimates contain an element of uncertainty. A physician seeing a new patient may have an educated guess as to how the patient's cancer course will proceed based on the physician's experience. Although this guess may often be correct, there is still a significant degree of uncertainty. Similarly, a statistical model that estimates the likelihood of recurrence in a group of patients has some degree of uncertainty. By incorporating as many strong prognostic factors into the estimate as possible, we can reduce this uncertainty. As new candidate prognostic factors are discovered, they can be tested in combination with the known prognostic factors to investigate whether the new combination provides more accurate estimates of prognosis than the old combination (i.e., the known prognostic factors).

In evaluating prognostic tests, researchers consider *prognostic accuracy*, defined as the extent to which the test is able to predict which individuals have a higher or lower likelihood of getting the outcome (discrimination) and the extent to which the probability of a certain outcome in the population predicted by the test matches the actual probability (calibration).[16] In a disease with multiple prognostic factors, it is important to know whether a prognostic estimate incorporating a molecular pathology test of interest has improved calibration and discrimination compared with a model using only previously known prognostic factors such as those in Table 1. Further, it may be even more informative to assess net reclassification[17-19] (i.e., how much more frequently appropriate reclassification occurs than inappropriate reclassification when using a new model [incorporating a molecular pathology test] than with a model only using previously known prognostic factors). Unfortunately, not all newly discovered molecular pathology prognostic tests are evaluated in this manner. Thus, when a novel prognostic marker is reported in the scientific literature, physicians and patients may not know whether to place more value in estimates of prognosis based on older information, or on estimates based on the novel marker.

This review evaluates the value of 11 tests that are potentially useful for assessing prognosis for patients with colorectal (CRC), breast, lung, or bladder cancers. The outcome of interest in this review is recurrence of the cancer after the initial resection and treatment.

In a preliminary literature search and discussion with clinical experts for this systematic review, no commercially available molecular prognostic tests were identified for prostate cancer; therefore, prostate cancer is excluded from further consideration for this review. The molecular pathology tests of interest for the remaining four most common solid tumors among Medicare beneficiaries (Table 2) were selected based on preliminary literature searches, consultation with clinical experts, and consultation with the funding agency.

Table 2. Descriptions of genomic tests evaluated in this report

Cancer Type	Vendor	Vendor Test, Kit, or LDT	FDA-Approved	Sample Requirements	Type of Analyte	Panel Size	Neoplasms to be Tested	Results Reported from Which Lab?
Colorectal ACa								
MSI	Promega	Kit	No	Ca and NL FFPE tissue	DNA	5 microsatellites	Colorectal ACa	MGP
MLH-1 PHM	Qiagen	Kit (Pyro seq)	No	FFPE tissue	DNA	1 locus	Colorectal ACa	MGP
BRAF mutation	(1) Qiagen (2) Roche	(1) Kit (Pyro seq) (2) Quant PCR	(1) No (2) Yes	FFPE tissue	DNA	1 locus	Colorectal ACa	MGP
KRAS mutation	Qiagen	Kit (Pyro seq)	No	FFPE tissue	DNA	1 locus	Colorectal ACa	MGP
Oncotype DX	Genomic Health	Vendor IVDMIA	No	FFPE tissue	mRNA	12 loci	Stg II CRC ACa	Vendor
Breast Ca								
Oncotype DX	Genomic Health	Vendor IVDMIA	No	FFPE tissue	mRNA	21 loci	ER+ LN− BrCa	Vendor
MammaPrint	Agendia	Vendor IVDMIA	Yes	Frozen or FFPE tissue	mRNA	70 loci	<5 cm LN− BrCa	Vendor
Lung Ca, NSCLC								
ALK transloc'n	Vysis	Kit	Yes	FFPE tissue	DNA (interphase chromosomes)	1 locus	NSCLC	Cytogenetics
EGFR mutation	(1) Illumina	(1) TruSeq Amplicon Cancer Panel	No	FFPE tissue	DNA	1 locus	NSCLC	(1) MGP
	(2) Ion Torrent	(2) Ion AmpliSeq Cancer Panel	No	FFPE tissue	DNA	1 locus	NSCLC	(2) MGP
	(3) Qiagen	(3) Kit (Pyro seq) Quant PCR	Yes	FFPE tissue	DNA	1 locus	NSCLC	(3) MGP
	(4) Integrated Onc/Lab Corp	(4) Ref lab Sanger sequencing	No	FFPE tissue	DNA	1 locus	NSCLC	(4) Vendor
	(5) Roche	(5) Cobas Real-time PCR	No	FFPE tissue	DNA	1 locus	NSCLC	(5) MGP
KRAS mutation	Qiagen	Kit (Pyro seq)	No	FFPE tissue	DNA	1 locus	NSCLC	MGP

Table 2. Descriptions of genomic tests evaluated in this report (continued)

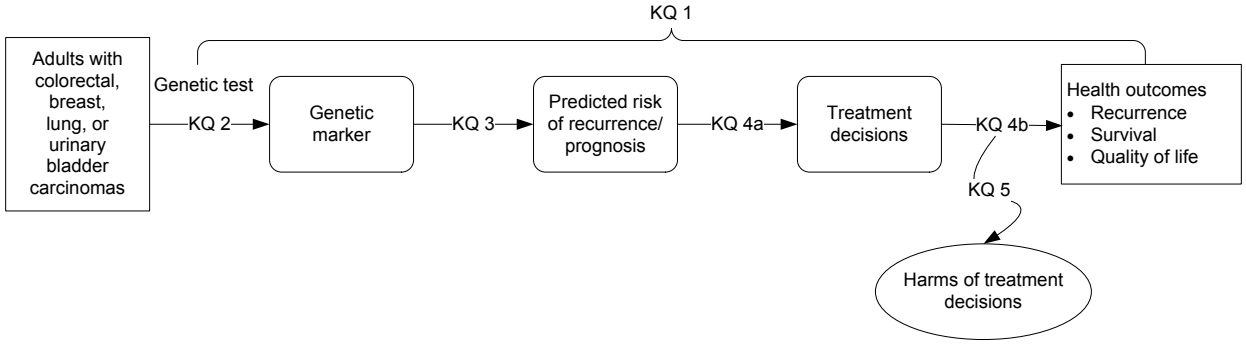
Cancer Type	Vendor	Vendor Test, Kit, or LDT	FDA-Approved	Sample Require-ments	Type of Analyte	Panel Size	Neo-plasms to be Tested	Results Reported from Which Lab?
Bladder (Urothe-lial) Ca								
UroVysion	Vysis	Kit	Yes	Alcohol-fixed	Cells	DNA (4 inter-phase chromo-somes)	Urothelial	Vendor

Abbreviations: ACa = adenocarcinoma; *ALK* = gene name; *BRAF* = gene name; Ca = cancer; BrCa = breast cancer; CRC = colorectal cancer; DNA = deoxyribonucleic acid; DX = diagnosis; *EGFR* = gene name; ER+ = estrogen receptor positive; FDA = Food and Drug Administration; FFPE = formalin-fixed paraffin-embedded tissue; IVDMIA = in vitro diagnostic multivariate index assays; *KRAS* = gene name; LDT = lab-developed test; LN- = lymph node; MGP = molecular genetic pathology; MLH-1 PHM = MLH-1 gene name promoter hypermethylation; mRNA = messenger ribonucleic acid; MSI = microsatellite instability; NL = normal; NSCLC = nonsmall cell lung carcinoma; PCR = polymerase chain reaction; seq = sequence; Stg = stage.

Scope and Objectives of the Review

These genetic tests are used in two different contexts. In one, the tests are used in a specific context of a diagnostic/therapy combination, where the diagnostic test is being used to predict response to a very specific treatment. In the second context, the genetic tests are used to estimate the patient's prognosis, and physicians use this prognostic information to choose from a variety of different treatment options. CMS requested this report to evaluate the second context. Therefore, studies that evaluate specific diagnostic/therapy combinations are excluded from this reporte thus did not include studies that were assessing tests for that purpose.The main purpose of this review is to determine whether the tests improve estimation of prognosis (for recurrence); affect physician decision-making; and thereby improve outcomes for patients with colorectal, breast, lung, or bladder cancer compared with traditional assessment of prognosis of recurrence. This technology assessment will use the analytic framework illustrated in Figure 2 and the associated Key Questions (KQs) to systematically review the evidence on 11 molecular pathology tests. In this review, we address the KQs listed below.

Figure 2. Analytic framework



Abbreviation: KQ = Key Question.

The following tests are under consideration for this assessment and apply to all KQs: microsatellite instability for CRC, MLH1 promoter methylation for CRC, *KRAS* mutation testing for CRC, *BRAF* mutation testing for CRC, Oncotype DX Colon for CRC, Oncotype DX Breast

for breast cancer, MammaPrint for breast cancer, *ALK* translocations for non-small cell lung carcinoma (NSCLC), *EGFR* for NSCLC, *KRAS* for NSCLC, and UroVysion for urinary bladder cancer.

KQ 1. Overarching Question: Is there direct evidence that the addition of the specified molecular pathology tests used alone or in combination with traditional prognostic factors changes physician decisionmaking and improves outcomes for adult patients with CRC, breast, lung, or bladder cancer compared with the use of traditional factors to predict risk of recurrence (RR) for adults with these cancers?

KQ 2. Analytic Validity: Does existing evidence establish the technical accuracy and reliability of these tests for detecting the relevant molecular analytes?

KQ 3. Clinical Validity: Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4. Clinical Utility: Does existing evidence support clinical utility of the molecular pathology tests?

KQ 4a. What is the evidence that the prognostic information provided by the molecular pathology tests modifies physician decisions regarding use of adjuvant antineoplastic chemo- and/or radiotherapy, enhanced diagnostic testing for recurrence, and/or surgery among adult patients with malignant tumors?

KQ 4b. What is the evidence that modified decisions lead to improved outcomes, including patient-centered outcomes such as improved quality of life, reduced disease recurrence, increased overall survival (OS) or disease-free survival (DFS), or reduced therapeutic side effects?

KQ 5. What are the harms associated with treatment decisions that are informed by the molecular pathology tests?

Methods

The methods for this systematic review follow the methods suggested in the Agency for Healthcare Research and Quality (AHRQ) *Methods Guide for Medical Test Reviews*.[3,20-23]

Literature Search Strategy

Search Strategy

To identify articles relevant to each Key Question (KQ), we searched PubMed®, the Cochrane Library, and EMBASE®. The full search strategy is presented in Appendix A. We used either Medical Subject Headings (MeSH) or major headings as search terms when available or key words when appropriate, focusing on terms to describe the relevant cancers and tests of interest. Searches were run by an experienced information scientist serving as the Evidence-based Practice Center (EPC) librarian and were peer reviewed by another information scientist.

We limited the electronic searches to English-language, adult, and human-only studies. Sources were searched from inception through November 2013. We manually searched reference lists of pertinent review articles and studies meeting our inclusion criteria to look for any relevant citations that our searches might have missed. We imported all citations into an EndNote® X5 (Thomson Reuters, New York, NY) electronic database.

We also searched for unpublished studies relevant to this review using test developers' Web sites, ClinicalTrials.gov, the Food and Drug Administration (FDA) Web site, Health Services Research Projects in Progress, and the European Union Clinical Trials Register. In addition, we requested unpublished information from the College of American Pathologists (CAP) and from the relevant companies, asking for data that they believe should be considered for the review. In cases in which relevant information was unclear or not reported, we contacted authors to get additional or unpublished information.

Any literature suggested by reviewers was investigated and, if appropriate, incorporated into the final review. We determined appropriateness for inclusion in the review by the same methods described in this chapter.

Eligibility Criteria

We developed eligibility (inclusion and exclusion) criteria for Populations, Interventions (i.e., tests for this report), Comparators, Outcomes, Timing, Settings (PICOTS), and study designs (Table 3). We included studies meeting the criteria described in Table 3 that were relevant to at least one of the KQs.

Table 3. Inclusion/exclusion criteria

Category	Inclusion	Exclusion
Population	Adult patients (19 years of age or older) with cancer of the colon/rectum, breast, lung, or urinary bladder (cancer types known to be of higher incidence in the core Medicare beneficiary population [people 65 years of age or older])	People 18 years of age or younger; all other tumor types and sites. Studies focused on a population with advanced/metastatic cancer.
Interventions (i.e., tests)	The following molecular pathology tests when used alone or in combination with "traditional" prognostic factors to measure the likelihood of malignant tumor recurrence (e.g., tumor size, tumor grade, estrogen receptor status; see Table 1): Lung cancer: *ALK*, *EGFR*, *KRAS* CRC: Microsatellite instability (MSI), MLH1 promoter methylation, *KRAS*, *BRAF*, Oncotype DX Colon Breast cancer: Oncotype DX Breast, MammaPrint Urinary bladder cancer: UroVysion	Molecular pathology tests used to measure the likelihood of malignant tumor recurrence that are not commercially available in the United States. Studies focused on predicting response to treatments (e.g., studies focused on response to gefitinib for people with advanced lung cancer).
Comparators	We will compare the molecular pathology tests listed above with standard tests or markers (see Introduction) used to measure the likelihood of malignant tumor recurrence. We will collect information about comparators as they are defined and reported in each study. For KQs 1, 4, and 5, we are looking for studies that compare an eligible molecular pathology test plus all known prognostic factors with all known prognostic factors alone to isolate whether the test adds any value (benefit) or introduces additional harms (KQ 5). Alternatively, studies that compare an eligible molecular pathology test alone with known prognostic factors are also eligible for inclusion. If a study uses most of the known prognostic factors but not all of them, it could also meet our inclusion criteria, and the comparison would be considered in the risk of bias (RoB) assessment (related mainly to confounding and approach to statistical adjustment). For KQ 2 (analytic validity), comparators also include intra- and interlaboratory repeat testing of samples. For KQ 3 (clinical validity), cohort studies that do the test on everyone, follow them over some period of time to capture relevant outcomes, and then do a multivariate analysis to adjust for known factors and to determine whether the tests have prognostic value for predicting recurrence are eligible. Such studies must compare those with different test results (e.g., those with a mutation versus those who are wild-type) to establish prognostic value, adjusted for known factors. To inform whether molecular pathology tests add value above standard prognostic factors, results must be adjusted for known prognostic factors (or they must be addressed in other ways, such as through inclusion/exclusion criteria of the study or stratification). Relevant case-control studies for KQ 3 could compare cases (people with recurrence) and controls (without recurrence) by rates of exposure (e.g., test positive vs. test negative), adjusting for known prognostic factors.	Unadjusted studies demonstrating associations between test results (e.g., those with a mutation versus those who are wild-type) and outcomes.

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Table 3. Inclusion/exclusion criteria (continued)

Category	Inclusion	Exclusion
Outcomes	KQ 1. Overarching question. Studies must provide direct evidence of the impact of a test of interest on physician decision-making (regarding the use of adjuvant anti-neoplastic chemo- and/or radiotherapy, enhanced diagnostic testing for recurrence, or preventive surgery) and also on health outcomes (overall survival, disease-free survival, time to recurrence, or quality of life). KQ 2. Analytic validity Tissue sample acceptance/rejection criteria. Sensitivity/specificity of testing for each marker at assay conditions. Degree of variation in the results if the same tumor is tested in multiple laboratories. Uniqueness of the markers used in the panel and their robustness to contamination. Quality control standards for considering the validity of the overall assay (for multiplex tests). Proportion of probes or antibodies that must return a valid result (for multiplex tests). KQ 3. Clinical validity Prognostic value for recurrence (e.g., time to recurrence and disease-free survival) and overall survival. Differences in prognostic value associated with patient characteristics. Differences in prognostic value between non-Medicare and Medicare population. KQ 4. Clinical utility 4a. Decision-making Modification of physician decisions regarding the use of the following: adjuvant anti-neoplastic chemo- and/or radiotherapy enhanced diagnostic testing for recurrence preventive surgery 4b. Health outcomes Overall survival, disease-free survival, time to recurrence, quality of life (must be assessed using a valid and reliable quality-of-life measurement tool) KQ 5. Harms Adverse effects of tests, adverse effects of adjuvant therapy, decreased quality of life	Prediction of response to treatment. Progression-free survival is not an eligible outcome.
Time Frame/ Duration	We will include studies of any duration that otherwise meet inclusion criteria.	

Table 3. Inclusion/exclusion criteria (continued)

Category	Inclusion	Exclusion
Study Designs	Include/eligible study designs, by KQ: KQ 1a. Randomized controlled trials (RCTs), non-RCTs, prospective cohort studies with eligible comparison groups, and case-control studies that directly assess the impact of a test of interest on physician decision-making (regarding the use of adjuvant antineoplastic chemo- and/or radiotherapy, enhanced diagnostic testing for recurrence, or preventive surgery) and also on health outcomes (overall survival, disease-free survival, time to recurrence, or quality of life). KQ 2. Collaborative studies using panels (ideally large panels) of well-characterized samples, data from external proficiency testing schemes, data from inter-laboratory comparison programs, test and retest of samples, or validation studies (including those from manufacturers). KQ 3. Prospective and retrospective cohort studies and case-control studies with eligible comparison groups. This could also include publications of validated clinical decision algorithms that were developed using cohort data to quantify the contributions of different variables (e.g., molecular pathology test result, tumor size, tumor grade) in order to determine classification/interpretation of a molecular pathology test result for prognosis. KQ 4a. RCTs, non-RCTs, prospective and retrospective cohort studies with eligible comparison groups, and case-control studies. Case series will be considered for KQ 4a for decision-making outcomes only if other eligible study designs are not available. KQ 4b.[a] RCTs, non-RCTs, prospective and retrospective cohort studies with eligible comparison groups, and case-control studies. KQ 5. RCTs, non-RCTs, prospective and retrospective cohort studies with eligible comparison groups, and case-control studies.	Non-English publications, systematic and nonsystematic reviews, case series (except as noted for KQ 4a; we determined that the RoB in case series is too high to provide valid and reliable information for the other KQs), letters, editorials, abstracts, and observational studies without comparison groups.
Settings	Any country (no limits)	

[a] An RCT that randomized subjects to use a molecular pathology testmolecular pathology test alone or in combination with standard prognostic factors compared with standard prognostic factors alone to inform decisions and that reported health outcomes would address the overarching question (KQ 1), whereas KQ 4b would include studies that evaluated the link between decisions and health outcomes.

Abbreviations: *ALK* = gene name; *BRAF* = gene name; DX = diagnosis; *EGFR* = gene name; KQ = Key Question; *KRAS* = gene name; MLH-1 = gene name promoter hypermethylation; MSI = microsatellite instability; RCT = randomized controlled trial; RoB = risk of bias.

Study Selection

We developed and pilot tested review forms for review of titles/abstracts and full texts. All references were tracked in a Microsoft Excel® Version 14.0 (Microsoft Corporation, Redmond, WA) database. Two trained members of the research team independently reviewed each title and abstract identified through searches for eligibility. Studies marked for possible inclusion by either reviewer underwent a full-text review. For studies that lacked adequate information to determine inclusion or exclusion, we retrieved the full text and then made the determination. If the necessary information in full-text articles was unclear or missing, we contacted authors of the publications.

Two trained members of the research team independently reviewed each full-text article for inclusion or exclusion based on the eligibility criteria described above. If both reviewers agreed that a study did not meet the eligibility criteria, we excluded it. If the reviewers disagreed, they resolved conflicts by discussion and consensus or by consulting a third member of the review

team. We recorded the principal reason that each excluded full-text publication did not satisfy the eligibility criteria (Appendix B).

Data Extraction

For studies that met our inclusion criteria, we extracted study characteristics and results into evidence tables. We designed, pilot tested, and used structured data extraction forms to gather pertinent information from each article; this included characteristics of study populations, settings, interventions, comparators, study designs, methods, and results. Trained reviewers extracted the relevant data from each included article into the data extraction forms. All data extractions were reviewed for completeness and accuracy by a second member of the team. All data extraction was performed using Microsoft Excel® software.

Risk-of-Bias Assessment of Individual Studies

We assessed the RoB (internal validity) in the reviewed studies using an approach supported by the *Methods Guide for Medical Test Reviews*.[3] For studies of analytic validity, we considered the potential for bias due to flaws in the sample selection, testing protocol, reference standards, verification procedures, interpretation, and/or analysis. We used the questions relevant for prognostic tests from the QUADAS-2[24,25] (which was developed for diagnostic tests) for assessing risk of relevant biases. For studies of clinical validity and clinical utility, we assessed the potential for selection bias, confounding, performance bias, attrition bias, and detection bias. We assessed these biases using relevant questions and predefined criteria based on guidance from the AHRQ *Methods Guide for Effectiveness and Comparative Effectiveness Reviews* and the RTI Question Bank.[26]

We summarized RoB for each study as low, medium, high, or unclear. In general, studies with a low RoB are those that have a low risk of selection bias, use valid and reliable outcome measures, use appropriate statistical and analytical methods, have low attrition, and report methods and outcomes clearly and precisely. Studies with a medium RoB are those that do not meet all criteria required for low RoB but do not have flaws that are likely to cause major bias. Missing information often led to ratings of medium as opposed to low RoB. Studies with inadequate reporting to allow assessment of RoB were described as having unclear RoB—this was often the case for abstracts from conference proceedings. Studies with a high RoB are those with at least one substantial flaw in the study's design, conduct, or analysis that could invalidate the results. Two independent reviewers assessed the RoB for each study. Disagreements were resolved by discussion and consensus or by consulting a third member of the team. Appendix C details the criteria used for evaluating the RoB of all included studies and explains the rationale for high RoB ratings.

Data Synthesis

For clinical validity, data were synthesized for each test by cancer type using meta-analytic techniques described in Hedges & Vevea.[27] The measure of the effect size in most studies was a hazard ratio (HR). A few studies had odds ratios. A summary HR was estimated for any outcome that had three or more independent HR estimates. In cases where subpopulations from one study were included in the sample for another study, we used the study with the largest included population in our meta-analyses (to avoid double-counting any data). If studies provided separate estimates for different subsamples (e.g., node negative and node positive), both estimates were

included if no overall HR was provided. If an overall HR for the entire sample and the separate HRs for subsamples were provided, then only the overall measure was used.

If the test is non-informative, we expect that the probability of experiencing the end point would be the same for either group, with an HR of 1. If the HR is greater than 1, the probability of the endpoint is higher in the group with the higher hazard. If the HR is lower than 1, the probability of experiencing the endpoint is lower in the group with the lower hazard. For example, an HR of 2 for CSS indicates that one group (e.g., those with high risk results for Oncotype DX Breast) has twice the rate per unit of time as the comparison group (e.g., those with low-risk test results).

The standard error (SE) of the HR was used to estimate the variance of the effect size. In most cases, the SE was estimated from the 95% confidence interval (CI). We tested the null hypothesis of homogeneity of effect sizes across the studies[27,28] for each of the outcomes. If the null hypothesis was rejected at a significance level of 0.05, the summary HR was estimated using a random effects model; if not, it was estimated using a fixed effects model. In both cases, the inverse of the variance was used to estimate a weighted summary HR. Forest plots with indicators of the weight assigned to each study were used to help visualize the contributions of each study and to show the summary HR.

We did not include studies with a high or unclear RoB in our main analyses/main data syntheses.

Strength of the Body of Evidence

We graded the strength of evidence (SOE) based on the guidance established for the Evidence-based Practice Center program Program.[29,30] Developed to grade the overall strength of a body of evidence, this approach incorporates four key domains: RoB (includes study design and aggregate quality), consistency, directness, and precision of the evidence. It also considers other optional domains that may be relevant for some scenarios, such as a dose-response association, plausible confounding that would decrease the observed effect, strength of association (magnitude of effect), and publication bias. Table 4 defines the grades of evidence that we assigned.

Table 4. Definitions of the grades of overall strength of evidence

Grade	Definition
High	High confidence that the evidence reflects the true effect. Further research is very unlikely to change our confidence in the estimate of effect.
Moderate	Moderate confidence that the evidence reflects the true effect. Further research may change our confidence in the estimate of the effect and may change the estimate.
Low	Low confidence that the evidence reflects the true effect. Further research is likely to change our confidence in the estimate of the effect and is likely to change the estimate.
Insufficient	Evidence either is unavailable or does not permit estimation of an effect.

Source: Owens et al., 2010[29]

For each test being evaluated in this review, two reviewers assessed each domain for each key outcome and determined an overall SOE grade based on domain ratings. In the event of disagreements on the domain or overall grade, they resolved differences by consensus discussion or by consulting with a third investigator.

We attempted to find studies that directly address the overarching question (KQ 1). If we did not find adequate direct evidence addressing the overarching question, we attempted to construct an indirect chain of evidence (using studies identified for the other KQs).

Appendix D includes tables showing our assessments for each domain and resulting SOE grades for each outcome, organized by type of cancer and test. We graded the SOE separately for analytic validity and for evidence on our overarching KQ. For analytic validity, we graded the SOE for the following: sensitivity and specificity, positive and negative predictive value, and cross-lab validity. For evidence on the overarching KQ, we graded the SOE for the following outcomes: risk of recurrence, cancer-specific survival, overall survival, and decisions about treatment.

Applicability

We assessed applicability of the evidence following guidance from the *Methods Guide for Comparative Effectiveness Reviews*.[28,31] We used the PICOTS framework to explore factors that affect applicability. Some factors identified that may limit the applicability of evidence include age, sex, and race or ethnicity of enrolled populations.

Peer Review and Public Commentary

Peer Review

The comments were generally favorable from the peer reviewers. Two main areas were identified as needing revisions.

1. Emphasizing that this report focused on the prognostic value of the tests and did not examine the predictive value of the tests (i.e., in predicting response to treatment).
2. Incorporating CAP's information into the description of the analytic validity for the tests and general comments on improving the description of analytic validity of the tests.

We have incorporated these suggestions in the document.

Public Comments

A lot of the public comments were from the manufacturers of the tests examined. Besides disputing some of the SOE grades and conclusions or suggesting revisions to KQs, one salient issue raised by a manufacturer was about including UroVysion in the report. Abbott Molecular pointed out that UroVysion was not designed or marketed as a test to assess prognosis and requested that the test be excluded from the report.

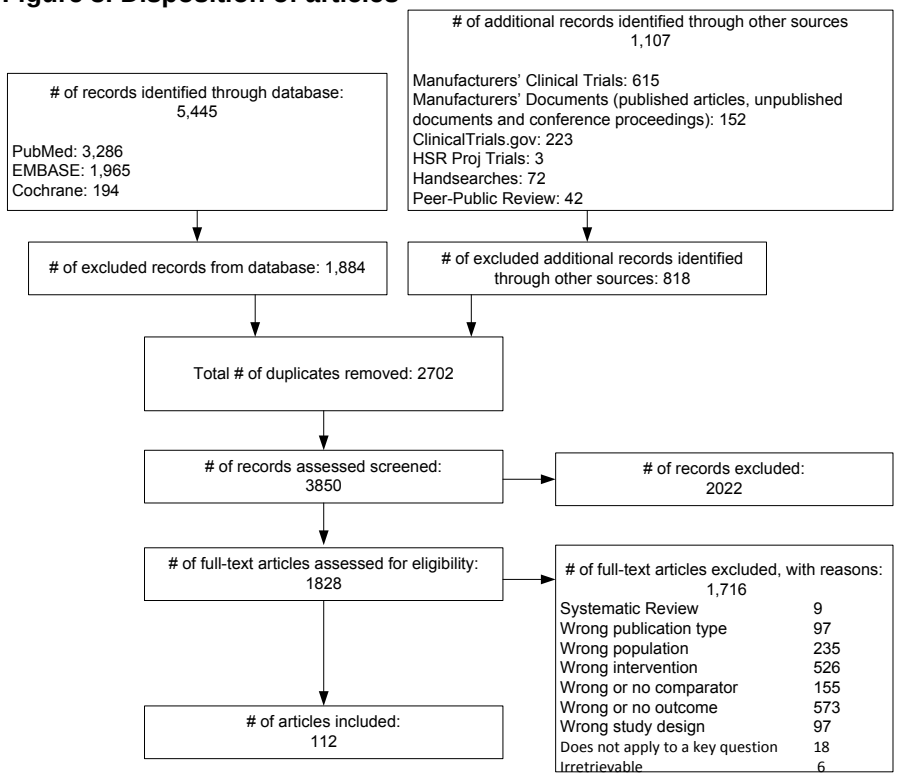
We have decided to keep UroVysion in the report but have added clear statements in various sections to clarify the intended use of the test.

Results

Literature Search

We included 112 publications reporting on the tests of interest (Figure 3). We included 21 publications for Key Question (KQ) 2, 70 for KQ 3, and 22 for KQ 4a. We found no eligible studies for KQ 1, KQ 4b, or KQ 5. Of the included studies, we rated 31 as low, 57 as medium, 12 as high, and 12 as unclear risk of bias (RoB).

Figure 3. Disposition of articles



Results of Included Studies

Key Question 1. Direct Evidence That Using the Tests Changes Physician Decisionmaking and Improves Outcomes

We found no eligible studies that addressed this overarching question.

Key Question 2. Analytic Validity

We included 21 studies, using a total of 23 different tests (Table 5) as well as data from the College of American Pathologists. We analyzed marker test performance and intra/interlab reproducibility.

Table 5. Characteristics of included studies reporting on analytic validity, by test and cancer

Author, Year, Design, Test (Primary Site), Risk of Bias	Vendor, Developer of Test	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Tissues in Sample
Abbot Labs, 2011[32] Validation *ALK* (lung) Unclear	Abbot Labs Vendor	FFPE DNA fluorescence in situ hybridization (FISH)	Nonsmall cell lung cancer Authors' lab(s) Chromosome rearrangements 136
Angulo, 2013[33] Test-retest *EGFR* (lung) Low	Qiagen Vendor	FFPE DNA PCR followed by *therascreen*® *EGFR* Mutation Kit	Nonsmall cell lung cancer Histology: Carcinomas NOS (23.5%) Squamous cell carcinomas (10.3%) Adenocarcinomas (64%) Large cell carcinomas (2.2%) Authors' lab(s) *EGFR* mutations at exons 19 to 21 136
Bando, 2010[34] Validation *KRAS* (colorectal) Medium	DxS Ltd.; Vienna Lab Diagnostics, Vendor	FFPE DNA Direct (Sanger) sequencing; *therascreen KRAS* Mutation Kit: Allele-specific PCR with Scorpion fluorescent probes (ARMS/S); *KRAS* Strip-Assay: Reverse hybridization (RH)	CRC Authors' lab(s) *KRAS* mutations at codons 12 and 13 100
Beau-Faller, 2011[35] Validation *EGFR* (lung) Low	NA Lab developed for each center	FFPE and frozen DNA Direct or nested sequencing; each center used their own standard procedure.	Nonsmall cell lung cancer NR *EGFR* mutations at exons 18 to 21 74
Beau-Faller, 2009[36] Test-retest *KRAS* (lung) Low	Roche (Germany), Eurogentec (Belgium), Timolbiol (Germany); Lab developed	Frozen DNA PNA-mediated PCR clamping	Nonsmall cell lung cancer Authors' lab(s) *KRAS* mutations at codons 12 and 13 114
Beau-Faller, 2011[35] Validation *KRAS* (lung) Low	NA Lab developed for each center	FFPE and frozen DNA Direct or nested sequencing; each center used their own standard procedure.	Nonsmall cell lung cancer NR *KRAS* mutations at exon 2 74

Table 5. Characteristics of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Vendor, Developer of Test	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Tissues in Sample
Clark-Langone 2010[37] Test-retest Oncotype DX (colorectal) Medium	Genomic Health Vendor	FFPE RNA RT-PCR of 12-gene Recurrence Score Assay	Stage II adenocarcinoma and mucinous carcinoma Authors' lab(s) Expression levels NR
Cronin, 2007[38] Test-retest Oncotype Dx (Breast) Unclear	Genomic Health Vendor	FFPE RNA RT-PCR of 21-gene Recurrence Score Assay	Node-negative, estrogen receptor–positive breast cancer Vendor's lab Expression levels NR
Delahaye, 2013[39] Test-retest MammaPrint (breast) Medium	Agendia NV, Amsterdam, The Netherlands Vendor	Fresh tissue RNA Microarray	Early-stage, invasive breast cancer Vendor's labs Expression levels N = 100 (cross lab validity tests)
Feigelson, 2012[40] KRAS (colorectal) Low	NA Lab developed for each center	FFPE DNA PCR amplification followed by Direct Sanger sequencing, PCR amplification followed by standard bidirectional sequencing, PCR amplification followed by sequencing, Single nucleotide primer extension with fragment analysis by capillary electrophoresis using a modified SNaPshot assay, or Qualitative real time PCR.	CRC (18 adenocarcinoma, 2 carcinoma) Genzyme, Clarient, Quest Diagnostics, Henry Ford Health System & Molecular and Medical Genetics-Oregon Health and Science University KRAS mutations at codons 12 and 13 20
Gao, 2010[41] Validation *KRAS* (colorectal) Unclear	Vienna Lab of Austria CND	FFPE DNA Dideoxy sequencing, *KRAS* StripAssay and pyrosequencing	CRC Authors' lab(s) *KRAS* mutation s at exon 2 NR

Table 5. Characteristics of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Vendor, Developer of Test	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Tissues in Sample
Gonzalez de Castro 2012[42] Test-retest *KRAS* (colorectal) Low	Roche Molecular Systems, Inc., Qiagen Vendor	FFPE DNA Cobas *EGFR* Mutation Test (AS-PCR) *therascreen EGFR*29 Mutation Kit (ARMS); 2× bidirectional; Sanger sequencing	CRC 2 clinical site labs *KRAS* mutations at codons 12 and 13 115
Hancer, 2011[43] Cohort *KRAS* (colorectal) Unclear	1) Big Dye Terminator kit v.3.1 (Applied Biosystems, Foster City, CA); 2) DxS Ltd., Manchester, United Kingdom; 3) Entrogen, Tarzana, CA Vendor	FFPE DNA Dideoxy sequencing, *therascreen KRAS* kit, and Entrogen *KRAS* mutation analysis kit	Adenocarcinomas of the colon and the rectum Authors' lab(s) *KRAS* mutations at codons 12 and 13 64
Jancik, 2012[44] Test-retest *KRAS* (lung) High	PyroMark KRAS assay test: Biotage, Uppsala, Sweden *therascreen* DxS *KRAS* Mutation Kits KR-21 and KR-22: QiaGen, Hilden, Germany KRAS StripAssay REF 5–590: ViennaLab Diagnostics GmbH, Vienna, Austria Direct sequencing and HRM analysis used components from various vendors Vendor	Frozen DNA Direct sequencing, Pyrosequencing, *therascreen* DxS, KRAS StripAssay kits, and HRM analysis	Nonsmall cell lung cancer Authors' lab(s) *KRAS* mutations at codons 12 and 13 131
Kobunai, 2010[45] Test-retest *KRAS* (colorectal) Low	Qiagen, Panagene Lab developed	Frozen DNA Peptide nucleic acid (PNA)-clamp real-time PCR	CRC Authors' lab(s) *KRAS* mutations at codons 12 and 13 224

Table 5. Characteristics of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Vendor, Developer of Test	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Tissues in Sample
Lopez-Rios 2013[46] Test-retest *EGFR* (lung) Low	Roche Molecular Systems, Inc., Qiagen, Vendor	FFPE DNA Cobas *EGFR* Mutation Test (AS-PCR) *therascreen EGFR*29 Mutation Kit (ARMS); 2× bidirectional; Sanger sequencing	Nonsmall cell lung cancer 2 clinical site labs *EGFR* mutations at exons 18 to 21 124
Mancini, 2010[47] Test-retest *BRAF* (colorectal) Medium	NA Lab developed	Frozen DNA PCR: Cold PCR—sensitivity then evaluated by high-resolution melting (HRM) analysis and sequencing	Sporadic colorectal cancer (CRC) Authors' lab(s) *BRAF* mutations at exon 15 117
Mancini, 2010[47] Test-retest *KRAS* (colorectal) Medium	NA Lab developed	Frozen DNA PCR: Cold PCR—sensitivity then evaluated by HRM analysis and sequencing	Sporadic CRC Authors' lab(s) *KRAS* mutations at codons 12 and 13 117
Naoki, 2011[33] Test-retest EGRF (lung) Low	NA Lab developed	FFPE and frozen DNA PCR: PCR-invader method	Nonsmall cell lung cancer BML *EGFR* mutations at exons 18 to 21 54
Pang, 2011[48] Validation *KRAS* (colorectal) High	Qiagen Vendor	FFPE DNA PCR amplification	Stage IV colorectal adenocarcinoma Authors' lab(s) *KRAS* mutations at exons 2 and 3 11
Pang, 2011[48] Validation *BRAF* (colorectal) High	Qiagen Vendor	FFPE DNA PCR amplification	Stage IV colorectal adenocarcinoma Authors' lab(s) *BRAF* mutations at exon 15 11

Table 5. Characteristics of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Vendor, Developer of Test	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Tissues in Sample
Poulet, 2012[47] Test-retest MammaPrint (breast) Unclear	NR NR	Frozen DNA Microarray	ER+, HER2-neg early breast cancer Authors' lab(s) Cluster analysis 67
Poulet, 2012[47] Test-retest Oncotype DX (breast) Unclear	NR NR	Frozen RNA RT-PCR of 12-gene Recurrence Score Assay	ER+, HER2-neg early breast cancer Authors' lab(s) Expression levels 67
Sriram, 2011[33] Cohort *EGFR* (lung) Low	NA Lab developed	Frozen DNA ME-PCR; HRM	Nonsmall cell lung cancer NR *EGFR* mutations(exon 19 deletion and exon 21 L858R) 522 tumor tissue and 64 matched serum samples
Van't Veer, 2002[49] Validation MammaPrint (breast) Medium	NA Lab developed	Frozen DNA Microarray	Breast cancer Authors' lab(s) Cluster analysis 78 in development set, 19 in validation set

Abbreviations: ARMS = Amplification Refractory Mutation System; ARMS/S = Amplification Refractory Mutation System and a Scorpion fluorescent primer/probe system; BML = Bio Medical Laboratories (Tokyo, Japan); *BRAF* = v-Raf murine sarcoma viral oncogene homolog B1; DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor gene; FFPE = formalin-fixed, paraffin-embedded; HRM = high-resolution melting; *KRAS* = V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; ME = mutant enriched; NA = not applicable; NOS = not otherwise specified; NR = not reported; PCR = polymerase chain reaction; PNA = peptide nucleic acid; RH = reverse hybridization.

Of the 11 potentially considered molecular pathology tests of interest, we found published studies rated as low or medium RoB for 6 tests: MammaPrint mRNA expression for breast cancer, *EGFR* mutations and *KRAS* mutations for lung cancer, *KRAS* mutations and *BRAF* mutations for colorectal cancer (CRC), and Oncotype DX Colon mRNA expression for CRC. The published studies that we identified reporting on *ALK* cytogenetics for lung cancer, Oncotype DX Breast mRNA expression for breast cancer, and MLH1 promoter methylation for CRC were rated as high or unclear RoB. Results of the included studies are provided in Table 6.

Table 6. Results of included studies reporting on analytic validity, by test and cancer

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Abbot Labs, 2011[32] Validation *ALK* (lung) Unclear	FFPE DNA Fluorescence in situ	Nonsmall cell lung cancer Abbot Labs Chromosome rearrangements 136	Vysis LSI 3′-*ALK* SO: 100% 95% CI, (98.5 to 100.0) Vysis LSI 5′-*ALK* SGn: 100% 95% CI, (98.5 to 100.0)	Vysis LSI 3′-*ALK* SO: 100% 95% CI, (97 to 100.0) Vysis LSI 5′-*ALK* SGn: 100% 95% CI, (97 to 100.0)	Yes Median Kappa score: 0.92 (range: 0.85 to 0.98)
Angulo, 2013[50] Test-retest *EGFR* (lung) Low	FFPE DNA PCR followed by *therascreen EGFR* Mutation Kit; Sanger sequencing	Nonsmall cell lung cancer Histology: Carcinomas NOS (23.5%) Squamous cell carcinomas (10.3%) Adenocarcinomas (64%) Large cell carcinomas (2.2%) Authors' lab(s) *EGFR* mutations at exons 19 to 21 136 samples	Exon 19 deletion: 100% Exon 21 L858R: 100% Exon 20 insertion: 67.75% All mutations :94.7%	Exon 19 deletion: 100% Exon 21 L858R: 100% Exon 20 insertion: 100% All mutations : 100%	No NA
Bando, 2010[34] Validation *KRAS* (colorectal) Medium	FFPE DNA Direct (Sanger) sequencing; *therascreen KRAS* Mutation Kit: Allele-specific PCR with Scorpion fluorescent probes (ARMS/S); *KRAS* Strip-Assay: Reverse hybridization (RH)	CRC Authors' lab(s) *KRAS* mutations at codons 12 and 13 100	RH: 51/74 (69%) ARMS/S: 56/74 (76%)	RH: 25/26 (96%) ARMS/S: 26/26 (100%)	No NA

Table 6. Results of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Beau-Faller, 2011[35] Validation *EGFR* (lung) Low	FFPE and frozen DNA Direct or nested Sanger sequencing; each center used its own standard procedure.	Nonsmall cell lung cancer NR *EGFR* mutations at exons 18 to 21 74	False positive results, median (range) Exon 19: 0% (0–27%) Exon 21: 17% (0–37%) False positive results, number of centers with false positive rate Exon 19: 0%–9/15 >0%–<10%: 1/15 ≥10%: 5/15 Exon 21: 0%: 7/15 >0–<10%: 0/15 ≥10%–8/15	False negative results, median (range) Exon 19: 2% (0–9%) Exon 21: 3% (0–6%) False negative results, number of centers with false positive rate Exon 19: 0%: 6/15 >0–<10%: 9/15 ≥10%: 0/15 Exon 21: 0%: 6/15 >0–<10%: 9/15 ≥10%: 0/15	Yes *EGFR* Exon 19: Median Kappa score: 0.52 (range: 0.23–0.73) *EGFR* Exon 21: Median Kappa score: 0.37 (range: 0.20–0.57)
Beau-Faller, 2009[36] Test-retest *KRAS* (lung) Low	Frozen DNA PNA-mediated PCR clamping Sanger sequencing	Nonsmall cell lung cancer Authors' lab(s) *KRAS* mutations at codons 12 and 13 114	NR	NR	No NA
Beau-Faller, 2011[35] Validation *KRAS* (lung) Low	FFPE and frozen DNA Direct or nested sequencing; each center used their own standard procedure.	Nonsmall cell lung cancer NR *KRAS* mutations at exon 2 74	False positive results, median (range) 8% (0–25%) False positive results, number of centers with false positive rate 0%–5/15 >0%–<10%: 4/15 ≥10%: 6/15	False negative results, median (range) 9% (2–17%) False negative results, number of centers with false positive rate 0%: 0/15 >0%–<10%: 8/15 ≥10%: 7/15	Yes Median Kappa score: 0.39 (range: 0.15–0.66)

Table 6. Results of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Clark-Langone, 2010[37] Test-retest Oncotype DX (colorectal) Medium	FFPE RNA RT-PCR of 12-gene Recurrence Score Assay	Stage II adenocarcinoma and mucinous carcinoma Authors' lab(s) Expression levels NR	NR	NR	No NA
Cronin, 2007[38] Test-retest Oncotype Dx (breast) Unclear	FFPE RNA RT-PCR of 21-gene Recurrence Score Assay	Node-negative, estrogen receptor–positive breast cancer Vendor's lab Expression levels NR	NR	NR	
Delahaye, 2013[39] Test-retest MammaPrint (breast) Medium	Fresh tissue RNA Microarray	Early-stage, invasive breast cancer Vendor's labs Expression levels N = 100 (cross-lab validation) N = 302 (PPV and NPV)	DMFS PPV (95% CI) (5 years): 0.22 (0.16 to 10.28) Overall survival PPV (95% CI) (10 years): 0.29 (0.22 to 20.35)	DMFS NPV (95% CI) (5 years): 0.95 (0.91 to 90.99) [see comment bubble] Overall survival NPV (95% CI) (10 years): 0.90 (0.85 to 80.96)	Yes 99%

Table 6. Results of included studies reporting on analytic validity, by test and cancer (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Feigelson, 2012[40] KRAS (colorectal) Low	FFPE DNA PCR amplification followed by Direct Sanger sequencing, PCR amplification followed by standard bidirectional sequencing, PCR amplification followed by sequencing, Single nucleotide primer extension with fragment analysis by capillary electrophoresis using a modified SNaPshot assay, or Qualitative real time PCR.	CRC (18 adenocarcinoma, 2 carcinoma) Genzyme, Clarient, Quest Diagnostics, Henry Ford Health System & Molecular and Medical Genetics-Oregon Health and Science University KRAS mutations at codons 12 and 13 20	False positive results, number of centers with false positive rate 0%: 3/5 5%: 2/5	False negative results, number of centers with false positive rate 0%: 4/5 5%: 1/5	Yes 90% were concordant across all five laboratories
Gao, 2010[41] Validation *KRAS* (colorectal) Unclear	FFPE DNA Dideoxy sequencing, *KRAS* StripAssay and pyrosequencing	CRC Authors' lab(s) *KRAS* mutation—exon 2 100	Pyrosequencing=5% *KRAS* strip assay=1% dideoxy=15%	NR	No NA

Table 6. Results of included studies reporting on analytic validity, by test and carcinoma (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Gonzalez de Castro, 2012[42] Test-retest *KRAS* (colorectal) Low	FFPE DNA Cobas *EGFR* Mutation Test (AS-PCR) *therascreen EGFR*29 Mutation Kit (ARMS); 2× bidirectional; Sanger sequencing	CRC 2 clinical site labs *KRAS* mutations at codons 12 and 13 115	Cobas *KRAS* and Sanger Sequencing (N=113): PPA=98.2%	Cobas *KRAS* and Sanger Sequencing (N=113): NPA=89.7%	Yes Cobas *KRAS* test reproducibility between the two sites:110/112 (98.2%) produced concordant results
Hancer, 2011[43] Cohort *KRAS* (colorectal) Unclear	FFPE DNA *therascreen KRAS* kit Entrogen *KRAS* mutation analysis kit Dideoxy sequencing,	Adenocarcinomas of the colon and the rectum Authors' lab(s) *KRAS* mutation—exon 2 64	NR	NR	No NA
Jancik, 2012[44] Test-retest *KRAS* (lung) High	Frozen DNA Direct sequencing, Pyrosequencing, and the *therascreen* DxS and KRAS StripAssay kits All of the above as well as high-resolution melting analysis (HRM)	Nonsmall cell lung cancer Authors' lab(s) *KRAS* mutations at codons 12 and 13 131	*therascreen* DxS kit: 95% KRAS StripAssay: 90% HRM: 70% Pyrosequencing: 48% Sequencing: 29%.	*therascreen* DxS kit, sequencing, and pyrosequencing: 100% HRM: 98% KRAS StripAssay: 95%	No NA

Table 6. Results of included studies reporting on analytic validity, by test and carcinoma (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Kobunai, 2010[45] Test-retest *KRAS* (colorectal) Low	Frozen DNA Peptide nucleic acid (PNA)-clamp real-time PCR Sanger sequencing	CRC Authors' lab(s) *KRAS* mutations at codons 12 and 13 224	NR	NR	No NA
Lopez-Rios, 2013[46] Test-retest *EGFR* (lung) Low	FFPE DNA Cobas *EGFR* Mutation Test (AS-PCR); *therascreen EGFR*29 Mutation Kit (ARMS); 2× bidirectional; Sanger sequencing	Nonsmall cell lung cancer 2 clinical site labs *EGFR* mutations at exons 18 to 21 124	AS-PCR test and Sanger: (N=113): PPA of 98.8%	AS-PCR test and Sanger: (N=113): NPA of 79.3%	Yes AS-PCR test reproducibility between the two sites: 122/123 (99.2%) produced concordant results
Mancini, 2010[47] Test-retest *BRAF* (colorectal) Medium	Frozen DNA PCR: Cold PCR—sensitivity then evaluated by HRM analysis and Sanger sequencing	Sporadic CRC Authors' lab(s) *BRAF* mutations at exon 15 117	NR	NR	No NA
Mancini, 2010[47] Test-retest *KRAS* (colorectal) Medium	Frozen DNA PCR: Cold PCR—sensitivity then evaluated by HRM analysis and sequencing	Sporadic CRC Authors' lab(s) *KRAS* mutations at codons 12 and 13 117	NR	NR	No NA

Table 6. Results of included studies reporting on analytic validity, by test and carcinoma (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Naoki, 2011[51] Test-retest EGRF (lung) Low	FFPE and frozen DNA PCR: PCR-invader method; Sanger sequencing	Nonsmall cell lung cancer BML *EGFR* mutations at exons 18 to 21 54	94.44% (derived from study)	79.17% (derived from study)	No NA
Pang, 2011[48] Validation *KRAS* (colorectal) High	FFPE DNA *KRAS* PCR	Stage IV colorectal adenocarcinoma Authors' lab(s) *KRAS* mutation—exons 2 and 3 11	NR	NR	No NA
Pang, 2011[48] Validation *BRAF* (colorectal) High	FFPE DNA *BRAF* PCR	Stage IV colorectal adenocarcinoma Authors' lab(s) *BRAF* mutation—exon 15 11	NR	NR	No NA
Poulet, 2012[52] Test-retest MammaPrint (breast) Unclear	Frozen DNA Microarray	ER+, HER2− early breast cancer Authors' lab(s) Cluster analysis 67	NR	NR	No NA

Table 6. Results of included studies reporting on analytic validity, by test and carcinoma (continued)

Author, Year, Design, Test (Primary Site), Risk of Bias	Tissue Sample Requirements, Type of Analyte, Measure Technique	Neoplasms, Lab Reporting Results, Test Measure, Sample Size	Marker Sensitivity	Marker Specificity	Cross-Lab Validity Checked, Degree of Cross-Lab Variation
Poulet, 2012[52] Test-retest Oncotype DX (breast) Unclear	Frozen RNA RT-PCR of 12-gene Recurrence Score Assay	ER+, HER2− early breast cancer Authors' lab(s) Expression levels 67	NR	NR	No NA
Sriram, 2011[53] Cohort *EGFR* (lung) Low	Frozen DNA ME-PCR; HRM Sanger sequencing	Nonsmall cell lung cancer NR *EGFR* mutations (exon 19 deletion and exon 21 L858R) 522 tumor tissue and 64 matched serum samples	ME-PCR:100% HRM: 100%	ME-PCR: 99% HRM: 100%	No NA
Van't Veer, 2002[49] Validation MammaPrint (breast) Medium	Frozen DNA Microarray	Breast cancer Authors' lab(s) Cluster analysis 78 in development set, 19 in validation set	NR	NR	No NA

Abbreviations: ARMS = Amplification Refractory Mutation System; AS = Allele-Specific; BML = Bio Medical Laboratories (Tokyo, Japan); *BRAF* = gene name; DNA = deoxyribonucleic acid; *EGFR* = gene name; ER = estrogen receptor; FFPE = formalin-fixed, paraffin-embedded; HER2 = Human Epidermal Growth Factor Receptor 2, also known as (Neu); HRM=high-resolution melting; *KRAS* = gene name; ME = mutant enriched; N = number of samples; NA = not applicable; NOS = not otherwise specified; NPA = negative percent agreement; NR = not reported; PCR = polymerase chain reaction; PNA= peptide nucleic acid; PPA = positive percent agreement; RH = reverse hybridization; SGn = SpectrumGreen; SO = SpectrumOrange.

Data from the College of American Pathologists' for *EGFR* mutations, *KRAS* mutations, *BRAF* mutations, microsatellite instability for colorectal cancer, and UroVysion cytogenetics for urinary bladder cancer support the analytic validity of these tests (see data in relevant sections below).

Breast Cancer: MammaPrint

Characteristics of Included Studies Assessing Analytic Validity of MammaPrint

MammaPrint uses a 70-gene signature to classify breast carcinoma patients into those with a good or poor prognosis, based on risk of distant metastasis.

One study by Poulet[52] was rated unclear in terms of RoB and is excluded from the description of results below.

Two MammaPrint studies were rated as low or medium RoB (Table 5).[39,49] van't Veer[49] developed the 70-gene signature prognostic assay (now known as MammaPrint) for risk of recurrence in early-stage breast cancer, and tested prediction to metastases in 97 patients. Delahaye,[39] affiliated with Agendia NV, studied the analytical performance of MammaPrint, including reproducibility, precision, repeatability, tumor heterogeneity, and inter-lab reproducibility.

MammaPrint: Analytic Validity

Both MammaPrint studies indicated that the MammaPrint test is precise, repeatable (98.5 to 99 percent), and reproducible for prediction of metastasis in breast cancer (Table 6). The largest contributor to assay variation was intratumor heterogeneity, which is significantly greater than the technical variation in the MammaPrint test.

Nonsmall Cell Lung Carcinoma: *EGFR* and *KRAS* Mutations

Characteristics of Included Studies Assessing Analytic Validity of *EGFR* Mutation

We reviewed the analytic validity of tests that detected mutations in the *EGFR* gene. All studies looked at mutations in the gene within exons 18, 19, and/or 21.

We rated 5 studies reporting analytic validity of *EGFR* testing for lung cancer as having low RoB (Table 5). Angulo[50] compared *therascreen EGFR* mutation screening to direct sequencing of *EGFR* in FFPE lung tumor tissue. Lopez-Rios[46] compared the Cobas *EGFR* Mutation Test and *therascreen EGFR* Mutation Test with direct sequencing of *EGFR* in FFPE lung carcinoma tissue. Sriram[53] compared mutant-enriched-PCR and high-resolution melting methods in frozen lung tissue, a subset (97 of 522 samples) of which were previously DNA sequenced in the *EGFR* gene. Naoki[51] compared the PCR-invader method with direct DNA sequencing in FFPE lung tissue and pleural and pericardial effusions. Beau-Faller[35] compared several PCR-based methods to direct DNA sequencing of *EGFR* in FFPE lung tissue across 15 centers.

Also, we received results of proficiency testing from The College of American Pathologists (CAP) to address intralab reproducibility of *EGFR* mutation testing.

EGFR Mutation: Analytic Validity

In general, all five *EGFR* testing studies for lung cancer compared various PCR-based methods against one another or against DNA sequencing in FFPE tissue (Table 6). All of these studies found that, compared with DNA sequencing, PCR-based methods of screening for *EGFR* mutations had a lower limit of detection and thus were more sensitive in samples with <20% carcinoma. However, because PCR-based methods are based on known mutations found between exons 18 and 21 of the *EGFR* gene, DNA sequencing is a better method for identifying novel mutations and mutations elsewhere in the gene.

The CAP sends proficiency test *EGFR* unknowns to CLIA-approved US clinical laboratories or International clinical laboratories for assessing nationwide interlab reproducibility. The three most recent surveys[54] found accuracy rates of 93 percent (122/131; EGFR-B, 2012), 96 percent (157/164; EGFR-A, 2013), and 96 percent (195/204; EGFR-B, 2013), for an average diagnostic accuracy of 94 percent (474/499).

Characteristics of Included Studies Assessing Analytic Validity of *KRAS* Mutation in Nonsmall Cell Lung Carcinoma

We reviewed the analytic value of tests that detected mutations in the *KRAS* gene. The studies looked at mutations at codons 12 and 13 or on exon 2 in the *KRAS* gene but not at codon 61.

One study by Jancik[44] was rated as having a high RoB and is excluded from the description of results below.

We rated two *KRAS* mutant lung cancer studies as having low RoB (Table 5). Beau-Faller (2009)[36] compared peptide-nucleic-acid-mediated PCR clamping to direct DNA sequencing in frozen lung tissue. Beau-Faller (2011)[35] compared several PCR-based methods to direct DNA sequencing of *KRAS* in FFPE lung tissue across 15 centers.

Also, we received results of proficiency testing from CAP to address intralab reproducibility of *KRAS* mutation testing.

KRAS Mutation for Lung Cancer: Analytic Validity

Both of the *KRAS* mutant lung cancer studies (Table 6) found that, compared with DNA sequencing, PCR-based methods of screening for *KRAS* mutations had a lower limit of detection, and thus were more sensitive when the samples contained <20% carcinoma.

CAP sends proficiency test *KRAS* unknowns to CLIA-approved US clinical laboratories or International clinical laboratories for assessing nationwide interlab reproducibility. The three most recent surveys[55] found accuracy rates of 98 percent (194/197; KRAS-B, 2012), 98 percent (206/209; KRAS-A, 2013), and 98 percent (211/215; KRAS-B, 2013), for an average diagnostic accuracy of 98 percent (611/621).

Characteristics of Included Studies Assessing Analytic Validity of *ALK* Translocation

There were no published studies that provided any information about the analytic validity of the ALK Translocation test. Abbot Labs, 2011[32] has information provided with their Vysis ALK Break Apart FISH Probe Kit literature on specific performance characteristics, including analytical sensitivity and specificity, tissue reproducibility, and external reproducibility (Tables 5 and 6). This work was rated unclear in terms of RoB and is excluded from the description of results.

Colorectal Carcinoma:

Characteristics of Included Studies Assessing Analytic Validity of *BRAF* Mutation

The *BRAF* mutation tests being examined in this report screen for the V600E mutation in the *BRAF* gene.

One study by Pang (2011)[48] was rated as having a high RoB and is excluded from the description of results below.

A single *BRAF* CRC study had a rating of medium RoB (Table 5). Mancini[47] compared a novel lower denaturation temperature PCR (COLD-PCR) with the more traditional high-resolution melting (HRM) PCR method in screening for *BRAF* c.1799 T>A (p.V600E) mutation.

Also, we received results of proficiency testing from CAP to address intralab reproducibility of *BRAF* mutation testing.

BRAF Mutation: Analytic Validity

Mancini[47] found that COLD-PCR was more sensitive than HRM-PCR for detecting the *BRAF* mutation (Table 6).

The College of American Pathologists sends proficiency test *BRAF* unknowns to CLIA-approved U.S. clinical laboratories or International clinical laboratories, an excellent mechanism for assessing inter-lab reproducibility. The three most recent surveys[56] found accuracy rates of 100% (140/140; BRAF-B, 2012), 98% (192/195; BRAF-A, 2013), and 98% (196/200; BRAF-B, 2013), for an average diagnostic accuracy of 99% (528/535).

Characteristics of Included Studies Assessing Analytic Validity of *KRAS* Mutation in Colorectal Carcinoma

The genetic test for *KRAS* mutations described here checks for mutations in codons 12 and 13 in the *KRAS* gene but not in codon 61.

One study by Pang[48] was rated as having a high RoB, and two studies—Gao[41] and Hancer[43]—were rated as having unclear RoB and are excluded from the description below.

We rated five *KRAS* mutant CRC studies as having low to medium RoB (Table 5). Mancini[47] compared COLD-PCR with the HRM PCR method in screening for *KRAS* mutations in frozen colorectal tissue. Kobunai[45] compared peptide nucleic acid-clamp PCR with direct sequencing in frozen colorectal tissue. Gonzalez de Castro[42] compared the cobas *KRAS* Mutation Test and the Qiagen *therascreen KRAS* Kit methods to direct *KRAS* sequencing in FFPE colorectal tissue. Bando[34] compared the *KRAS* StripAssay and *therascreen KRAS* Mutation kit with direct *KRAS* sequencing. Feigelson[40] compared five different *KRAS* mutation testing regimens for comparability and consistency across five laboratories.

Also, we received results of proficiency testing from CAP to address intralab reproducibility of *KRAS* mutation testing.

KRAS Mutation for Colorectal Carcinoma: Analytic Validity

All of these studies found that, compared with DNA sequencing, PCR-based methods of screening for *KRAS* mutations had a lower limit of detection, and thus were more sensitive in samples with <20% carcinoma (Table 6).

CAP sends proficiency test *KRAS* unknowns to CLIA-approved US clinical laboratories or International clinical laboratories for assessing nationwide interlab reproducibility. The three

most recent surveys[55] found accuracy rates of 98 percent (194/197; KRAS-B, 2012), 98 percent (206/209; KRAS-A, 2013), and 98 percent (211/215; KRAS-B, 2013), for an average diagnostic accuracy of 98 percent (611/621).

Characteristics of Included Studies Assessing Analytic Validity of MSI

We found no individual published studies meeting our inclusion criteria that reported eligible information on analytic validity of the microsatellite instability (MSI) test for CRC.

We received results of proficiency testing from CAP to address intralab reproducibility of MSI testing.

MSI for Colorectal Carcinoma: Analytic Validity

CAP sends proficiency test MSI unknowns to CLIA-approved US clinical laboratories or International clinical laboratories for assessing nationwide interlab reproducibility. The three most recent surveys[57] found accuracy rates of 100 percent (89/89; MSI-B, 2012), 99 percent (96/97; MSI-A, 2013), and 98 percent (103/105; MSI-B, 2013), for an average diagnostic accuracy of 99 percent (288/291).

Characteristics of Included Studies Assessing Analytic Validity of Oncotype DX (for Breast and Colon)

Oncotype DX Breast and Oncotype DX Colon are mRNA abundance assays performed on formalin-fixed paraffin-embedded tissue. They are not intended to act as surrogate tests for identifying underlying gene mutations. Selected mRNA abundance differences have been correlated with known clinical outcomes and are now used to predict risk of recurrence and to guide adjuvant therapy use in new patients who have undergone resection of their primary carcinomas for cure. Oncotype DX Breast uses a 21-gene mRNA expression signature and assigns a recurrence risk score (RS) ranging from 0 to 100 for each patient. Oncotype DX Colon uses a 12-gene mRNA expression signature and assigns an RS ranging from 0 to 100 for each patient.

The Cronin lab at Genomic Health measured intralaboratory detectability, dynamic range, and reproducibility for assays on this set of 21 mRNAs for Oncotype DX Breast.[38] This study was rated as having unclear RoB (Table 5). Published data on Oncotype DX Colon by Clark-Langone,[37] affiliated with the Cronin lab and referencing the above paper, provided similar analytical validity data for the 12-gene Oncotype DX Colon assay.

Oncotype DX: Analytic Validity

The data from Cronin's group at Genomic Health[38] found high intralab reproducibility for Oncotype quantitative RT-PCR mRNA abundance measurements relevant to the Oncotype DX tests for breast and colon carcinoma. Clark-Langone[37] reported high analytic validity and precision for the Oncotype DX Colon assay (Table 6). No references were found to estimate interlaboratory reproducibility, because this assay is not performed outside of Genomic Health.

Bladder Cancer: UroVysion

Characteristics of Included Studies Assessing UroVysion

UroVysion is a fluorescence in situ hybridization (FISH) test of exfoliated urothelial cells that has been shown to be sensitive in terms of diagnosing urothelial cancer.

We found no individual published studies meeting our inclusion criteria that reported eligible information on analytic validity of the UroVysion test for bladder cancer.

We received results of proficiency testing from CAP to address intralab reproducibility of UroVysion testing.

UroVysion: Analytic Validity

CAP sends proficiency test UroVysion unknowns to CLIA-approved US clinical laboratories or International clinical laboratories for assessing nationwide interlab reproducibility. The four most recent surveys[58] found accuracy rates of 99 percent (168/170; UroVysion-A, 2013), 99 percent (168/170; UroVysion-B, 2013), 99 percent (191/192; UroVysion-C, 2013), and 100 percent (193/193; UroVysion-D, 2013), for an average diagnostic accuracy of 100 percent (552/555).

Clinical Validity: Breast Cancer: MammaPrint and Oncotype DX

Characteristics of Included Studies Assessing Clinical Validity of MammaPrint

We found 11 studies[49,59-68] that reported on the prognostic value of the RS from MammaPrint for RR, CSS, or OS in patients with breast cancer (Table 7). All were cohort studies. Sample sizes ranged from 89 to 964. Median followup was 3 to 10 years. Eight out of 10 studies were conducted in the Netherlands either exclusively by the group that developed the signature or in collaboration with other European countries. One study was conducted in the United States,[61] and one was conducted in France, Sweden, and the United Kingdom.[60]

All studies compared outcomes of patients who had a good prognosis signature with those who had a poor prognosis signature. Age of the women in the studies for which age is reported was approximately 50 years, although a couple of studies included women who were in their twenties. Race or ethnicity was not reported in any of the studies.

There were some differences in the populations enrolled in the included studies. Differences were in stage and ER, node positivity, and age. Three studies restricted the sample to node-negative cancers,[59,60,65] a couple included cases with one to three positive nodes,[63,67] one looked at patients with four to nine positive nodes[68]and two included stages I through III,[62,63] whereas the others restricted the study to stage I or II.

Table 7. Characteristics of included studies: MammaPrint for breast cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Bueno-de-Mesquita 2009[59] Cohort Low	151 Median 10.2 years (0.7–21.3)	The Netherlands	Early stage Node-negative breast cancer, tumor diameter pT1–2.	Good prognosis signature Poor prognosis signature	NR NR	Mean (range): 47 (27–55)
Buyse, 2007[60] Cohort Unclear	307 Median: 13.6 years	France, Sweden, and United Kingdom	I–II Node negative	Good prognosis Poor prognosis	NR NR	Median: 58 100%
Iwamoto, 2011[61] Cohort Unclear	228 Median 56.4 (5.9 to 60) months	United States	I–III ER-positive and -negative cancers as well as low and high histologic grade tumors	Good prognosis Poor prognosis	NR NR	51.9 (26–79) NR
Knauer, 2010[62] Cohort Low	89	The Netherlands, Austria, and Belgium	I–III Unilateral HER2 positive invasive breast carcinoma, no chemotherapy, no trastuzumab	Good prognosis Poor prognosis	NR NR	Median (range): 50 (28–79) 100%
Mook, 2009[63] Cohort Low	241	The Netherlands, Italy	II–III (T stage 1–3 with 1–3 positive lymph nodes)	Good prognosis Poor prognosis	NR NR	NR NR
Mook, 2010[64] Pooled analysis of multiple cohorts Low	964 Median (range): 7.1 (0.2–25.2) years	The Netherlands	pT1 including 1a, 1b, and 1c tumors	Good prognosis Poor prognosis	NR NR	NR NR
Mook, 2010[65] Cohort Medium	148 Median (range): 375.1 (41.7 to 922.2) weeks	The Netherlands	I–II Node negative	Good prognosis Poor prognosis	NR NR	Range 55–70 100%
Nuyten, 2008[66] Cohort Medium	144 Median (range): 10.2 (0.05 to 21.7) years	The Netherlands	II Node positive	Good prognosis Poor prognosis	NR NR	NR NR

Table 7. Characteristics of included studies: MammaPrint for breast cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Saghatchian, in press[68] Cohort Medium	173 Median, 7.9 years	The Netherlands, Italy	Primary invasive breast cancer with 4 – 9 positive nodes, T1-3, estrogen receptor +/-, progesterone receptor +/-, HER2 +/-	Genomic high risk Genomic low risk		Age for each group, mean (SD): GHR = 49.1 (9.7); GLR = 51.4 (9.5 100%
Van't Veer, 2002[49] Cohort Medium	98	The Netherlands	Sporadic breast cancer 55 years or younger, 18 cases of BRCA1 and 2 BRCA2	Good prognosis Poor prognosis	NR NR	All patients were younger than 55 years at diagnosis 100%
Van de Vijver, 2002[67] Cohort Low	295 Median (range): 7.8 years (0.05 to 18.3) years (207 patients without metastasis as the first event) Median (range): 2.7 (0.3 to 14.0) years (88 patients with metastasis as the first event). Median (range): 6.7 (0.05 to 18.3) years (all 295 patients)	The Netherlands	I-II	Good prognosis Poor prognosis	NR NR	All patients were younger than 53 years 100%

Abbreviations: HER2 = Human Epidermal Growth Factor Receptor 2, also known as Neu; MUT = mutation; N = number; NR = not reported; SD = standard deviation.

MammaPrint: Risk of Recurrence

Six cohort studies rated as low or medium RoB reported RR (total N=1,913).[59,62-64,67,68] All studies used distant metastasis-free survival (DMFS) instead of recurrence-free survival (local and distant recurrence) as their outcome. All six reported on the prognostic value of MammaPrint after controlling for traditional prognostic factors such as stage, size, and differentiation, for example. One study used the composite score from Adjuvant! Online—an online program that combines traditional prognostic factors to calculate an RS.[69] All studies concluded that patients with a poor prognosis signature had a higher risk of distant metastasis compared with patients with a good prognosis signature. The hazard ratios (HRs) in these studies ranged from 2.43 to 5.8, and all the studies had overlapping 95% confidence intervals (Table 8).

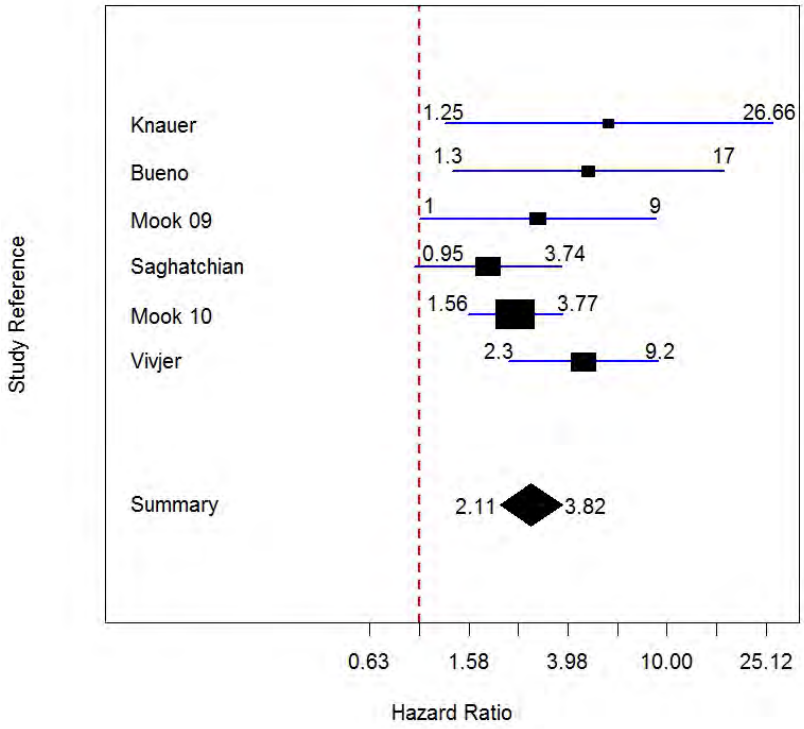
Table 8. Breast Cancer: MammaPrint risk of distant metastasis-free survival

Author, Year, Design, N, Risk of Bias	Adjusted HR (95% CI), p-value	Variables Used in Multivariate Model
Knauer, 2010[62] Cohort 89 Low	DMFS HR: 5.8 (1.25 to 26.66) p=0.025	Age at diagnosis, tumor size, number of positive lymph nodes, histological grade (1/2 vs. 3), ER status, progesterone receptor (PR) status, adjuvant hormonal therapy
Bueno-de-Mesquita 2009[59] Cohort 151 Low	DMFS RS in NKI-RdGG series HR: 4.8 (1.3 to 17) RS in updated validation series HR: 5.3 (95% CI: 2.4 to 12)	Performance of 70-gene prognosis signature vs. performance of predictor with results for 4 different models; results from Adjuvant! Online
Mook, 2009[63] Cohort 241 Low	DMFS HR: 3.0 (1.0 to 9.0) p=0.05	Age (years); number of positive nodes: 2 vs. 1 and 3 vs. 1; tumor size (20 mm vs. ≤20 mm); histological grade: moderate vs. good, poor vs. good; ER status; HER2 receptor status; surgery (mastectomy vs. breast conservation therapy [BCT]) chemotherapy; endocrine therapy; prognosis signature (poor vs. good signature)
Mook, 2010[64] Pooled analysis of several cohorts 964 Medium	DMFS HR: 2.43 (1.56 to 3.77) p<0.001	Age, histology, tumor size, nodal status, grade, ER status, HER2 status, type of surgery, hormonal therapy (yes/no), chemotherapy (yes/no), 70-gene signature
Saghatchian, in press[68] Cohort 173 Medium	DMFS MammaPrint, low risk vs. high risk, main analysis HR: 1.888 (0.953 to 3.739) p=0.068 DMFS MammaPrint, low risk vs. high risk, subgroup analysis (N=129 women with 4 to 9 positive nodes) 2.698 (1.267 to 5.748) p=0.010	Main analysis: Age, invasive ductal carcinoma, number of positive nodes, grade, ER status, PR status, and HER2 status Subgroup analysis: Age, invasive ductal carcinoma, number of positive nodes, grade
Van de Vijver, 2002[67] Cohort 295 Low	DMFS HR: 4.6 (2.3 to 9.2) p<0.001	Age, lymph node status, diameter of tumor, tumor grade, vascular invasion, estrogen receptor status, mastectomy, chemotherapy, hormonal treatment

Abbreviations: BCT = breast conservation therapy; CI = confidence interval; ER =estrogen receptor; HER2 = Human Epidermal Growth Factor Receptor 2, also known as Neu; HR = hazard ratio; mm = millimeter; N = number; N+ = number positive; NKI-RdGG = the name of a clinical trial; p = probability; PR = progesterone receptor; RS = risk score.

Using a fixed effects model, our meta-analysis found a greater risk of recurrence for the poor prognosis group than for the good prognosis group (HR, 2.84; 95% CI, 2.11 to 3.82; Figure 4).

Figure 4. Prognostic value of MammaPrint for risk of recurrence, meta-analysis of adjusted hazard ratios



MammaPrint: Cancer-Specific Mortality

Five studies (N=1,615) rated as medium RoB reported on CSS for MammaPrint.[62-65,68]

The reported HRs are from models that controlled for traditional prognostic factors such as histology, size, and nodal status. Mook (2010)[65] used Adjuvant! Online that combines various clinic pathological factors to create an RS; the remaining studies included the clinic pathological factors in the model. All studies reported that patients with a poor prognosis signature had a higher cancer-specific mortality rate than those with a good prognosis signature (Table 9).

Table 9. Breast cancer: MammaPrint cancer-specific survival

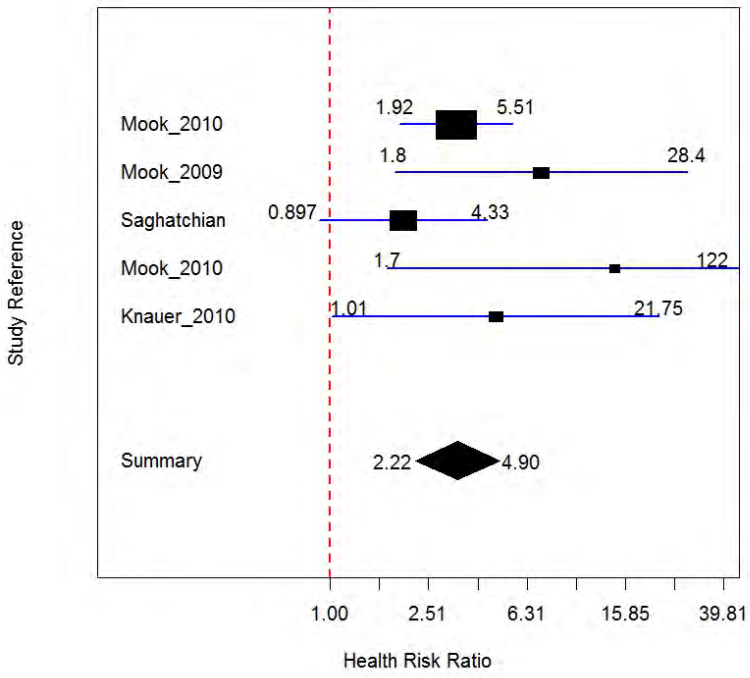
Author, Year, Design, Number of Patients, Risk of Bias	HR (95% CI),	p-value	Variables Used in the Multivariate Model
Knauer, 2010[62] Cohort 89 Medium	4.7 (1.01 to 21.75)	p=0.048	Age at diagnosis, tumor size, number of positive lymph nodes, histological grade (1/2 vs. 3), ER status, PR status, adjuvant hormonal therapy
Mook, 2010[65] Cohort 148 Moderate	14.4 (1.7 to 122) at 5 years	p=0.01	Adjuvant! Online score, which incorporates age, tumor size and grade, lymph node status, health status, hormone receptors
Mook, 2010[64] Pooled analysis of several cohorts 964 Moderate	3.25 (1.92 to 5.51)	p<0.001	Age, histology, tumor size, nodal status, grade, ER status, HER2 status, type of surgery, hormonal therapy (yes/no), chemotherapy (yes/no), 70-gene signature
Mook, 2009[63] Cohort 241 Low	7.2 (1.8 to 28.4)	p=0.005	Age (years), number of positive nodes: 2 vs. 1 and 3 vs. 1, tumor size (20 mm vs. ≤20 mm) Histological grade: moderate vs. good, poor vs. good, ER status, HER2 receptor status, surgery (mastectomy vs. BCT) chemotherapy, endocrine therapy, prognosis signature (poor vs. good signature)
Saghatchian, in press[68] Cohort 173 Medium	MammaPrint, low risk vs. high risk, main analysis 1.971 (0.897 to 4.330)	p=0.091	Main analysis: Age, invasive ductal carcinoma, number of positive nodes, grade, ER status, PR status, and HER2 status
	MammaPrint, low risk vs. high risk, subgroup analysis (N = 129 women with 4 to 9 positive nodes 2.674 (1.069 to 6.685)	p=0.035	Subgroup analysis: Age, invasive ductal carcinoma, number of positive nodes, grade

[a] calculated from years by abstractor

Abbreviations: BCT = breast conservation therapy; CI = confidence interval; ER = estrogen receptor; HER2 = Human Epidermal Growth Factor Receptor 2, also known as Neu; HR = hazard ratio; p = probability; PR = progesterone receptor.

Using a fixed effects model, our meta-analysis found a greater RR for the poor prognosis group than for the good prognosis group (HR, 3.3; 95% CI, 2.22 to 4.9) (Figure 5).

Figure 5. Prognostic value of MammaPrint for cancer-specific survival, meta-analysis of adjusted hazard ratios



MammaPrint: Overall Survival

Only one study[66] with a moderate RoB rating was available to assess the prognostic value of MammaPrint for overall survival (Table 10). The study included 144 patients and did not find a statistically significant association between test result and survival.

Table 10. Breast cancer: MammaPrint overall survival

Author, Year, Design, N, Risk of Bias	Adjusted Ratios (95% CI), p-value	Variables Used in Multivariate Model
Nuyten, 2008[66] Cohort N=144 Moderate	Death: HR:1.67 (0.73 to 3.82) p=0.23	Diameter, grade age; ER status; angio invasion; chemotherapy; hormonal therapy hypoxia-wound signature combination

Abbreviations: CI = confidence interval; ER = estrogen receptor; HR = hazard ratio; p = probability; N = number.

Characteristics of Included Studies Assessing the Clinical Validity of Oncotype DX

We included eight studies[70-77] that reported on the prognostic value of Oncotype DX Breast for RR, CSS, and OS in patients with breast cancer. Six were cohort studies with sample sizes that ranged from 338 to 1,231. Median followup was between 8 and 10 years. Two studies were case-control studies. Five studies were conducted in the United States, one in Japan, and one in

the United Kingdom. Race and ethnicity were not reported for most studies; one study reported enrolling 80 percent non-Hispanic White subjects,[71,72] and one reported enrolling 92 percent White subjects.[76]

All of the cohort studies used tissue collected during large randomized clinical trials. Dowsett[70] used tissue from the ATAC trial; Habel sampled cases and controls from the Kaiser Permanente Tumor registry. Paik (2004)[74] and Tang[76] report on the same 668 patients from the NSABP-14 trial. Mamounas[72] includes the 668 patients used in Paik (2004)[74] and Tang[76] in the 895 patients they report on in their paper. Solin[75] used tissue from the E2197 trial. One of the case-control studies identified 165 cases as those who died from breast cancer, and 401 controls matched by age, race, and calendar year[71]; the other case-control study used 10 cases who had distant metastases and age-matched controls who did not have metastases during the observation period.

Most of the studies included only ER+ patients.[72-74,77] Half of the included studies also restricted enrollment to node-negative subjects[72-74,76] (Table 11).

Table 11. Characteristics of included studies: Oncotype DX for breast cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Dowsett, 2010[70] Cohort Medium	1,231 Median 8.5 years	United Kingdom	Stage NR ER+ breast cancer	RS was a continuous var[a]	NR NR	Mean 64.1 100%
Habel, 2006[71] Case-control Low	566 NR	United States	Stage NR Invasive breast cancer	Low (RS<18) Intermediate (RS 18–30) High (RS>31)	80% non-Hispanic White	NR 100%
Mamounas, 2010[72] Cohort Moderate	895 Median: 12.5 years	United States	Stage NR Node negative, ER+ breast cancer	Low (RS<18) Intermediate (RS 18–30) High (RS≥31)	NR NR	NR NR
Mamounas, 2012[73] Cohort Unclear	1,065 Median 11.2 years	NR	Stage NR Node positive, ER+ breast cancer	Low (RS<18) Intermediate (RS 18–30) High (RS≥31)	NR NR	NR NR
Paik 2004[74] Cohort Medium	668 NR	United States	Stage NR Node negative, ER+ breast cancer	Low (RS<18) Intermediate (RS 18–30) High (RS≥31)	NR NR	NR NR
Solin 2012[75] Cohort Medium	388 Median (range): 9.7 (3.7 to 11.6) years	United States	Stage NR 1–3 positive lymph nodes or negative lymph nodes with tumor size >1.0 cm	Local recurrence Local-regional recurrence	NR NR	NR 100%
Tang, 2011[76] Cohort Low	668 Median 14.3 years	United States	NR Node negative, ER+ breast cancer, tamoxifen treated	RS percentile[b]	White: 615 (92%) Black: 31 (5%) Others: 22 (3%) NR	NR 100%

Table 11. Characteristics of included studies: Oncotype DX for breast cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Yorozuya, 2010[77] Case-control Medium	40 (10 cases), 30 controls Median: 232.3 weeks (cases)	Japan	Stage I or IIa, ER+ primary breast cancer	Low (RS<18) Intermediate (RS 18–30) High (RS≥31) (within 2 years)	NR NR	Cases mean (range): 49.1 (37 to 76) Controls mean (range): 50.9 (37 to 78) 100%

[a] Risk score was a continuous variable, with the HR for distant recurrence calculated relative to an increment of 50 units (e.g., the HR for Risk Score=55 vs. Risk Score=5), chosen to be consistent with prior clinical validation studies.

[b] RI-PCT had a range from 0 to 100; it was by 50 and rescaled to a range from 0 to 2 in regression analyses so that the associated HRs were comparable to those HRs associated with clinicopathologic factors.

Abbreviations: cm = centimeter; ER = estrogen receptor; MUT = mutation; N = number; NR = not reported; RS = risk score.

Oncotype DX: Risk of Recurrence

Six cohort studies[70,72,74-77] with low or medium RoB rating (total N=3,222 patients) and a case-control study with 40 patients (10 cases with recurrence and 30 controls) reported on the prognostic value of Oncotype for RR in breast cancer.

Dowsett,[70] Paik (2004),[74] Tang,[76] and Yorozuya[77] all used distant recurrence as the primary endpoint. Solin[75] and Mamounas[72] used loco or loco-regional recurrence (LRR) as the primary end point. All of the included studies reported on the prognostic value of Oncotype DX after controlling for traditional prognostic factors such as stage, size, and differentiation, for example. The number of variables in the models varied across the studies, and a couple looked at the effect of controlling for several combinations of prognostic factors (Table 12). A high RS was consistently shown to be a predictor of distant recurrence but not for LRR.

Table 12. Breast cancer: Oncotype DX risk of cancer recurrence (disease-free survival, recurrence-free survival, distant metastasis-free survival)

Author, Year, Design, Number of Patients, Risk of Bias	Adjusted Ratios(95% CI), p-values	Variables Used in Multivariate Model
Dowsett, 2010[70] Cohort 1,231 Moderate	Distant recurrence N0 patients: HR: 5.25 (2.84 to 9.73) p<0.001 N+ patients: HR: 3.47 (1.64 to 7.38) p=0.002	Tumor size (>2 vs. ≤2 cm); central grade (moderate vs. well, and poor vs. well); age (<65 vs. ≥65); positive nodes (≥4 vs. 1–3 nodes); positive nodes variable only included in analyses for N+ patients
Mamounas, 2010[72] Cohort 895 qwModerate	RS as a continuous variable calculated relative to an increment of 50 units: LRR HR: 2.16 (1.26 to 3.68) p=0.005 Intermediate vs. low risk: HR: 2.16 (0.98 to 4.75) p=0.143 High vs. low risk: HR: 1.67 (0.78 to 3.58) p=NR	Age (≥50 vs. <50); mastectomy vs. lumpectomy and radiation therapy; clinical tumor size (>2 vs. ≤2 cm); tumor grade (moderate vs. well); tumor grade (poor vs. well)

Table 12. Breast cancer: Oncotype DX risk of cancer recurrence (disease-free survival, recurrence-free survival, distant metastasis-free survival) (continued)

Author, Year, Design, Number of Patients, Risk of Bias	Adjusted Ratios(95% CI), p-values	Variables Used in Multivariate Model
Paik 2004[74] Cohort 668 Medium	RS Distant recurrence HR:3.21 (2.23 to 4.61) p<0.001	Age, tumor size, RS
Solin 2012[75] Cohort 388 Medium	NR NS for local or LRR	Chemotherapy arm, patient age, HR status, pathologic axillary lymph node status, histologic grade, pathologic T stage, biologic subtype, and 21-gene RS
Tang, 2011[76] Cohort 668 Low	Distant recurrence RS-PCT/50 Model 4: HR: 2.83 (95% CI: 1.91 to 4.18) p<0.001 Model 5: HR: 2.37 (95% CI, 1.58 to 3.55) p<0.001 Model 6: HR: 2.34 (95% CI, 1.56 to 3.5) p<0.001	Model 4: Adjuvant! Online score (RI) as a composite covariate representing common prognostic factors Model 5: included Adjuvant! Online score (RI); also included common prognostic factors as individual covariates including: age (>50 vs. ≤50); tumor size (cm); grade (moderate vs. well); grade (poor vs. well); RI-PCT/50 Model 6: included only individual prognostic factors as covariates including age (>50 vs. ≤50); tumor size (cm); grade (moderate vs. well); grade (poor vs. well); RI-PCT/50
Yorozuya, 2010[77] Case-control 40 (10 cases), 30 controls) Moderate	OR: 2.85 (95% CI: 0.07 to 115.5) p=0.579	Age at diagnosis; ER score; PgR score; RS 50 or more vs. RS<50; histological grade lymphatic invasion

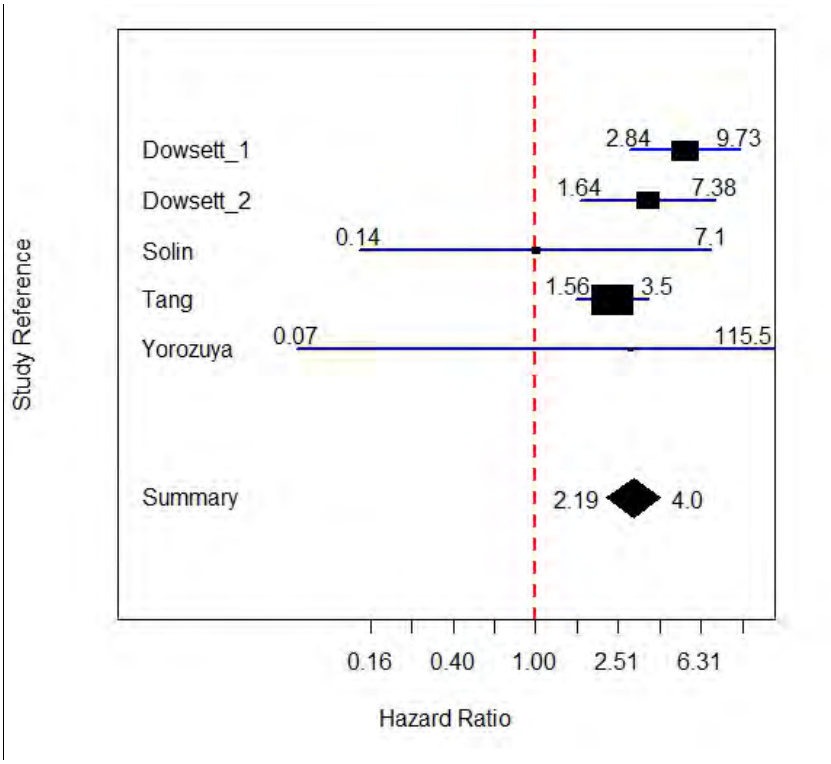
[a] Calculated from years by abstractor

Abbreviations: CI = confidence interval; cm = centimeter; ER = estrogen receptor; HR = hazard ratio; LRR = loco-regional recurrence; N+ = number positive; N0 = node negative; NR = not reported; OR = odds ratio; p = probability; PgR =progesterone receptor; RI = risk index; RI-PCT = risk index percentile; RS = risk score; RS-PCT = risk score percentile; vs. = versus.

Mamounas[72] and Solin[75] reported that the RS was not a significant predictor of the risk of LRR. Paik and Tang used the same set of patients but fitted different models to assess the prognostic value of the Oncotype DX RS in predicting distant recurrence. Models reported in Paik[74] and Tang[76] found statistically significant HRs between 2.3 and 3.1. Dowsett[70] reported a significant HR for both node-negative (5.25) and node-positive patients (3.47). The case-control study reported that cases having a distant recurrence had a higher RS than age-matched controls with no distant metastasis.

In a fixed effects model, our meta-analysis found an overall HR for RR for the high RS group compared with the intermediate/low RS group of 2.97 (95% CI, 2.19 to 4.0) (Figure 6).

Figure 6. Prognostic value of Oncotype DX Breast for risk of recurrence, meta-analysis of adjusted hazard ratios



Oncotype DX: Cancer-Specific Survival

We found two included studies rated as low RoB that reported on the prognostic value for CSS. One was a cohort study, Tang,[76] that included 668 patients, and one was a case-control study, Habel,[71] that included 566 patients. Tang[76] fit several models to the data and reported HRs ranging from 2 to 2.45, depending on the prognostic variables controlled for. Habel[71] reported that both tamoxifen-treated and tamoxifen-untreated breast cancer patients who died had higher RSs than matched patients who survived (Table 13).

Table 13. Breast cancer: Oncotype DX cancer-specific survival

Author, Year, Design, Number of Patients, Risk of Bias	Adjusted Risk (95% CI), p-value	p-value	What Variables Were Used in the Multivariate Model?
Habel, 2006[71] Case-control 566 Low	RS: Tamoxifen treated RR: 5.3 (1.6 to 17.2) p=0.003 Tamoxifen untreated RR: 2.4 (1.1 to 5.2) p=0.03	NR	Tumor size continuous (cm); grade (well, moderate, poor); RS (continuous)
Tang, 2011[76] Cohort 668 Low	RS-PCT/50 Model 4: HR: 2.45 (1.66 to 3.61) p<0.001 Model 5: HR: 2.02 (1.35 to 3.00) p<0001 Model 6: HR: 2.01 (1.35 to 2.98) p<0.001	NR	Model 4: Adjuvant! Online score (RI) as a composite covariate representing common prognostic factors but did not also include those factors individually Model 5: included Adjuvant! Online score (RI); also included common prognostic factors as individual covariates including age (>50 vs. ≤50); tumor size (cm); grade (moderate vs. well); grade (poor vs. well); RI-PCT/50 Model 6: included only individual prognostic factors as covariates including age (>50 vs. ≤50), tumor size (cm), grade (moderate vs. well), grade (poor vs. well), RI-PCT/50

[a] calculated from years by abstractor

Abbreviations: cm = centimeter; HR = hazard ratio; p = probability; RI = risk index; RI-PCT = risk index percentile; RR = relative risk; RS = risk score; RS-PCT = risk score percentile.

One cohort study, Tang,[76] rated as low RoB, was available. Tang fitted models with different combinations of adjustments for traditional prognostic factors. The HRs were of a similar magnitude in all three models, and the association between high RS and earlier death from all causes was positive and significant (Table 14).

Table 14. Breast cancer: Oncotype DX—overall survival

Author, Year, Design, N, Risk of Bias	Median Length of Followup to Determine Recurrence/Survival	Adjusted Ratio (95% CI), p-values	Variables Used in Multivariate Model
Tang, 2011[76] Cohort N=688 Low	14.3 years (range: NR)	RS-PCT/50 Model 4: HR: 1.77 (1.35 to 2.33) p<0.001 Model 5: HR: 1.65 (1.24 to 2.19) p<0.001 Model 6: HR: 1.65 (1.24 to 2.19) p<0.001	Model 4: Adjuvant! Online score (RI) as a composite covariate representing common prognostic factors but did not also include those factors individually Model 5: included Adjuvant! Online score (RI); also included common prognostic factors as individual covariates including age (>50 vs.≤50), tumor size (cm), grade (moderate vs. well), grade (poor vs. well), RI-PCT/50 Model 6: included only individual prognostic factors as covariates including: age (>50 vs.≤50), tumor size (cm), grade (moderate vs. well), grade (poor vs. well), RI-PCT/50

Abbreviations: CI = confidence interval; HR = hazard ratio; p = probability; NR = not reported; RI = risk index; RI-PCT = risk index percentile; RS = risk score; RS-PCT = risk score percentile.

Lung Cancer: *EGFR* and *KRAS* Mutations

Characteristics of Included Studies Assessing the Clinical Validity of *EGFR* Mutation Testing

We found 12 studies[78-89] that examined the added prognostic value of *EGFR* mutations in patients with nonsmall cell lung carcinoma (NSCLC). All were cohort studies. Sample sizes ranged from 50 to 524 patients. Median followup was not reported in all studies. Followup varied from 4 to 14 years. Studies were conducted in Japan, South Korea, Taiwan, China, various European countries, and the United States.

All studies primarily compared outcomes of patients with *EGFR* wild-type to those with *EGFR* mutations. A few looked at specific mutations such as the exon 19 deletion or the exon 21 L858R. The representation of female patients in the studies ranged from a low of 16 percent to a high of 65 percent. Race or ethnicity was reported only in two studies[84,89] conducted in the United States and Canada; Mak[84] and Tsao[89] had samples with 93 percent and 44 percent white patients, respectively.

Populations represented in the studies varied somewhat. The differences were both in cancer stage and cancer type. Four studies included all four stages,[78-80,88] three included stages I to III,[82,83,86] two included only stage I,[81,85] and the remaining included stages I and II. Not all studies specify the type of tumors included (Table 15).

Table 15. Characteristics of included studies: *EGFR mutation testing for lung* cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Study groups	Race Ethnicity	Overall Age (years) % Female
An, 2012[78] Cohort Medium	524	China	I–IV		NR NR	Mean (range)=59.3 (23 to 88) 31%
Hiramitsu, 2010[79] Cohort High	193 470.4 weeks	Japan	I–IV Primary lung cancer	*EGFR*-WT *EGFR*-MUT	NR NR	Mean=63 50.3%
Kim, 2008[80] Cohort Medium	71 Mean (SD): 25.3 (11.1) months Range: 2.9 to 47.5 months	Republic of Korea (South Korea)	I–IV	*EGFR*-WT *EGFR*-MUT	NR NR	Median (range)=59.6 (38 to 85) 59.2%
Kim,2013[88] Cohort Medium	863 23.6 ± 0.7 months	Korea	I–IV	*EGFR*-MUT	NR	Mean=63 (27 to 87) 40%
Kobayashi, 2008[81] Cohort Medium	127 Median (range): 67 (12 to 134) months	Japan	IA Adenocarcinoma of the lung ≤20 mm diameter	*EGFR*-WT *EGFR*-MUT	NR NR	Median (range)=65 (38 to 83) 54.3%
Koh, 2010[82] Cohort High	136 Median: 491.3 weeks	Korea	IA–IIB	*EGFR*-WT *EGFR*-MUT	NR NR	Median (range)=61 (33 to 80) 26%
Liu, 2010[83] Cohort Medium	164 Range: 3.9 to 687 weeks	China (Taiwan)	I–IIIA Adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma	*EGFR*-WT *EGFR*-MUT	NR NR	NR 32.9%
Mak, 2011[84] Cohort Medium	123 Median (95% CI): 40.4 (30.5 to 52.6) months	United States	IIB–IIIB	*EGFR*-WT *EGFR*-MUT	White:93% Asian: 6% Other: 2% Ethnicity NR	Median (range)=62 (39 to 85) 65%
Matsumoto, 2006[85] Cohort Medium	107 Median (range) 63 (4 to 110) months	Japan	I	*EGFR*-WT *EGFR*-MUT	NR NR	NR 43%
Rouquette, 2012[86] Cohort Low	50 Median (interquartile range [IQR]): 35 (29 to 49) months	France	I–III Adenocarcinomas, squamous cell carcinomas, bronchioloalveolar carcinomas	*EGFR*-WT *EGFR*-MUT	NR NR	

Table 15. Characteristics of included studies: *EGFR mutation testing for lung* cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Study groups	Race Ethnicity	Overall Age (years) % Female
Scoccianti, 2012[87] Cohort Medium	250 Up to 48 months	France, Germany, Ireland, Italy, the Netherlands, Poland, Spain, and the United Kingdom	T1–T4 Primary lung cancers	*EGFR*-WT *EGFR*-MUT	NR NR	NR 16%
Tsao, 2011[89] Cohort Low	436 NR	Canada and United States	IB–II	*EGFR*-WT *EGFR*-MUT	White=44.7% Other=6% Unknown=49%	NR *EGFR*-MUT: 13.4% *EGFR*-WT: 86.6%

Abbreviations: *EGFR* = gene name; IQR = interquartile range; mm = millimeter; MUT = mutation; N = number; NR = not reported; SD = standard deviation; WT = wild-type.

EGFR Mutation: Risk of Recurrence

Six cohort studies[80,81,84,87-89] (total N=1,870) assessed the prognostic value of this test for RR. Kobayashi[81] used recurrence as the outcome; Mak[84] looked at LRR and distant recurrence but provided adjusted results only for LRR. Scoccianti[87] used time to disease progression as the outcome. Tsao[89] used relapse-free survival as the outcome. Kim[80] used freedom from recurrence as the outcome. Three[80,81,89] of the five studies did not specify whether the outcome was loco–regional or distant or both.

Although the HRs for *EGFR* mutation suggest a protective effect (i.e., were all <1), none of the findings were statistically significant. Thus, the test for *EGFR* mutations does not appear to have prognostic value in predicting recurrence in NSCLC (Table 16).

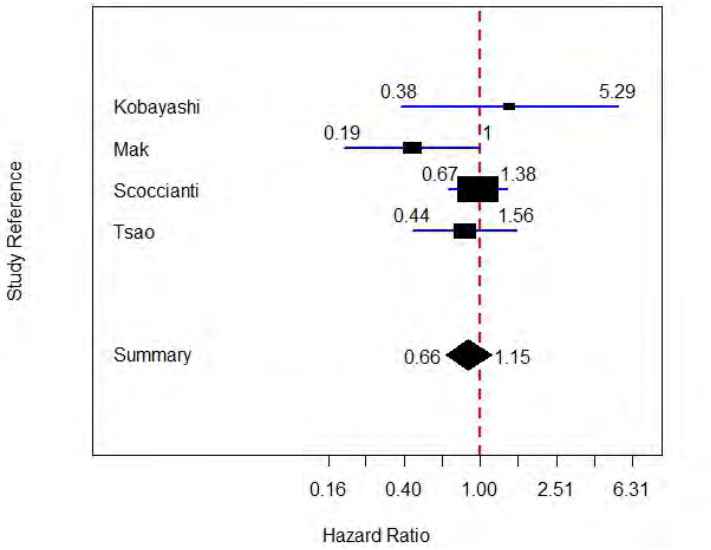
Table 16. Lung cancer: Risk of cancer recurrence/disease-free survival/recurrence-free survival for *EGFR*

Author, Year, Design, N, Risk of Bias	Adjusted Ratios, (95% CI) p-value Test Positive, Test Negative	Variables Used in Multivariate Model
Kim, 2008[80] Cohort 71 Medium	Freedom from Recurrence HR: NR, p=NS	Male, age >60 years, smoking+, BAC feature+, ≥Stage IIIA, *KRAS*+, EFGR−
Kim,2013[88] Cohort863 Medium	Freedom from recurrence EGFR NS	BAC, age, smoking, pathologic stage, cell type
Kobayashi, 2008[81] Cohort 127 Medium	Risk of cancer recurrence: HR: 1.42 (0.38 to 5.29) p=0.60 DFS: HR: 1.55 (0.54 to 4.49) p=0.42	Age (≥64 vs. <64); sex; smoking status (ever vs. never); CEA level (≥5.0 vs. <5.0); tumor size ≥15 vs. <15); non-BAC component (>50% v. ≤50%); *EGFR* mutation (yes or no)
Mak, 2011[84] Cohort 123 Medium	LRR HR: 0.44 (0.19 to 1.00) p=0.05	*EGFR* mutation status, stage, use of surgery, chemotherapy use, RT dose, primary tumor size
Soccianti, 2012[87] Cohort 250 Medium	Time to Disease Progression HR: 0.97 (0.67 to 1.38) p=0.68	Pathological tumor score, pathological node score
Tsao, 2011[89] Cohort 436 Low	RFS: HR: 0.83 (0.44 to 1.56) p=0.56	Sex, age, performance status, stage, histology, smoking status, baseline anemia, resection, serum LDH, p53 mutation status, and immunohistochemistry

Abbreviations: BAC = bronchioloalveolar cancer; CEA = carcinoembryonic antigen; CI = confidence interval; CISH = cytokine-inducible SH2-continaing protein; DFS = disease-free survival; *EGFR* = gene name; HR = hazard ratio; *KRAS* = gene name; LDH = lactate dehydrogenase; LRR = loco-regional recurrence; NR = not reported; NS = not statistically significant; p = probability; RFS = recurrence-free survival; RT = radiation therapy.

Using a fixed effects model, our meta-analysis, which included the four studies that reported HRs, found no added prognostic value for the *EGFR* mutation in predicting risk of recurrence HR: 0.87 95% CI (0.65 to 1.15) (Figure 7).

Figure 7. Prognostic value of *EGFR* mutation testing for risk of recurrence in lung cancer, meta-analysis of adjusted hazard ratios



EGFR Mutation: Cancer-Specific Survival

We found no studies meeting our inclusion criteria.

EGFR Mutation: Overall Survival

Six studies[81,83-85,88,89] representing 1,820 patients reported on the prognostic value of *EGFR* mutation for this outcome. One of the studies did not report any quantitative measures and therefore did not contribute to our strength of evidence determination. All studies looked at the prognostic value of the test in patients with NSCLC.

None of the included studies found a statistically significant HR for overall survival. The magnitude ranged from 0.6 to 1.6 in studies that reported quantitative measures. The other studies only reported that the result was not statistically significant (Table 17).

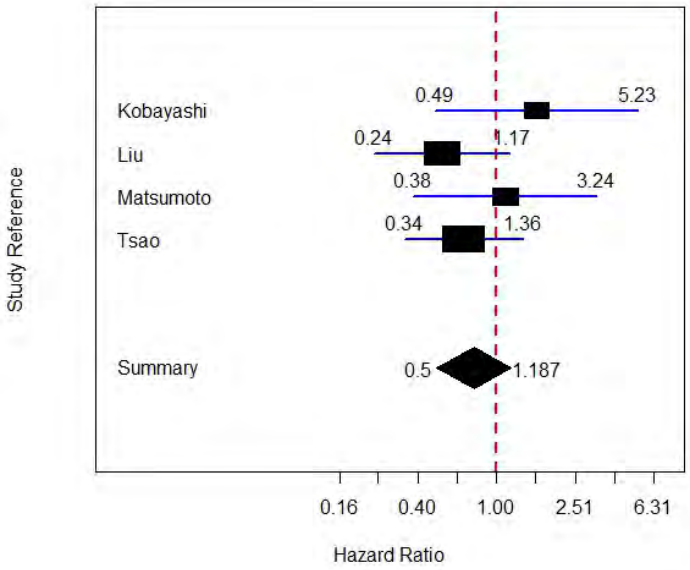
Table 17. Lung Cancer: *EGFR* mutation testing overall survival

Author, Year, Design, Risk of Bias	Adjusted Ratios, (95% CI) p-value Test Positive, Test Negative	Variables Used in Multivariate Model
Kim, 2013[88] Cohort 863 Medium	EGFR NR p=NS	BAC, Gender, age, pathologic stage, and smoking status
Kobayashi, 2008[81] Cohort 127 Medium	HR: 1.60 (0.49 to 5.23) p=0.44	Age (≥64 v. <64); sex; smoking status (ever vs. never); CEA level (≥5.0 vs. <5.0); tumor size (≥15 vs. <15); non-BAC component (>50% vs. ≤50%); *EGFR* mutation (yes or no)
Liu, 2010[83] Cohort 164 Medium	HR: 0.53 (95% CI, 0.24 to 1.17), p=NR	Year group, gender, age, pathology, stage, smoking status, CISH, *KRAS* mutation status, *EGFR* mutation expression
Mak, 2011[84] Cohort 123 Medium	HR: NR p=NS	*EGFR* mutation status, age, stage, PS score, smoking status, use of surgery, chemotherapy use, RT dose, primary tumor size (i.e., all outcomes that were significantly different between groups)
Matsumoto, 2006[85] Cohort 107 Medium	HR: 1.11 (0.38 to 3.24) p=0.85	Adjusted by all potential prognostic factors including gender, age, smoking status, tumor stage, tumor size, differentiation, *KRAS* status
Tsao, 2011[89] Cohort 436 Low	HR: 0.68 (0.34 to 1.36) p=0.28	Sex, age, performance status, stage, histology, smoking status, baseline anemia, type of resection, serum lactate dehydrogenase, p53 mutation status, and p53 immunohistochemistry

Abbreviations: BAC = bronchioloalveolar cancer; CI = confidence interval; CISH = cytokine-inducible SH2-continaing protein; *EGFR* = gene name; HR = hazard ratio; *KRAS* = gene name; NR = not reported; NS = not statistically significant; OS = overall survival; p = probability; PS = performance status; RT = radiation therapy.

Using a fixed effects model, our meta-analysis, which included the four studies that reported HRs, found no added prognostic value for the *EGFR* mutation in predicting overall survival HR, 0.77; 95% CI (0.50 to 1.19) (Figure 8).

Figure 8. Prognostic value of *EGFR* mutation testing for overall survival in lung cancer, meta-analysis of adjusted hazard ratios



Characteristics of Included Studies Assessing the Clinical Validity of *KRAS* Mutation testing

We found six studies[79,80,83,86,87,90] that examined the added prognostic value of *KRAS* mutations on the RR, CSS, and OS in patients with NSCLC. All were cohort studies with sample sizes that ranged from 100 to 250 patients. Studies were conducted in Japan, South Korea, Taiwan, and various European countries. Race/ethnicity was not reported in any of the studies. All studies compared outcomes of patients with *KRAS* wild-type with those with *KRAS* mutations. The representation of female patients in the studies ranged from a low of 16 percent to a high of 59 percent. Median length of follow-up was not available in all studies. Length of follow-up ranged from 4 to 14 years.

Populations represented in the studies varied somewhat. The differences were both in cancer stage and cancer type. Three studies included all stages.[79,80,87] Two included stages I to III,[83,86] and one[90] included only stage I (Table 18).

Table 18. Characteristics of included studies: *KRAS* mutation testing for nonsmall cell lung cancer

Author, Year Study Type Risk of Bias	N Length of followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Guan, 2012[91] Cohort Medium	182 Median (all groups): 22.0 months Median (KRAS/WT): 22.7/20.6 months	China	I–IV Primary lung cancer	KRAS-WT KRAS-MUT	NR	Median: 59 37%
Hiramitsu, 2010[79] Cohort High	193 Median: 295.1 week	Japan	I–IV Primary lung cancer	*KRAS*-WT *KRAS*-MUT	NR	Mean=63 50.3%*
Kim, 2008[80] Cohort Medium	71 Mean (SD): 25.3 (11.1) months Range: 2.9 to 47.5 months	Republic of Korea (South Korea)	I–IV	*KRAS*-WT *KRAS*-MUT	NR	Median (range)=59.6 (38–85) 59.2%
Liu, 2010[83] Cohort Medium	164 Range: 3.9 to 687 weeks	China (Taiwan)	I–IIIA Adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma	*KRAS*-WT *KRAS*-MUT	NR	NR 32.9%
Rouquette, 2012[86] Cohort Low	100 Median (IQR): 35 (29 to 49) months	France	I–III Adenocarcinomas, squamous cell carcinomas, bronchioloalveolar carcinomas	*KRAS*-WT *KRAS*-MUT	NR	NR 50%
Scoccianti, 2012[87] Cohort Medium	250 Up to 48 months	France, Germany, Ireland, Italy, the Netherlands, Poland, Spain, and the United Kingdom	T1–T4 Primary lung cancers	*KRAS*-WT *KRAS*-MUT	NR	NR 16%
Woo, 2009[90] Cohort Medium	190 Median (range): 35.9 (1.1 to 82.5) months	Japan	IA–IB	*KRAS*-WT *KRAS*-MUT	NR	Mean (SD) (range)=67.8 (8.4) (45–85) 48.4%

Abbreviations: IQR = interquartile range; *KRAS* = gene name; MUT = mutation; N = number; NR = not reported; SD = standard deviation; WT = wild-type.

KRAS Mutation: Risk of Recurrence

Four studies[80,86,87,90] examining five cohorts(total N=611) rated as low or medium RoB assessed the prognostic value for RR (Table 19). Kim[80] and Scoccianti[87] studied

NSCLC, whereas Woo[90] studied the effect of the mutation specifically on stage I adenocarcinomas.

Table 19. Lung cancer: *KRAS* risk of cancer recurrence/disease-free survival/recurrence-free survival

Author, Year, Design, N, Risk of Bias	Adjusted Ratios, (95% CI) p-value Test Positive, Test Negative	Variables Used in Multivariate Model
Kim, 2008[80] Cohort 71 Medium	Freedom from recurrence HR: 2.8 (1.060 to 7.391) p=0.038	Multifactors chosen by step-forward method: male; age >60 years; smoking+; BAC feature+; ≥ Stage IIIA; *KRAS*+; *EGFR*−
Rouquette, 2012[86] Cohort 100 Low	DFS HR: NR, p=NS	*EGFR* mutation status, *KRAS* mutation status, HER2 expression, progesterone receptor expression, presence of lymphatic embols, tumor stage III
Scoccianti, 2012[87] Cohort 250 Medium	Time to Progression HR: 1.19 (0.75 to1.90) p=0.46	Pathological tumor score; pathological node
Woo, 2009[90] Cohort 1A N = 131 Cohort 1B N = 59 Medium	DFS For stage IA: HR: 4.55, (1.61 to 12.82) p=0.004 For Stage IB: HR: 14.99 (1.55–145.21) p=0.019	Age, tumor size, vessel invasion, Ki-67 expression

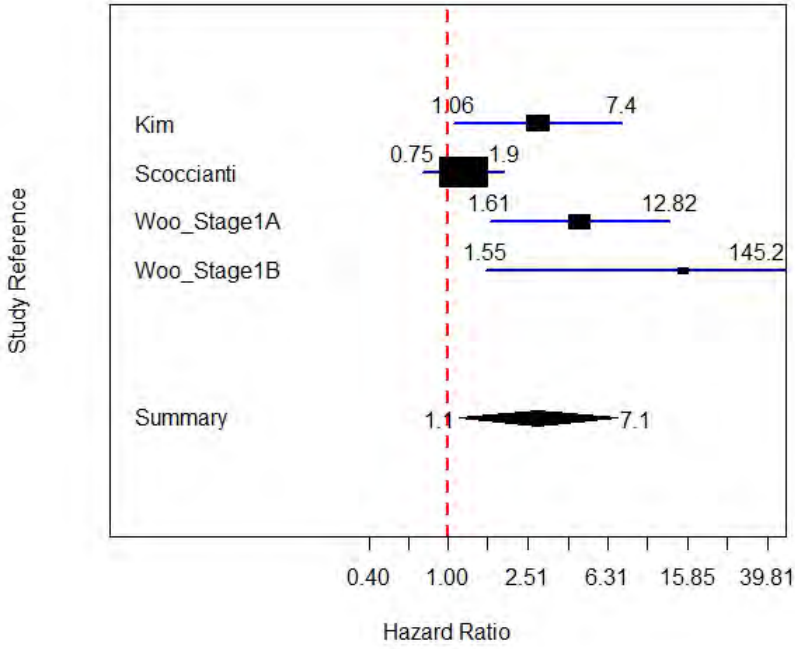
Abbreviations: BAC = bronchioloalveolar cancer; CI = confidence interval; CISH = cytokine-inducible SH2-containing protein; *EGFR* = gene name; HER2 = Human Epidermal Growth Factor Receptor 2, also known as Neu; HR = hazard ratio; *KRAS* = gene name; NR = not reported; OR = odds ratio; p = probability.

Kim[80] used freedom from recurrence as the outcome; the publication did not specify whether the recurrence was restricted to loco-regional or distant recurrence. Scoccianti[87] used time to disease progression as the outcome. Woo[90] also used disease-free recurrence as the outcome and did not specify if this was restricted to loco-regional or distant recurrence. Rouquette[86] used disease-free survival as the outcome and defined it as relapse, metastases, or death by any cause, whichever occurred first.

Rouquette[86] and Scoccianti[87] reported that *KRAS* mutation did not have added prognostic value. However, Kim[80] and Woo[90] reported that *KRAS* is a significant predictor for RR after controlling for other prognostic factors; the HRs range between 2.8 and 4.55.

Using a random effects model, our meta-analysis found added prognostic value of *KRAS* for risk of recurrence (HR, 2.84; 95% CI, 1.14 to 7.10) (Figure 9).

Figure 9. Prognostic value of *KRAS* mutation testing for risk of recurrence in lung cancer, meta-analysis of adjusted hazard ratios



KRAS Mutation: Cancer-Specific Survival

We found no studies meeting our inclusion criteria.

KRAS Mutation: Overall Survival

Two studies[80,91] with 253 patients reported that *KRAS* testing had added prognostic value for OS (Table 20).

Table 20. Lung cancer: *KRAS* overall survival

Author, Year, Design, N, Risk of Bias	Adjusted Ratios, (95% CI) p-value Test Positive, Test Negative	Variables Used in Multivariate Model
Kim, 2008[80] Cohort 71 Medium	*KRAS*+ HR: 3.339 (1.027 to10.862) p=0.045 *KRAS*+ 7.0%[a] (N=5) *KRAS*− 93.0%[a] (N=66)	Multifactors chosen by step-forward method: male; age >60 years; smoking+; BAC feature+; ≥ Stage IIIA; *KRAS*+
Guan, 2012[91] Cohort 182 Medium	KRAS(wild/mutation) (N=91/ N=91) HR: 2.69, 95 % CI 1.91–3.80; P< 0.001	Gender, age, KRAS/EGFR mutation status, pathology, TNM stage, PS, comorbidities, and smoking status

[a] calculated by abstractor

Abbreviations: BAC = bronchioloalveolar cancer; CI = confidence interval; HR = hazard ratio; *KRAS* = gene name; N = number; OR = odds ratio; p = probability.

Colorectal Cancer—*BRAF* Mutation

Characteristics of Included Studies Assessing the Clinical Validity of *BRAF* Mutation Testing

We included 17 studies[92-108] that examined the added prognostic value of *BRAF* mutations for RR, CSS, and OS in patients with CRC. All were cohort studies. Sample sizes ranged from 69 to 1,680. Median followup ranged from 3 to 8 years. Studies were conducted in Australia, China, various European countries, and the United States.

All studies compared outcomes of patients with *BRAF* wild-type to those with *BRAF* V600 mutations. The representation of female patients in the studies ranged from 2 percent to 53.6 percent. Race or ethnicity was not reported in most studies; one reported enrolling 98 percent white patients.[102]

Populations represented in the studies varied somewhat. The differences were both in cancer stage and the inclusion or exclusion of rectal cancers. Two studies[92,109] examined a sample of stage I and stage III colon cancers. Nine[93,94,99,102-106,108] included all stages and colon and rectal cancers. Two[95,102] included all stages of colon (not rectal) cancers. In the rest, one study[96] included both colon and rectal cancers stages I through III, another[97] had only stage III colon cancer patients, one study[98] included patients with only proximal colon cancer stages I through IV, and two[100,107] included stages II and III colon cancer (Table 21).

Table 21. Characteristics of included studies: *BRAF* mutation testing for colorectal cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Eklof, 2013[108] Cohort Medium	197 (NSHDS) 414 (CRUMS) Median (CRUMS): 113 months Median (NSHDS): 102 months	Sweden	I–IV	*BRAF*-MUT *BRAF*-WT	NR	NR 43.7% (CRUMS) 56.9% (NSHDS)
Farina-Sarasqueta, 2010[92] Cohort Low	364 Stage II median (range): 239.1 (0 to 473.9) weeks Stage III median (range): 200 (8.7 to 578.3) weeks	The Netherlands	II–III	*BRAF*-WT *BRAF*-MUT	NR NR	Stage II mean age: 71.5 Stage III mean age: 62.5 45.6%
Kakar, 2008[93] Cohort High	69 NR	United States	I–IV	*BRAF*-WT *BRAF*-MUT	NR NR	NR 41%
Kalady, 2012[94] Cohort Medium	475 NR	United States	I–IV	*BRAF*-WT *BRAF*-MUT	NR NR	NR *BRAF*-WT: 85% *BRAF*-MUT: 15%
Liou, 2011[95] Cohort Medium	314 Median (range) 57 (49 to 82) months	China	I–IV	*BRAF*-WT *BRAF*-MUT	NR NR	Median WT 66 Median MUT 75 39%

Table 21. Characteristics of included studies: *BRAF* mutation testing for colorectal cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Lochhead, 2013[105] Cohort Medium	1253 Median: 8.2 years, range(3.5–13.1)years	United States	I-IV	*BRAF*-WT BRAF-MUT	NR	Mean age +/− SD 68.5 ±8.7 55%:
Maestro, 2007[96] Cohort Medium	324 Median (IQR): 43 (27 to 63) months	Spain	Duke's Stages A–D	*BRAF*-WT *BRAF*-MUT	NR NR	Mean (range): 69.6 (30 to 95) 47.9%
Ogino, 2012[97] Cohort Medium	506 Median: 91.2 months	United States	III	*BRAF*-WT *BRAF*-MUT	NR NR	Median (SD): 59.7 (11.5) 46%
Pai, 2012[98] Cohort High	181 Median (range): 52 (0 to 331) weeks	United States	I–IV	*KRAS*-MUT *BRAF*-MUT *KRAS* and *BRAF*-WT	NR NR	Mean (range): 67 (33 to 98) 47%
Phipps, 2012[99] Cohort Medium	1980 Median (range): 7.4 (0.4 to 13.8) years	United States	Stages categorized according to SEER conventions (localized, regional, distant, or unknown)	*BRAF*-WT *BRAF*-MUT	NR NR	NR 54.5%
Roth, 2010[100] Cohort Medium	1307 Median: 296 weeks	Switzerland, Belgium, Italy, Hungary, Spain, France, and United Kingdom	II–III	*BRAF*-WT *BRAF*-MUT	NR NR	NR 42.2%
Roth, 2012[107] Cohort Medium	1404 Median: 69 months	Switzerland	II-III	*BRAF*-MUT *BRAF*-WT	NR	Median (range): 60 (21-76) 42.8%
Samadder, 2013[106] Cohort Medium	563 NR	United States	I-IV	*MSS* *MSI-L/H* *BRAF*-MUT *BRAF*-WT	NR	Mean (SD): 73.9 (5.92) 100%
Samowitz, 2005[101] Cohort High	803 NR	United States	I–IV	*BRAF*-WT (microsatellite stable tumors) *BRAF*-MUT (V600E) (microsatellite stable tumors)	NR NR	Range: 30 to 79 45.8%
Shaukat, 2010[102] Cohort Medium	194 NR	United States	I–IV	*BRAF*-WT *BRAF*-MUT	White: 98% NR	NR 2%

Table 21. Characteristics of included studies: *BRAF* mutation testing for colorectal cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Tie, 2011[103] Cohort Medium	525 38.5 months (median recurrence) 12.9 months (median overall survival)	Australia	I–IV	*BRAF*-WT *BRAF*-MUT	NR NR	Median 70.5 50%
Zlobec, 2010[104] Cohort Medium	404 Median: 234.8 weeks (all patients) Median: 260.9 weeks (CSS)	Switzerland	T1–T4	*BRAF*-WT *BRAF*-MUT	NR NR	Mean (range): 69.5 (40–95) 53.6%

Abbreviations: *BRAF* = gene name; CSS = cancer-specific survival; IQR = interquartile range; *KRAS* = gene name; MUT = mutation; NR = not reported; SD = standard deviation; SEER = Surveillance, Epidemiology, and End Results; WT = wild-type.

BRAF: Risk of Recurrence

Five cohort studies[92,97,100,103,107] rated as low or medium RoB (total N=4,106) examined the prognostic significance of the *BRAF* mutation for RR (Table 22). The definitions for the outcomes differed a little between the studies. Farina[92] defined disease-free survival (DFS) as time to recurrence of disease or distant metastasis; Ogino[97] defined DFS as time to disease recurrence or distant metastasis or death from any cause; Roth (2010),[100] Roth (2012)[107] and Tie[103] defined their outcome as recurrence of CRC and did not specify if recurrence includes local and distant.

Table 22. Colorectal cancer: *BRAF* risk of cancer recurrence/disease-free survival/recurrence-free survival

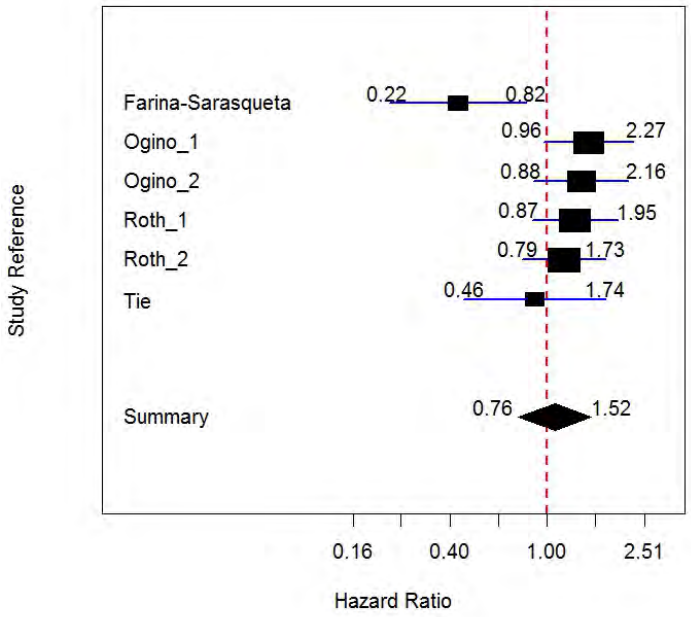
Author, Year, Design, N Test (Cancer), Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Farina-Sarasqueta, 2010[92] Cohort 364 Low	DFS HR=0.43 (0.22 to 0.82) p=NR	Differentiation grade, age, sex, tumor location, tumor stage, node stage, *KRAS* status, *BRAF* status, and MSI status
Ogino, 2012[97] Cohort 506 Medium	DFS HR: 1.48 (0.96 to 2.27) p=NR RFS HR: 1.38 (0.88 to 2.16) p=NR	Age, sex, baseline body mass index (BMI), family history of CRC in first-degree relatives, performance status, presence or absence of perforation and/or obstruction at surgery, treatment arm, tumor location, tumor and node stage, *KRAS* status, MSI status
Roth, 2010[100] Cohort 1307 Medium	RFS, All subjects HR: 1.30 (0.87 to 1.95) p=0.21 Stage II subjects HR: 1.93 (0.67 to 5.60) p=0.23 Stage III subjects HR: 1.23 (0.79 to 1.92) p=0.35	Treatment arm, age, sex, grade, tumor site, nodal stage, MSI, *KRAS*, *BRAF* mutation status
Roth, 2012[107] Cohort 1404 Medium	RFS, HR: 1.17 (0.79 to 1.73) p=0.44	Age, sex, grade, stage, number of examined lymph nodes, site, treatment group, MSI status, SMAD4 expression, 18qLOH status, and KRAS
Tie, 2011[103] Cohort 525 Medium	RR HR: 0.89 (0.46 to 1.74) p=0.74	Age, gender, stage, tumor site, use of adjuvant therapy

Abbreviations: BMI = body mass index; *BRAF* = gene name; CI = confidence interval; CRC = colorectal cancer; DFS = disease-free survival; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; N = number; NR = not reported; p = probability; RFS = recurrence-free survival; RR = risk of recurrence.

The included studies reported on the prognostic value of testing for *BRAF* after controlling for certain traditional prognostic factors such as stage, sex, and age. Three of the four studies found no association between *BRAF* and prognosis in adjusted analyses. Farina[92] reported a significant association (HR, 2.32; 95% CI, 1.21 to 4.54) between RR and the *BRAF* mutation.

Using a random effects model, our meta-analysis found no added prognostic value of *BRAF* for risk of recurrence (HR, 1.07; 95% CI, 0.76 to 1.52) (Figure 10).

Figure 10. Prognostic value of *BRAF* mutation testing for risk of recurrence in colorectal cancer, meta-analysis of adjusted hazard ratios



BRAF for Colorectal Cancer: Cancer-Specific Mortality

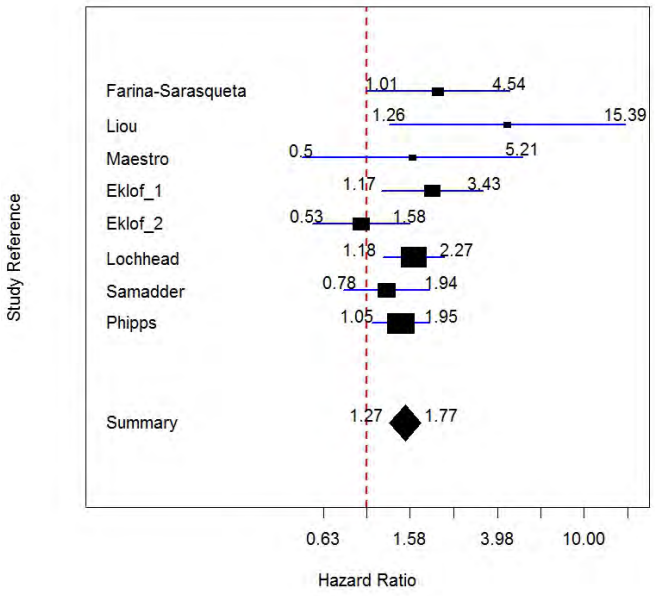
Seven studies reporting on eight different cohorts with low or medium RoB (total N=5,409) reported on *BRAF* mutation and CSS.[92,95,96,99,105,106,108] Five out of seven cohorts had statistically significant HRs, suggesting a positive association between mutation and greater risk of cancer-specific death (Table 23). A summary measure was estimated using a fixed effects model. The overall HR for CSS for the patients with wild-type compared with mutated was 1.50, 95% CI (1.26 to 1.77) (Figure 11).

Table 23. Colorectal cancer: *BRAF* cancer-specific mortality

Author, Year, Design, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Farina-Sarasqueta, 2010[92] Cohort 364 Low	HR: 2.12 (1.01 to 4.54) p=0.05	Differentiation grade, age, sex, tumor location, tumor stage, node stage, *KRAS* status, *BRAF* status, and MSI status
Eklof, 2013[108] Cohort 197 (NSHDS) 414 (CRUMS) Medium	(NSDHS, N = 196) HR: 1.998 (1.165 to 3.426) p=NR (CRUMS, N = 377) HR: 0.94 (0.529 to1.576) p=NR	Sex, age at diagnosis, stage, tumor site
Liou, 2011[95] Cohort 314 Medium	HR: 4.41 (1.26 to 15.39) p=0.02	Age, sex, stage, lymphatic invasion, venous invasion, tumor differentiation, CEA level (>5 vs. ≤5 ng/mL)
Lochhead, 2012[105] Cohort 1,253 Medium	*BRAF*-MUT HR:1.64 (1.18 to 2.27) p =0.003	Age at diagnosis, year of diagnosis, BMI, differentiation grade LINE-1 methylation, stage, KRAS and P1K3CA status
Maestro, 2007[96] Cohort 324 Medium	HR: 1.62 (0.50 to 5.21) p=0.38	Age, sex, Duke's stage, tumor site, differentiation grade, histology, adjuvant chemotherapy, MSI, and *BRAF*
Phipps, 2012[99] Cohort 1980 Medium	HR: 1.43 (1.05 to 1.95) p=NR	Age at diagnosis, sex, time from diagnosis to enrollment, SEER stage, MSI status
Samadder, 2013[106] Cohort 563 Medium	HR: 1.23 (0.78 to 1.94) p=0.38	Age at diagnosis, anatomic subsite, tumor grade, SEER stage, chemotherapy exposure, and radiation therapy exposure

Abbreviations: *BRAF* = gene name; CEA = carcinoembryonic antigen; CI = confidence interval; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; ng/mL = nanograms/milliliter; NR = not reported; p = probability; SEER = Surveillance, Epidemiology, and End Results.

Figure 11. Prognostic value of *BRAF* mutation testing for cancer-specific survival in colorectal cancer, meta-analysis of adjusted hazard ratios



BRAF for Colorectal Cancer: Overall Survival

Eleven studies with twelve cohorts rated as low or medium RoB reported on prognostic value of *BRAF* for OS in patients with CRC[92,94,95,97,99,100,102,103,105-107] (total N=7,610) (Table 24).

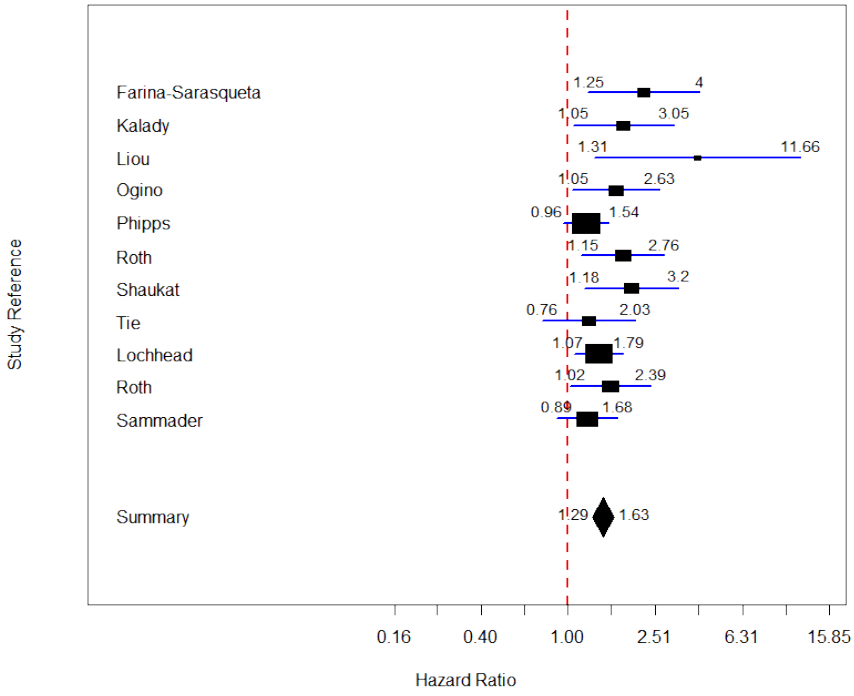
Nine out of twelve cohorts reported significant associations between *BRAF* mutation status and survival. The summary HR for overall survival used a fixed effects model for patients with wild-type compared with those with *BRAF* mutations of 1.45 (1.3 to 1.62) (Figure 12).

Table 24. Colorectal cancer: *BRAF* overall survival

Author, Year, Design, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Farina-Sarasqueta, 2010[92] Cohort 364 Low	*BRAF* HR: 2.22 (1.25 to 4) p<0.05	Differentiation grade, age, sex, tumor location, tumor stage, node stage, *KRAS* status, *BRAF* status, and MSI status
Kalady, 2012[94] Cohort 475 Medium	HR: 1.79 (1.05 to 3.05) p=0.031	*BRAF* mutation, MSI status, TNM stage, sex, age (>55 years), differentiation, proximal tumor site
Liou, 2011[95] Cohort 314 Medium	HR: 3.91 (1.31 to 11.66) p=0.014	Age, sex, stage, lymphatic invasion, venous invasion, tumor differentiation, CEA level (>5 vs. ≤5 ng/mL)
Lochhead, 2012[105] Cohort 1,253 Medium	*BRAF*-Mut HR: 1.38 (1.07-1.79) p = 0.013	Age at diagnosis, Year of diagnosis, BMI, differentiation grade LINE-1 methylation, stage, KRAS and P1K3CA status
Ogino, 2012[97] Cohort 506 Medium	HR: 1.66 (1.05 to 2.63) p=NR	Age, sex, baseline BMI, family history of CRC in first-degree relatives, performance status, presence or absence of perforation and/or obstruction at surgery, treatment arm, tumor location, T and N stage, *KRAS* status, MSI status
Phipps, 2012[99] Cohort 1980 Medium	HR: 1.21 (0.96 to1.54) p=NR	Age at diagnosis, sex, time from diagnosis to enrollment, SEER stage, MSI status
Roth, 2010[100] Cohort 1307 Medium	All subjects HR: 1.78 (1.15 to 2.76) p=0.010 Stage II subjects: HR: 3.83 (1.09 to 13.5) p=0.036 Stage III subjects: HR: 1.67 (1.04 to 2.68) p=0.035	Treatment arm, age, sex, grade, tumor site, nodal stage, MSI, *KRAS*, *BRAF* mutation status
Roth, 2012[107] Cohort 1404 Medium	HR: 1.56 (1.02 to 2.39) p=0.04	Age, sex, grade, stage, number of examined lymph nodes, site, treatment group, MSI status, SMAD4 expression, 18qLOH status, and KRAS
Samadder, 2013[106] Cohort 563 Medium	HR: 1.22 (0.89 to 1.68) p=0.21	Age at diagnosis, anatomic subsite, tumor grade, SEER stage, chemotherapy exposure, and radiation therapy exposure
Shaukat, 2010[102] Cohort 194 Medium	HR: 1.95 (1.18 to 3.20) p=0.008	Age, tumor grade, TNM (tumor-node-metastasis) stage, and MSI status
Tie, 2011[103] Cohort 503 Medium	*BRAF* mutant for Stages I–III HR: 1.24 (0.76 to 2.03) p=0.40	Age, gender, stage, tumor site, use of adjuvant therapy

Abbreviations: BMI = body mass index; *BRAF*= gene name; CEA = carcinoembryonic antigen; CI = confidence interval; HR= hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; NR = not reported; p = probability; SEER = Surveillance, Epidemiology, and End Results; TNM= tumor, node, metastases.

Figure 12. Prognostic value of *BRAF* mutation testing for overall survival in colorectal cancer, meta-analysis of adjusted hazard ratios



Colorectal Cancer: *KRAS* Mutation

Characteristics of Included Studies Assessing the Clinical Validity of *KRAS* Mutation Testing

The genetic test for *KRAS* mutations described here checks for mutations in codons 12 and 13 in the *KRAS* gene. We included 14 cohort studies[92,95,98,100,103,106-114] that examined the effect of *KRAS* mutations on the RR, CSS, or OS in patients with CRC (Table 25). Sample sizes ranged from 135 to 1,404. Median followup was between 3 to 12 years. Studies were conducted in Australia, China, various European countries, and the United States.

Table 25. Characteristics of included studies: *KRAS* mutation testing for colorectal cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Bazan, 2005[109] Cohort Medium	160 Median (range): 71 (34 to 115) months	Italy	Duke's stages A–D	*KRAS*-WT *KRAS*-MUT	NR NR	Median (range): 66 (31 to 88) 52.5%
Deschoolmeester, 2010[110] Cohort Unclear	164 Median: 242 weeks (overall survival): Median: 232 weeks (disease-free survival)	Belgium	I–IV	*KRAS*-WT *KRAS*-MUT	NR NR	Median: 65 +/− 13 48.8%
Eklof, 2013[108] Cohort Medium	197 (NSHDS) 414 (CRUMS) Median (CRUMS): 113 months Median (NSHDS): 102 months	Sweden	I-IV	*KRAS*-MUT *KRAS*-WT	NR	NR 43.7% (CRUMS) 56.9% (NSHDS)
Farina-Sarasqueta, 2010[92] Cohort Low	364 Stage II median (range): 239.1 (0–473.9) weeks Stage III median (range): 200 (8.7–578.3) weeks	The Netherlands	II–III	*KRAS*-WT *KRAS*-MUT	NR NR	Stage II mean age: 71.5 Stage III mean age: 62.5 45.6%
Geido, 2002[111] Cohort Low	135 Mean (range): 72 (3 to103) months	Italy	Duke's Stages A–D Sporadic CRC: proximal (i.e., cecum, ascending and transverse colon sites) and distal (i.e., descending and sigmoid colon and rectum)	*KRAS*-WT *KRAS*-MUT *KRAS*-MUT (G-->A) *KRAS*-MUT (G-->C/T)	NR NR	Median: 67 44%
Imamura, 2012[112] Cohort High	1075 Median (IQR): 611 (433 to 840) weeks	United States	I–IV	*KRAS*-WT *KRAS*-MUT codon 12 *KRAS*-MUT codon 13	NR NR	Mean (SD): 68.5 (8.7) 55%

Table 25. Characteristics of included studies: *KRAS* mutation testing for colorectal cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Li, 2012[114] Cohort Medium	78 Max FU was 43 months; Min was 12	China	I-IV	KRAS-WT KRAS-MUT	NR	Overall NR: Range: 28-70y 44.9% overall KRAS-WT:74.3% KRAS-MUT:25.7%
Liou, 2011[95] Cohort Medium	314 Median (range) 57 (49–82) months	China	I–IV	*KRAS*-WT *KRAS*-MUT	NR NR	*KRAS*-WT mean (SD): 64.8 (12.7) *KRAS*-MUT mean (SD): 65.7 (13.1) *KRAS*-WT: 42.6% *KRAS*-MUT: 57%
Ogino, 2009[113]; Cohort Medium	508 Median: 323.6 weeks	United States	III	*KRAS*-WT *KRAS*-MUT	NR NR	Median (SD): 59.8 (11.5) 46%
Pai, 2012[98] Cohort High	181 Median (range): 12 (0 to 76) months	United States	I–IV	*KRAS*-MUT *KRAS* WT	NR NR	Mean (range): 67 (33 to 98) 47%
Roth, 2012[107] Cohort Medium	1404 Median: 69 months	Switzerland	II-III	*KRAS*-MUT *KRAS*-WT	NR	Median (range): 60 (21-76) 42.8%
Roth, 2010[100] Cohort Medium	1,299 Median: 296 weeks	Switzerland, Belgium, Italy, Hungary, Spain, France, and United Kingdom	II–III	*KRAS*-WT *KRAS*-MUT	NR NR	NR 42.3%
Samadder, 2013[106] Cohort Medium	563 NR	United States	I-IV	*KRAS*-MUT *KRAS*-WT	NR	Mean (SD) [age at diagnosis]: 73.9 (5.92) 100%

Table 25. Characteristics of included studies: *KRAS* mutation testing for colorectal cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Tie, 2011[103] Cohort Medium	510 Median 38.5 months (recurrence) Median 12.9 months (overall survival)	Australia	I–IV	*KRAS*-WT *KRAS*-MUT	NR	*KRAS*-WT median: 70.7 *KRAS*-MUT median: 70.4 *KRAS*-WT: 68% *KRAS*-MUT: 32%

Abbreviations: IQR = interquartile range; *KRAS* = gene name; MUT = mutation; N = number; NR = not reported; SD = standard deviation; WT = wild-type.

All studies compared outcomes of patients with *KRAS* wild-type with those who had *KRAS* mutations. Some also looked at mutations in codon 13 and the mutation from G to C/t and G to A. The representation of female patients in the studies ranged from 42 percent to 55 percent. Race or ethnicity was not reported in any of the studies.

Populations represented in the studies varied somewhat. The differences were both in cancer stage and the inclusion or exclusion of rectal cancers. Five studies[103,107-112,114] included all stages and colon and rectal cancers. Among the rest, one[95] included all stages of colon (not rectal) cancers, another[92] examined a sample of stage II and stage III colon cancers, and Maestro[96] included stages I to III and both colon and rectal cancers. Ogino[97] had only stage three colon cancer patients, Pai[98] studied proximal colon stages I through IV, and Roth[100] included stage II and III colon cancer.

KRAS for Colorectal Cancer: Risk of Recurrence

Five cohort studies (total N=4,085) rated as low or medium RoB[92,100,103,107,113] examined RR as an outcome (Table 26). Farina[92] defined DFS as time to recurrence of disease or distant metastasis; Ogino[113] defined DFS as time to disease recurrence or distant metastasis or death from any cause; Roth[100] did not define their outcome of relapse-free survival but presumably included loco-regional and distant recurrence; and Tie[103] defined their outcome as recurrence of CRC, which presumably means loco-regional and distant recurrence.

Table 26. Colorectal cancer: *KRAS* risk of cancer recurrence/disease-free survival/recurrence-free survival

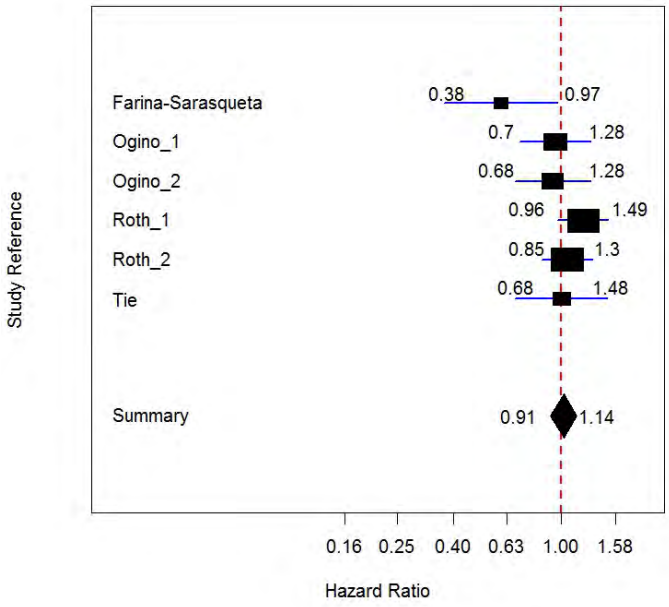
Author, Year, Design, N, Test (Cancer), Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Farina-Sarasqueta, 2010[92] Cohort 364 Low	DFS HR: 0.6 (0.38 to 0.97) p=NR	Differentiation grade, age, sex, tumor location, tumor stage, node stage, *KRAS* status, *BRAF* status, and MSI status
Ogino, 2009[113] Cohort 508 Medium	DFS: HR: 0.95 (0.70 to 1.28) p=NR RFS: HR: 0.93 (0.68 to 1.28) p=NR	Age, sex, BMI, tumor location, tumor and node stage, presence or absence of perforation and/or obstruction at diagnosis, performance status, MSI status, and treatment arm
Roth, 2010[100] Cohort 1,299 Medium	RFS All subjects HR:1.20 (0.96 to 1.49) p=0.11 Stage II subjects HR: 1.26 (0.75 to 2.13) p=0.38 Stage III subjects HR: 1.21 (0.95 to 1.54) p=0.12	Treatment arm, age, sex, grade, tumor site, nodal stage, MSI, *KRAS*, *BRAF* mutation status
Roth, 2012[107] Cohort 1404 Medium	RFS HR: 1.05 (0.85 to 1.30) p=0.65	Age, sex, grade, stage, number of examined lymph nodes, site, treatment group, MSI status, SMAD4 expression, 18qLOH status, and BRAF
Tie, 2011[103] Cohort 510 Medium	RR HR: 1.00 (0.68 to 1.48) p=1.00	Age, gender, tumor site, stage

Abbreviations: BMI = body mass index; *BRAF* = gene name; CI = confidence interval; DFS = disease-free survival; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; N = number; p = probability; RFS = recurrence-free survival.

The included studies reported on the prognostic value of testing for *KRAS* after controlling for certain traditional prognostic factors such as stage, sex, and age. Three out of the four studies reported no statistically significant association between *KRAS* and prognosis in adjusted analyses. Farina reported a significant association between the *KRAS* wild-type and improved survival.

Our meta-analysis found no significant added prognostic value of *KRAS* mutation testing for RR in patients with CRC HR, 1.02 (95% CI, 0.91 to 1.14) (Figure 13).

Figure 13. Prognostic value of *KRAS* mutation testing for risk of recurrence in colorectal cancer, meta–analysis of adjusted hazard ratios



KRAS for Colorectal Cancer: Cancer-Specific Mortality

We found two studies (N = 1,174) that looked at the prognostic effect of KRAS mutation on CSS. Eklof[108] examined the effect of KRAS on two different populations; the Colorectal Cancer in Umea Study (CRUMS) and the Northern Sweden Health Disease Study (NSHDS). They report a significant HR in CRUMS and a nonsignificant HR in the NHSDS. Sammader reports a nonsignificant HR (Table 27).

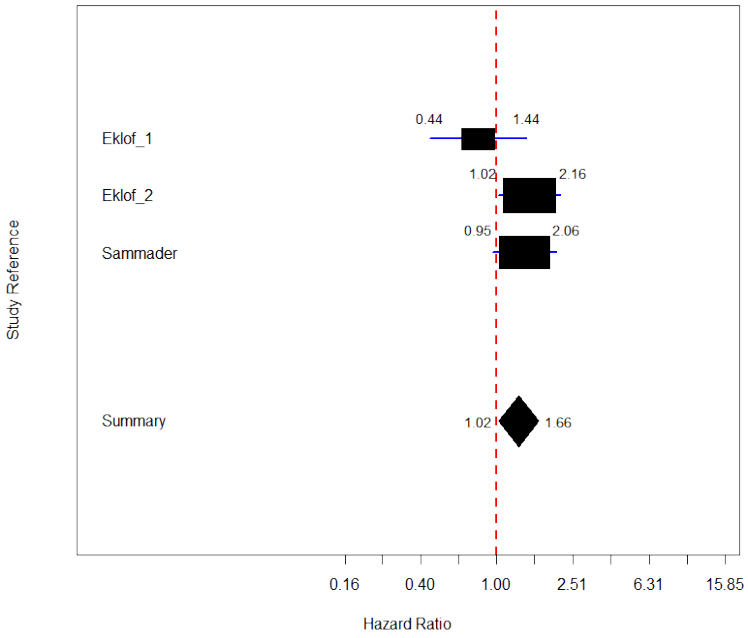
Our meta-analysis found that KRAS mutation has a significant prognostic effect on CSS with a HR of 1.30 (95% CI, 1.02 to 1.66) (Figure 14).

Table 27. Colorectal cancer: *KRAS* cancer-specific survival

Author, Year, Design, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Eklof, 2013[108] Cohort 197 (NSHDS) 414 (CRUMS) Medium	(NSDHS, N = 179) HR: 0.798 (0.443 to1.438) p=NR (CRUMS, N = 378) HR: 1.485 (1.023 to 2.15 Colorectal Cancer in Umeå Study (CRUMS)5) p=NR	Sex, age at diagnosis, stage, tumor site
Samadder, 2013[106] Cohort 563 Medium	HR: 1.40 (0.95 to 2.06) p=.08	Age at diagnosis, anatomic subsite, tumor grade, SEER stage, chemotherapy exposure, and radiation therapy exposure

Abbreviations: CRUMS = Colorectal Cancer in Umea Study; HR = hazard ratio; *KRAS* = gene name; N = number; p = probability. NSHDS= Northern Sweden Health Disease Study

Figure 14. Prognostic value of *KRAS* mutation testing for CSS in colorectal cancer, meta–analysis of adjusted hazard ratios



KRAS for Colorectal Cancer: Overall Survival

Ten cohort studies with low or medium RoB[92,95,100,103,106,107,109,111,113,114] (total N=5,328) examined overall survival as an outcome (Table 28). The included studies reported on the prognostic value of testing for *KRAS* after controlling for certain traditional prognostic factors such as stage, sex, and age. Six out of seven studies reported no statistically significant association between *KRAS* mutation status and OS in adjusted analyses. Bazan and Geido[109,111] reported significant associations between *KRAS* gene mutation status and survival.

Our meta-analysis found no significant added prognostic value of *KRAS* mutation testing for OS in patients with CRC HR: 1.22 (0.95, 1.55) (Figure 15).

Table 28. Colorectal cancer: *KRAS* overall survival

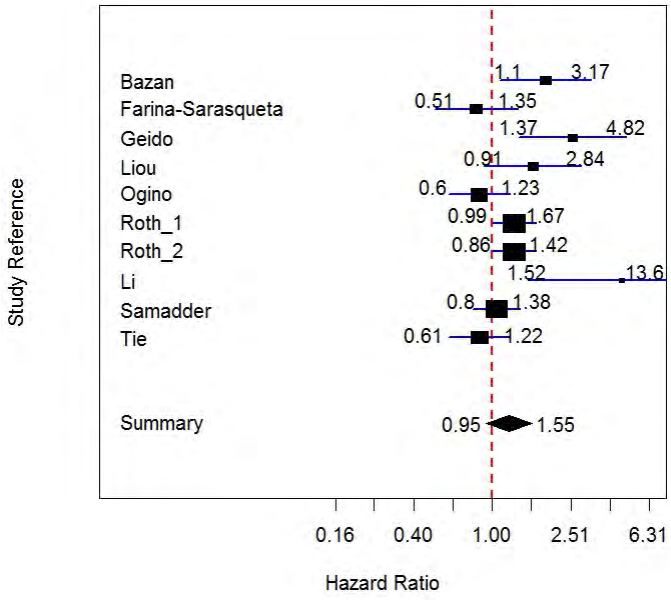
Author, Year, Design, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Bazan, 2005[109] Cohort MSI (colorectal) Medium	HR: 1.86 (1.10 to 3.17) p<0.05	Duke's stage, surgical resection, DNA-aneuploidy, high SPF
Farina-Sarasqueta, 2010[92] Cohort 364 Low	HR = 0.83(0.51 to 1.35) p=NR	Differentiation grade, age, sex, tumor location, T stage, nodal stage, *KRAS* status, *BRAF* status, and MSI status
Geido, 2002[111] Cohort 135 Low	Overall RR: 2.57 (1.37 to 4.82) p=0.003 G-A transition mutation subtype RR: 2.29 (1.12 to 4.66) p=0.02 G-C/T transversion mutation subtype RR: 3.34 (1.34 to 8.36) p=0.009	Model #1 (used to calculate RR of overall *KRAS* mutation): age, site, Duke's stages B and C, DNA ploidy, wild-type vs. mutated *KRAS*-2 status Model #2 (used to calculate RRs of G-->A and G-->C/T *KRAS* mutations): Same as Model #1, but with *KRAS* mutations subdivided into two specific types named above
Li, 2012[114] Cohort 78 Medium	KRAS mutation RR: 4.552 (1.522 -13.610) p=0.007	Sex, KRAS mutation, age, growth pattern, location of tumor, tumor differentiation, lymph node metastasis, liver metastasis, TNM stage
Liou, 2011[95] Cohort 314 Medium	HR: 1.61 (0.91 to 2.84) p=0.101	Age, sex, stage, use of adjuvant chemotherapy
Ogino, 2009[113] Cohort 508 Medium	HR: 0.86 (0.60 to 1.23) p=NR	Age, sex, BMI, tumor location, tumor and node stage, presence or absence of perforation and/or obstruction at diagnosis, performance status, MSI status, and treatment arm
Roth, 2010[100] Cohort 1,299 Medium	All subjects: HR: 1.29 (0.99 to 1.67) p=0.060 Stage II subjects: HR: 1.51 (0.79 to 2.90) p=0.21 Stage III subjects: HR:1.28 (0.97 to 1.71) p=0.086	Treatment arm, age, sex, grade, tumor site, nodal stage, MSI, *KRAS*, *BRAF* mutation status

Table 28. Colorectal cancer: *KRAS* overall survival (continued)

Author, Year, Design, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Roth, 2012[107] Cohort 1,404 Medium	HR: 1.10 (0.86 to 1.42) p=0.45	Age, sex, grade, stage, number of examined lymph nodes, site, treatment group, MSI status, SMAD4 expression, 18qLOH status, and BRAF
Samadder, 2013[106] Cohort 563 Medium	HR: 1.05 (0.80 to 1.38) p=0.70	Age at diagnosis, anatomic subsite, tumor grade, SEER stage, chemotherapy exposure, and radiation therapy exposure
Tie, 2011[103] Cohort 503 Medium	HR: 0.86 (0.61 to1.22) p=NR	Age, gender, tumor site, stage

Abbreviations: BMI = body mass index; *BRAF* = gene name; CI = confidence interval; DNA = deoxyribonucleic acid; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; NR = not reported; RR = relative risk; SPF = S-phase Fraction.

Figure 15. Prognostic value of *KRAS* mutation testing for overall survival in colorectal cancer, meta–analysis of adjusted hazard ratios



Colorectal Cancer: Microsatellite Instability (MSI)

Characteristics of Included Studies Assessing the Clinical Validity of MSI

The test for MSI described here uses the five reference microsatellite markers D5S345, D2S123, BAT25, BAT26, and D17S250 (Bethesda markers) to establish microsatellite instability. We included 17 cohort studies[96,103-107,115-125] that examined the added prognostic value of MSI for RR, CSS, or OS in patients with CRC (Table 29). Sample sizes ranged from 55 to 1,175. Median follow up ranged from 3 to 14 years. Studies were conducted in Australia, China, various European countries, Taiwan, and the United States.

Table 29. Characteristics of included studies: MSI for colorectal cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Chang, 2006[115] Cohort Medium	213 Median (range): 208.8 (17.4 to 334.8) weeks	China (Taiwan)	I–IV	MSI-H MSS	NR NR	Median (range): 67.6 (23 to 86)
Donada, 2011[116] Cohort Low	55 Median: 396.72 weeks, 25th–75th percentile=193.1 weeks to 443.5 weeks	Italy	II–III	MSI-H Non-MSI-H	NR NR	Mean (SD) (range): 62.9 (9.1) (35 to 79)
Guibodoni, 2001[117] Cohort Medium	109 Median Recurrence: 74 (50 to 120) months Overall survival: 78 (46 to 120) months	Italy	II–III	MSI-H MSS/MSI-L	NR NR	NR 58.7%
Gyrfe, 2000[118] Cohort Medium	587 Mean (SD): 7.2 (0.1) years	United States	T1–T4	MSI MSS	NR NR	Mean (SD): 43.1 (0.3) 50%
Hong, 2012[119] Cohort Medium	1,125 Median: 42 months	South Korea	I–IV	MSI MSS	NR NR	Median (range): 61 (25 to 92) 3.6%
Jensen, 2009[120] Cohort Medium	311 Median (range): 6.1 (4.1 to 11.3) years	Denmark	II–IV	MSI MSS	NR NR	NR 48.8%
Kim, 2007[121] Cohort Low	542 Censored at 5 years	United States	Duke's Stages B or C	MSI-H MSS/MSI-L	NR NR	NR NR
Lin, 2012[122] Cohort Medium	709 Median (range): 204.9 (4 to 796) weeks	China	I–III	MSI-H MSS/MSI-L	NR NR	Mean (SD): 68.8 (11.5) 32.4%

Table 29. Characteristics of included studies: MSI for colorectal cancer (continued)

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Lochhead, 2012[105] Cohort Medium	1,253 Median:8.2 years, range(3.5-13.1)years	United States	I-IV	MSI-H MSS	NR NR	NR NR
Maestro, 2007[96] Cohort Medium	324 Median (IQR): 43 (27-63) months	Spain	Duke's Stages A–D	MSS MSI-L MSI-H	NR NR	Mean (range): 69.6 (30 to 95)
Roth, 2012[107] Cohort Medium	1,404 Median: 69 months	Switzerland	II-III	MSI-H MS-L/S	NR	Median (range): 60 (21-76) 42.8%
Samadder, 2013[106] Cohort Medium	563 Mean(SD) 73.9 (5.92)	United States	Grade I-IV	MSS MSI-L MSI-H	NR NR NR	NR NR NR
Shia, 2008[123] Cohort High	130 Median (range): 98 (12 to 168) months	United States	I–IV	MSI-H Non-MSI-H	NR NR	Median (range): 65.5 (31 to 85) 47.7%
Soreide, 2009[124] Cohort Medium	186 Range: 291.45 to 495.9 weeks	Norway	I–III	MSS MSI-H	NR NR	Median (IQR): 67 (59 to 73)
Tie, 2011[103] Cohort Medium	503 Recurrence: 38.5 months Cancer specific survival: 12.9 months	Australia	I–IV	MSI MSS	NR NR	MSI median: 75.8 MSS median: 69.4 MSI: 15% MSS: 85%
Yoon, 2011[125] Cohort Low	2,028 43 (1 to 85) months	Korea	II–III	MSI-H MSI-L MSS	NR NR	Mean (SD): 56.7 (12) MSI-H: 10% MSI-L: NR MSS: 90%
Zlobec, 2010[104] Cohort Medium	404 Median: 234.8 weeks (all patients) Median: 260.9 weeks (cancer-specific survival)	Switzerland	T1–T4	MSI-H MSS/MSI-L	NR NR	Mean (range): 69.5 (40 to 95) 53.6%

Abbreviations: IQR = interquartile range; MSI = microsatellite instability; MSI-H = microsatellite instability high; MSS = microsatellite stability; N = number; NR = not reported; SD = standard deviation; WT = wild-type.

All studies compared outcomes of patients with MSI-H with those with MSS/MSI-L. The representation of female patients in the studies where sex is reported ranged from 3.6 percent to 53 percent. Race or ethnicity was not reported in any of the studies.

There were some differences in the populations examined in the different publications. The differences were with respect to stage and whether rectal cancers were included. Eight studies[103-106,115,118,119,123] included patients with colon and rectal cancer at all stages. Four studies[96,107,121,124] included patients with stage II or III colon or rectal cancers. Guidoboni[117] examined a sample of patients with stage I or II proximal colon cancer only; Jensen[120] included patients with stages II, III, and IV colon and rectal cancer; Lin[122] had a sample of only stage III colon cancer; and Yoon[125] included patients with colon and rectal cancer in stages I through III.

MSI for Colorectal Cancer: Risk of Recurrence

Ten cohort studies with low or medium RoB (total N=7,130)[103,107,115,117,119-122,124,125] examined RR as an outcome (Table 30). Several studies[103,115,117,122,125] had DFS or recurrence as an outcome but did not specify whether they were looking at loco-regional or distant recurrence. Hong[119] defined DFS as disease recurrence or progression; Jensen[120] looked at recurrence-free survival, which was defined as relapse of primary disease or death; Kim[121] had RFS as an outcome, which they defined as time to first occurrence of a tumor recurrence; and Soreido[124] looked at any recurrence and separately at loco-regional and distant metastasis.

Table 30. Colorectal cancer: MSI risk of recurrence

Author, Year, Design, N, Test (Cancer), Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Chang, 2006[115] Cohort 213 Medium	4 year DFS MSI-H vs. MSS HR: 0.49 (0.18 to 1.38) p=0.179	TNM stage, P53 mutation, CEA level, differentiation, MSI, location
Guidoboni, 2001[117] Cohort MSI (colorectal) 109 Medium	DFS HR: 0.32 (0.15 to 0.72) p=0.002	Sex, age as continuous, TNM stage
Hong, 2012[119] Cohort 1,125 Medium	DFS Overall HR: 0.57 (0.32 to 0.96) p=0.034 Colon HR: 0.61 (0.33 to 1.10) p=0.100 Rectum HR: 0.48 (0.12 to1.97) p=0.311	Stage, sex, age
Jensen, 2009[120] Cohort 311 Medium	RR HR: 0.3 (0.2 to 0.7) p=0.0007	Thymidylate synthase, dihydropyrimidine, gender, age, stage, differentiation, peripheral invasion, vascular invasion, perforation ileus

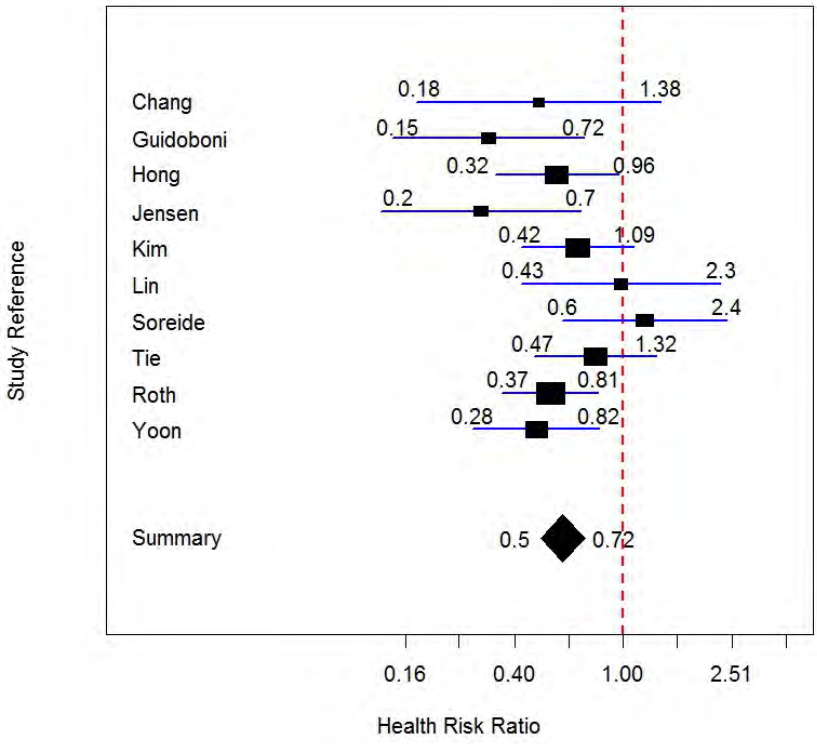
Table 30. Colorectal cancer: MSI risk of recurrence (continued)

Author, Year, Design, N, Test (Cancer), Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Kim, 2007[121] Cohort 542 Low	RR Overall: RR: 0.68 (0.42 to1.09) p=0.1 RR Untreated group: HR: 0.77 (0.40 to1.48) p=NR RR Treated group: HR: 0.60 (0.30 to 1.19) p=NR	Treatment and MSI/treatment interaction, age, sex, stage, tumor site
Lin, 2012[122] Cohort 709 Medium	DFS HR: 0.982 (0.425 to 2.268) p=0.965	Age, gender, stage, differentiation
Roth, 2012[107] Cohort 1404 Medium	RFS MSI-H vs. MS-L/S HR: 0.54 (0.37 to 0.81) p=0.003	Age, sex, grade, stage, number of examined lymph nodes, site, treatment group, SMAD4 expression, 18qLOH status, BRAF, and KRAS
Soreide, 2009[124] Cohort 186 Medium	LRR MSI adjusted for both age and sex OR: 2.5 (1.0 to 6.1) p=0.047 MSI adjusted for tumor location OR: 3.1 (1.1 to 8.8) p=0 .026 Any recurrence OR 1.2 (0.6 to 2.4) p=0.64	Age, sex, MSI and variables that had p-values <0.1 on univariate analysis
Tie, 2011[103] Cohort 503 Medium	DFS HR: 0.79 (0.47 to1.32) p=0.36	Age, gender, tumor site, stage
Yoon, 2011[125] Cohort 2,028 Low	DFS MSI-H: HR: 0.48 (0.28 to 0.82) p=0.007	Age, location, gender, CEA, node, distant metastases, histology, lympho-vascular invasion, growth type, adjuvant

Abbreviations: CEA = carcinoembryonic antigen; CI = confidence interval; DFS = disease-free survival; HR = hazard ratio; LRR = loco-regional recurrence; MSI = microsatellite instability; MSI-H = microsatellite instability high; MSI-L = microsatellite instability low; MSS = microsatellite stability; N = number; NR = not reported; OR = odds ratio; p = probability; RR = relative risk; TNM = tumor, node, metastases.

The included studies reported on the prognostic value of testing for MSI after controlling for certain traditional prognostic factors such as stage, sex, and age. All but two studies reported point estimates, suggesting an association between MSI-H and lower RR. Four out of nine studies reported statistically significant associations between MSI and RR in adjusted analyses—finding that MSI-H was associated with better prognosis. Our meta-analysis found a lower RR for the patients with MSI-H compared with those with MSS (HR, 0.60; 95% CI, 0.50 to 0.72) (Figure 16).

Figure 16. Prognostic value of microsatellite instability for risk of recurrence in colorectal cancer, meta-analysis of adjusted hazard ratios



MSI for Colorectal Cancer: Cancer-Specific Survival

Six cohort studies rated as medium RoB[96,104-106,122,124] (total N =3,439) reported on CSS as an outcome (Table 31).

Table 31. Colorectal cancer: MSI cancer-specific mortality

Author, Year, Design, N, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Lin, 2012[122] Cohort 709 Medium	HR: 0.891 (0.363 to 2.183) p=0.8	Age, sex, stage, differentiation, MSI status
Lochhead, 2013[105] Cohort 1253 Medium	MSI-H vs. MSS HR: 0.28 (0.17-0.46) p<0.001	Age, year of diagnosis, BMI, tumor differentiation LINE-1 methylation),disease stage. KRAS and P1K3CA status
Maestro, 2007[96] Cohort 324 Medium	MSI-L vs. MSS HR: 0.72 (0.29 to 1.80) p=NR MSI-H vs. MSS HR: 0.33 (0.12 to 0.92) p=NR Any MSI vs. MSS: p=0.036	Age, sex, Duke's stage, tumor site, differentiation grade, histology, adjuvant chemotherapy, MSI, and *BRAF*

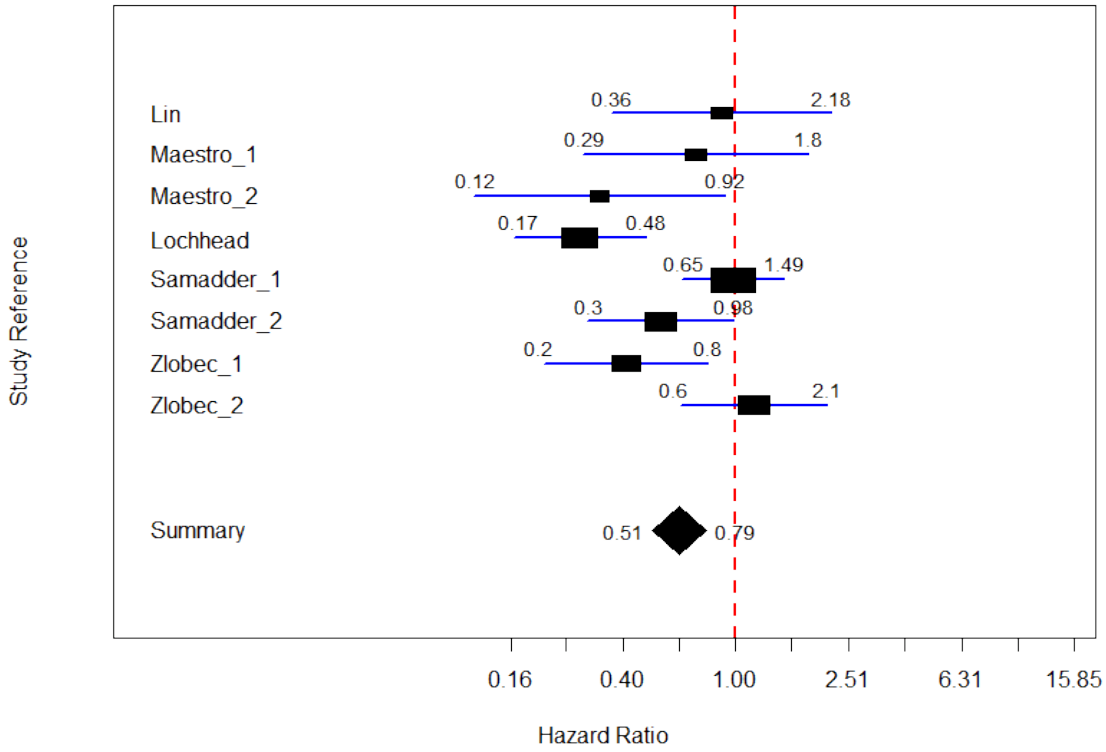
Table 31. Colorectal cancer: MSI cancer-specific mortality (continued)

Author, Year, Design, N, Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Samadder, 2013[106] 2013 Cohort 563 Medium	MSI –L HR: 0.98 (0.65-1.49) p=.08 MSI-H HR: 0.54 (0.30-0.98) p=.08	Age at diagnosis, anatomic subsite, tumor grade, SEER stage, chemotherapy exposure and radiation therapy exposure
Soreide, 2009[124] Cohort 186 Medium	HR: NR (NR) p=0.722	Age, sex, MSI status, and variables that had p-values <0.1 on univariate analysis
Zlobec, 2010[104] Cohort 404 Medium	Right-sided location MSI-H HR: 0.41 (0.2 to 0.8) p=0.015 Left-sided location MSI-H HR: 1.16 (0.6 to 2.1) p=0.622	pT stage, pN stage, vascular invasion and MSI in right- and left-sided CRC

Abbreviations: *BRAF* = gene name; CI = confidence interval; CRC = colorectal cancer; HR = hazard ratio; MSI = microsatellite instability; MSI-H = microsatellite instability high; MSI-L = microsatellite instability low; MSS = microsatellite stability; NR = not reported; p = probability; pN = pathological node score; pT = pathological tumor score.

The included studies reported on the prognostic value of testing for MSI after controlling for certain traditional prognostic factors such as stage, sex, and age. Two of the four studies reported a statistically significant association between MSI and prognosis of CSS in adjusted analyses. Zlobec[104] reported a positive association (HR 0.41 [0.2 to 0.8]) between MSI-H for right-sided tumors for CSS. Maestro[96] reported a significant HR association between MSI-H and CSS (HR 0.33 [0.12, 0.92]). Our meta-analysis found an overall HR for CSS for patients with MSI-H tumors compared with MSS tumors of 0.63; 95% CI, (0.51 to 0.79) (Figure 17).

Figure 17. Prognostic value of microsatellite instability for cancer specific survival in colorectal cancer, meta-analysis of adjusted hazard ratios



MSI for Colorectal Cancer: Overall Survival

Twelve cohort studies[103,105,107,115-122,125] (total N =8,839) rated as low or medium RoB examined overall survival (Table 32). Populations examined in the different publications varied somewhat.

The included studies reported on the prognostic value of testing for MSI after controlling for certain traditional prognostic factors such as stage, sex, and age. Four out of 10 studies reported a statistically significant association between MSI and prognosis for OS in adjusted analyses. All 4 found that MSI-H is a good prognostic marker for OS.

Our meta-analysis found an overall HR for OS for patients with MSI-H compared with MSS of 0.57; 95% CI (0.43 to 0.77) (Figure 18).

Table 32. Colorectal cancer: Overall survival with MSI

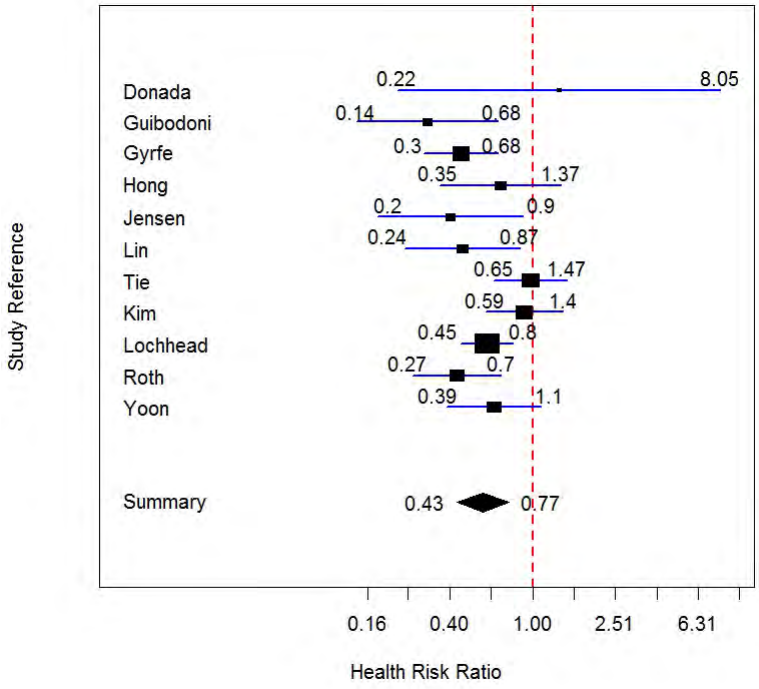
Author, Year, Design, N Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Chang, 2006[115] Cohort 213 Medium	4-year OS HR: NR MSI-H vs. MSS: p=0.858	TNM (I, II, III, IV), p53 mutation (yes vs. no), CEA level 2 (high vs. low), grade of differentiation (poor vs. well/moderate), MSI status (MSI-H vs. MSS), location (right, left, rectum)
Donada, 2011[116] Cohort 55 Low	MSI-H vs. non-MSI-H HR: 1.34 (0.22 to 8.05) p=0.75	Age, sex, tumor location (left-transverse-right), stage (III vs. II), grading (G3-G2-G1), TS expression (high vs. low), TP expression (high vs. low), DPD expression (high vs. low), MSI status (MSI-H vs. non-MSI-H)
Kim, 2007[121] Cohort 542 Low	MSI-H vs. MSS/MSI-L Stratified by treatment RR=0.91 (0.59, 1.4) Treated group HR=1.02 (0.56 to 1.85) Untreated group HR=0.82 (0.44 to 1.51)	Stratified by treatment: surgery alone vs. surgery + chemotherapy Age, sex, stage, tumor site
Guibodoni, 2001[117] Cohort 109 Medium	HR: 0.31 (0.14 to 0.68) p=NR	Sex, age, TNM stage
Gyrfe, 2000[118] Cohort 587 Medium	MSI vs. MSS HR: 0.45 (0.30 to 0.68) p<0.0001	Lymph-node metastases, microsatellite status (MSS, MSI); tumor invasion (T1-4); distant-organ metastases—microsatellite status (MSS, MSI); tumor invasion (T1–4)
Hong, 2012[119] Cohort 1125 Medium	Overall 0.7 (0.35 to 1.37) p=0.292 Colon 0.62 (0.29 to 1.4) Rectum 1.07 (0.26 to 1.4)	Stage, sex, age
Jensen, 2009[120] Cohort 311 Medium	HR: 0.4 (0.2 to 0.9) p=0.02	Microsatellite instability, thymidylate synthase , dihydropyrimidine dehydrogenase , sex, age, stage, differentiation, perineural invasion, vascular invasion, perforation, ileus
Lin, 2012[122] Cohort 709 Medium	HR: 0.459 (0.241 to 0.872) p=0.017	Age, sex, stage, differentiation (well to moderate vs. poor to undifferentiated), MSI status (MSI-H vs. MSI L/S)
Lochhead, 2013[105] Cohort 2,012 Medium	MSI-high vs. MSS HR: 0.60 (0.45-0.80) p<0.001	Age, year of diagnosis, BMI, tumor differentiation LINE-1 methylation, stage, KRAS and P1K3CA status
Roth, 2012[107] Cohort 1404 Medium	HR: 0.43 (0.27 to 0.70) p=0.001	Age, sex, grade, stage, number of examined lymph nodes, site, treatment group, SMAD4 expression, 18qLOH status, BRAF, and KRAS
Tie, 2011[103] Cohort 503 Medium	HR: 0.98 (0.65 to 1.47) p=0.92	Age, gender, tumor site, stage

Table 32. Colorectal cancer: Overall survival with MSI (continued)

Author, Year, Design, N Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Yoon, 2011[125] Cohort 2028 Low	HR: 0.65 (0.39 to1.10) p=0.11	Age, gender, location, MSI status, CEA, lymph node metastasis, distant metastasis, lymphovascular invasion, histology, adjuvant chemotherapy

Abbreviations: CEA = carcinoembryonic antigen; CI = confidence interval; DPD = dihydropyrimidine dehydrogenase; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; MSI-H = microsatellite instability high; MSI-L = microsatellite instability low; MSI-L/S = microsatellite instability low or stable; MSS = microsatellite stability; N = number; p = probability; RR = relative risk; TNM = tumor, node, metastases; TP = thymidine phosphorylase; vs. = versus.

Figure 18. Prognostic value of microsatellite instability for overall survival in colorectal cancer, meta-analysis of adjusted hazard ratios



Colorectal Cancer: Oncotype DX

Characteristics of Included Studies Assessing the Clinical Validity of Oncotype DX Colon

We included one cohort study[126] that examined the added prognostic value of Oncotype DX Colon for RR, CSS, or OS in patients with CRC (Table 33).

Table 33. Characteristics of included studies: Oncotype DX for colorectal cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age years) % Female
Venook 2013[126] Cohort Medium	690 NR	United States	II	Recurrent patients Nonrecurrent patients	White: 92% NR	NR 41.9%

Abbreviations: N = number; NR = not reported.

Oncotype DX: Risk of Recurrence

One included cohort study rated as medium RoB (N=690) reported this outcome.[126] The study examined a 12-gene RS as the outcome in stage II CRC patients who were part of the CALGB trial. All patients with recurrence and a random sample of patients with no recurrence were included in the study.

The study reported that the RS from Oncotype DX (treated as a continuous variable) was a significant predictor (HR 1.68; 95% CI, 1.18 to 2.39) after controlling for standard prognostic markers (Table 34).

Table 34. Colorectal cancer: Oncotype DX risk of cancer recurrence/disease-free survival/recurrence-free survival

Author, Year, Design, N, Test (Cancer), Risk of Bias	Adjusted Ratios, (95% CI) p-value	Variables Used in Multivariate Model
Venook 2013[126] Cohort 690 Medium	RS: HR: 68 (1.18 to 2.38) p=0.004	MMR, tumor state, number of nodes examined, grade , LVI, RS

Abbreviations: CI = confidence interval; HR = hazard ratio; LVI = lymphovascular invasion; MMR = mismatch repair; N = number; p = probability; RS = risk score.

Oncotype DX: Cancer-Specific Mortality

We found no studies meeting our inclusion criteria.

Oncotype DX: Overall Survival

We found no studies meeting our inclusion criteria.

Bladder Cancer: UroVysion

Characteristics of Included Studies Assessing the Clinical Validity of UroVysion

UroVysion is a fluorescence in situ hybridization (FISH) test of exfoliated urothelial cells that has been shown to be sensitive in terms of diagnosing urothelial cancer. It was designed as a diagnostic and not a prognostic test. In this report the test is being evaluated for its prognostic value with respect to predicting recurrence.

We included 3 studies[127-129] that examined the added prognostic value of UroVysion for RR, CSS, or OS in patients with bladder cancer (Table 35). Sample sizes ranged from 42 to 138. Studies were conducted in the United States and Switzerland. Race or ethnicity

was not reported in any of the studies. One study was rated as high RoB and was not included in our main data synthesis.[129]

Table 35. Characteristics of included studies: UroVysion for urinary bladder cancer

Author, Year Study Type Risk of Bias	N Length of Followup	Country(ies)	Disease Stage(s) Other Tumor/ Disease Characteristics	Study Groups	Race Ethnicity	Overall Age (years) % Female
Kamat, 2012[127] Cohort Low	126	United States	TA or T1 Nonmuscle invasive bladder cancer (primary or recurrent)	UroVysion+ UroVysion−	NR NR	Median: 67.5 24%
Whitson, 2009[128] Cohort Low	42 Median (range): 91.3 (21.7 to 230.5) weeks	United States	T1 High-risk superficial bladder tumor	UroVysion+ UroVysion−	NR NR	Median (range): 68 (41 to 97) NR
Zellweger, 2006[129] Cohort High	138 Median (range): 19.2 (4 to 52) months	Switzerland	Stage pTa to pT3; 9 tumors without information on stage were also included	UroVysion+ UroVysion−	NR NR	Median (range): 68.5 (33.4 to 92.5) 15.9%

Abbreviations = N = number; NR = not reported.

UroVysion: Risk of recurrence

Two cohort studies (total N = 168) reported on UroVysion and RR. Whitson[128] retrospectively studied patients who underwent intravesical therapy (IVT), and Kamat[127] prospectively enrolled patients who were scheduled to undergo IVT. Both studies reported that the test is a significant predictor after controlling for traditional prognostic factors (Table 36).

Table 36. Key Question 3: Bladder cancer: UroVysion risk of cancer recurrence/disease-free survival/recurrence-free survival

Author, Year, Design, N, Risk of Bias	Adjusted Ratio (95% CI), p-value	Variables Used in Multivariate Model
Kamat, 2012[127] Cohort 126 Low	HR: NR p<0.01	Stage, grade, history of prior tumors, concomitant CIS, use of postoperative mitomycin
Whitson, 2009[128] Cohort 42 Low	Multivariate HR: 6.7 (2.1 to 22.1) p<0.01	Age, smoking, radiotherapy, diabetes mellitus, hypertension, hyperlipidemia, CRI, immunosuppression, recurrence rate, whether the tumor was primary or recurrent, multiple tumors, tumor >3 cm, stage T1, high grade, CIS, EORTC score >6, positive cytology, and positive UroVysion

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; cm = centimeter; CRI = chronic renal insufficiency; EORTC = European Organization for Research and Treatment of Cancer; HR = hazard ratio; N = number; p = probability.

UroVysion Cancer-Specific Survival

We found no studies meeting our inclusion criteria.

UroVysion Overall Survival

We found no studies meeting our inclusion criteria.

Key Question 4a: Influence on Physician Decision

We identified studies evaluating the influence of molecular pathology tests for RR on treatment decisionmaking for three of the tests of interest, the Oncotype DX and MammaPrint assays for breast cancer, and Oncotype DX Colon. For Oncotype DX Colon, none of the included studies were rated as low or medium RoB. For Oncotype DX Breast and MammaPrint, the studies available for review included a mix of prospective and retrospective cohort studies and uncontrolled trial designs, with a larger body of literature examining the use of Oncotype DX than MammaPrint. No studies examined the effect of the two assays compared with each other.

Characteristics of Included Studies Assessing Oncotype DX Breast and Treatment Decisions

For Oncotype DX Breast, we included six cohort studies (total N=990 patients) and ten uncontrolled trials (total N=1,261 patients) rated as low or medium RoB (Table 37). One additional uncontrolled trial[130,131] was excluded from our main analysis because of an unclear RoB rating and is not included in this summary. Most of the studies were performed at single academic centers or in multicenter studies in the United States, but studies performed at centers in Europe, Canada, Australia, Mexico, Japan and Israel were also found. The studies uniformly examined cohorts with hormone-receptor positive breast cancer, and most were limited to women with node-negative cancers. The mean age of patients in the studies varied from 48 to 61 years.

Table 37. Characteristics of included studies: Oncotype DX Breast and treatment decisions

Author, Year Test (Cancer Type) Risk of Bias	N Study Type	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Overall Age (years) % Female
Ademuyiwa, 2011[132] Oncotype DX (breast) Medium	276 patients 2 oncologists Pre-post-test design and uncontrolled trial	United States	Node negative, ER+, HER2/neu−	Median: 55; Mean (range): 54.8 (29 to 82) 100%
Albanell, 2011[133] Oncotype DX (breast) Low	107 Uncontrolled trial	Spain	Node negative, ER+, HER2−	NR 100%
Bargallo, 2012[134] Oncotype DX (breast) Medium	96[a] Uncontrolled trial	Mexico	ER+, HER2-, stage I-IIIa, node-negative or –positive (1-3 nodes)	NR 100%
Davidson, 2013[135] Oncotype DX (breast) Medium	150 Uncontrolled trial	Canada	I–II ER+, HER2−, node negative	Mean (range): 53 (23 to 78) 100%

Table 37. Characteristics of included studies: Oncotype DX Breast and treatment decisions (continued)

Author, Year Test (Cancer Type) Risk of Bias	N Study Type	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Overall Age (years) % Female
de Boer, 2013[136] Oncotype DX (breast) Medium	151 Uncontrolled trial	Australia	Early stage, T1 – T3 Unifocal ER+/-, PR+/-, HER2− , node negative or node positive (1–3 positive nodes, including micrometastases and isolated tumor cells)	Mean: 56.2 100%
Eiermann, 2013[137] Oncotype DX (breast) Low	366 Cohort	Germany	Early breast cancer ER+, HER2−, node negative or histologically verified lymph node metastases in up to three lymph nodes	Mean: 56 100%
Geffen, 2011[138] Oncotype DX (breast) Medium	135 Cohort	Israel	I–II ER+ or ER−, PR+ or PR−, HER2+ or HER−	Median 58 (33–75) years 97.8%
Gligorov, 2012[130] Oncotype DX (breast) Unclear	92 patients NR oncologists Uncontrolled trial	France	ER+, HER2−, node negative or pN1 (mi) breast cancer	NR NR
Henry, 2009[139] Oncotype DX (breast) Low	29 Cohort	United States	I–II Node negative, ER+	Median (range): 51 (31 to 74) 96.5%
Holt, 2013[140] Oncotype DX (breast) Medium	142 Uncontrolled trial	United Kingdom	T1 – T3 ER+, node negative invasive breast cancer or minimal node involvement	Median (range): 55 (34 – 72) 100%
Joh, 2011[141] Oncotype DX (breast) Low	95 patients' records Survey participants: 4 surgical oncologists; 4 medical oncologists; 4 pathologists Pre-post-test design; Uncontrolled trial	United States	Early stage, ER-positive breast cancer	Median overall age of patients is NR Patients with RS<18: Median: 54 years; Mean (SD) 55.5 (1.1) Patients with RS 18–30: Median 51 years, Mean (SD) 53.4 (1.3) Patients with RS >30: Median 58.5, Mean (SD) 59.1 (3.3) 100%
Kamal, 2011[142] Oncotype DX (breast) Medium	31 Uncontrolled trial	United States	Early stage ER+, node negative	Median (range): 53 (42–82) NR
Klang, 2010[143] Oncotype DX (breast) Low	313 Cohort	Israel	NR	Mean (range): 57 (29–81) 99%
Lo, 2010[144] Oncotype DX (breast) Low	17 oncologists 89 patients Uncontrolled trial	United States	I-II ER+, node negative	Mean (range): 55 (35–77) 100%

Table 37. Characteristics of included studies: Oncotype DX Breast and treatment decisions (continued)

Author, Year Test (Cancer Type) Risk of Bias	N Study Type	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Overall Age (years) % Female
Rayhanabad, 2012[145] Oncotype DX (breast) Low	58 Cohort	United States	T1–T2 Node-negative, ER+	Mean (range): 54 (26–78)
Schneider, 2012[146] Oncotype DX (breast) Medium	89 Cohort	United States	ER+, node negative	NR 100%
Yamauchi, 2013[147] Oncotype DX (breast) Medium	124 patients 17 physicians Uncontrolled trial	Japan	Invasive early breast cancer ER+, HER2- 0 to 3 positive lymph nodes	Mean: 51.4 100%

[a]100 were enrolled; 96 were analyzed; 2 did not have assay results and outcome data were missing for 2.

Abbreviations: ER = estrogen receptor; HER = Human Epidermal Growth Factor; HER2 = Human Epidermal Growth Factor Receptor 2, also known as Neu; HR = hazard ratio; N = number; NR = not reported; PR = progesterone receptor; RS = risk score; SD: standard deviation.

Results: Oncotype DX and Treatment Decisions

Across studies, the percentage of patients whose recommendation for treatment was changed by the Oncotype test result was generally around 30 percent, ranging from 14 percent to 40 percent. Although treatment decision changes were observed in both directions for individual patients, all studies consistently showed a pattern of a shift to less-intensive treatment recommendations (more patients moving from chemotherapy plus hormonal therapy to hormonal therapy alone than from hormonal therapy to combination therapy) as a result of including the Oncotype score in decisionmaking information (Table 38).

One cohort study[139] also included a nested uncontrolled trial in which a panel of five experts provided treatment recommendations with and without the Oncotype score for patients included in the observational cohort; results were consistent with the other studies.

Table 38. Impact of test results on treatment decisions

Author, Year, Design, N, Test (Cancer), Risk of Bias	Decisions Based on Molecular Pathology Test Results Combined With Standard Prognostic Markers	Decisions Based on Standard Prognostic Markers Alone
Ademuyiwa, 2011[132] Pre-post-test design an uncontrolled trial 276 Oncotype DX (breast) Medium	ODX score OR: 1.23, 95% CI 1.16 to 1.29, p<0.001 Overall change: 38%	71/188 no CTX patients: recommended for CTX based on ODX blinded records review. 34/88 CTX patients; recommended for no CTX based on ODX blinded records review.

Table 38. Impact of test results on treatment decisions (continued)

Author, Year, Design, N, Test (Cancer), Risk of Bias	Decisions Based on Molecular Pathology Test Results Combined With Standard Prognostic Markers	Decisions Based on Standard Prognostic Markers Alone
Albanell, 2011[133] Uncontrolled trial 107 Oncotype DX (breast) Low	Overall change: 34 (32%; 95% CI 26% to 34%) CTX to no CTX: 22 (21%; 95% CI 19.6% to 21.6%) No CTX to CTX: 12 (11%; 95% CI 10.6% to 11.8%) In each group: No CTX to CTX: 12 of 68 (18%) CTX to no CTX: 22 of 39 (56%) CTX in each RS group: Total CTX: 29 of 107 (27%) Low RS: 2/62 (3%) Intermediate RS: 17/35 (49%) High RS: 10/10 (100%)	CTX: 39 (36%) No CTX: 68 (64%) CTX in each RS group: Low RS: 22 (35%) Intermediate RS: 9 (26%) High RS: 8 (80%)
Bargallo, 2012[134] Uncontrolled trial 96 Oncotype DX (breast) Medium	Overall change: 14% Proportion recommended CTX decreased from 48% to 34% (p=0.024). Decrease by subgroups: 6% decrease in N0 patients, and 26% in N_{mic} and N1-3 patients. Net decrease in chemotherapy recommendations: 13/96 = 14% (95% CI 7%-22%).	HT: 50 (52%) CHT: 46 (48%)
Davidson, 2013[135] Uncontrolled trial 150 Oncotype DX (breast) Medium	Overall change: 45 (30%) (95% CI: 22.8 to 38.0%) CTX to no CTX: 20% (95% CI 13.9 to 27.3%) No CTX to CTX: 10% (95% CI 5.7 to 16.0%) CTX in each RS group: Total CTX: 47 (31.3%) [percentage change overall=−10] Low RS: 0 [percentage change=−29] Intermediate RS: 13 [percentage change=−11] High RS: 34 [percentage change=+28]	CTX: 62 (41.3%) CTX in each RS group: Low RS: 20 Intermediate RS: 18 High RS: 24
De Boer, 2013[136] Uncontrolled trial 151 Oncotype DX (breast) Medium	Node negative: Overall change: 24% HTX to CTX+HTX: 12/71 (17%) CTX+HTX to HTX: 12/30 (40%) Node positive: Overall change: 26% HTX to CTX+HTX: 1/13 (8%) CTX+HTX to HTX: 12/37 (32%)	Node negative: Treatment plan pre-RS HTX=71 (70%) CTX+HTX=30 (30%) Node positive: Treatment plan pre-RS HTX=13 (26%) CTX+HTX=37 (74%)

Table 38. Impact of test results on treatment decisions (continued)

Author, Year, Design, N, Test (Cancer), Risk of Bias	Decisions Based on Molecular Pathology Test Results Combined With Standard Prognostic Markers	Decisions Based on Standard Prognostic Markers Alone
Eiermann, 2013[137] Cohort 366 Oncotype DX (breast) Low	Overall change: 33% 95 CI (28.3, 38.1); CTX=HTX to HTX: 22% HTX to CTX+HTX: 11% Other: 1% Node negative overall change: 30% change 95% CI (24.6, 36.5) CTX+HTX to HTX: 18% HTX to CTX+HTX: 12% CTX+HTX to CTX: 1 patient Node positive: Overall change: 39% 95% CI (29.9, 47.8); CTX+HTX to HTX: 28% HTX to CTX+HTX: 9% Observation to HTX or CTX+HTX: 2%	CTX+HTX overall: 209 (57%) CTX+HTX node negative: 48% CTX+HTX node positive: 75%
Geffen, 2011[138] Cohort 135 Oncotype DX (breast) Medium	Overall change: 34 (25.2%; 95% CI 17.9% to 32.5%) CTX to HTX: 24 (17.8%; 95% CI 11.3% to 24.2%) HTX to CTX: 10 (7.4%; 95% CI 2.9% to 11.8%) CTX in each risk group Low risk: 10% Intermediate risk: 55% High risk: 35% No CTX in each risk group Low risk: 72% Intermediate risk: 28% High risk: 0%	CTX: 63 (47%) No CTX: 72 (53%) CTX in each risk group: Low risk: 41% Intermediate risk: 41% High risk: 17% No CTX in each risk group Low risk: 57% Intermediate risk: 35% High risk: 8%
Henry, 2009[139] Cohort 29 Oncotype DX (breast) Low	Treating physicians: Overall change: 9 (31%) CTX to no CTX: 7 of 13 patients (54%) No CTX to CTX: 2 of 16 (13%) Expert panel: Overall change: 7 (24%) CTX to no CTX: 5 of 12 patients (42%) No CTX to CTX: 2 of 17 (12%)	Treating physicians: CTX: 13 (45%) HTX: 16 (55%) Expert panel: CTX: 12 (41%) HTX: 17 (59%)
Holt, 2013[140] Uncontrolled trial 142 Oncotype DX (breast) Medium	Overall change: 38 (26.76%) CTX + HT to HT: 26/57 (45.61%) HT to CTX+HT: 12/85 (14.12%)	Overall: CTX + HT: 57 (40.14%) HT: 85 (59.86%)

Table 38. Impact of test results on treatment decisions (continued)

Author, Year, Design, N, Test (Cancer), Risk of Bias	Decisions Based on Molecular Pathology TestResults Combined With Standard Prognostic Markers	Decisions Based on Standard Prognostic Markers Alone
Joh, 2011[141] Pre-post-test design and uncontrolled trial 95 patients' records Survey participants: 4 surgical oncologists, 4 medical oncologists, 4 pathologists Oncotype DX (breast) Low	Overall change: 24.9% % overtreated: 69.0% +/− 6.9% (range 5.9 to 85.7) p=0.0322 (compared with treatment decisions made with standard prognostic markers alone)	% Over treated: 82.3% +/− 1.3% (range 75.5 to 89.0)
Kamal, 2011[142] Uncontrolled trial 31 Oncotype DX (breast) Medium	Overall change: 19% of 31 cases changed 5 oncologists recommended chemotherapy in 10%–19% of 31 cases; 1 oncologist recommended chemotherapy in 58% of 31 cases Type of changes in chemotherapy recommendation (N=186 scenarios): No change, 151 (81.2%) CTX to no CTX: 20 (10.8%) No CTX to CTX: 15 (8.1%) Odds ratio of actual Oncotype DX RS changing chemotherapy recommendation: High vs. low: OR=82.650 (95% CI, 8.959 to 762.496) Intermediate vs. low: OR=6.463 (95% CI, 1.682 to 24.841)	5 oncologists recommended chemotherapy in 16% to 23% of the 31 cases; 1 oncologist recommended chemotherapy in 52% of 31 cases
Klang, 2010[143] Cohort 313 Oncotype DX (breast) Low	Overall change: 125 (40%) CTX to no CTX: 105 (34%)	CTX: 174 (56%)

Table 38. Impact of test results on treatment decisions (continued)

Author, Year, Design, N, Test (Cancer), Risk of Bias	Decisions Based on Molecular Pathology TestResults Combined With Standard Prognostic Markers	Decisions Based on Standard Prognostic Markers Alone
Lo, 2010[144] Uncontrolled trial 17 oncologists; 89 patients Oncotype DX (breast) Low	Overall change: 28 (31.5%) No CTX to no CTX: 40 (44.9%) No CTX to CTX: 3 (3.4%) CTX to no CTX: 20 (22.5%) CTX to CTX: 20 (22.5%) No CTX to equipoise: 3 (3.4%) CTX to equipoise: 2 (2.2%) Equipoise to equipoise: 1 (1.1%) HER2/neu-positive subset (N=6) CTX to no CTX: 1 (16.6%) CTX to CTX: 4 (66.7%) No CTX to no CTX: 1 (16.6%) (Note: equipoise is defined as equal options of either CTX, no CTX, or enrollment onto the TAILORx clinical trial, where patients would be randomly assigned between CTX followed by no CTX or no CTX alone)	CTX: 42 (47%), No CTX: 46 (51.7%) Either CTX or no CTX to one patient (1.1%)
Rayhanabad, 2012[145] Cohort 58 Oncotype DX (breast) Low	Overall change: 15/58 patients (26%) 23 (40%) low risk by RS 30 (51%) intermediate risk by RS 5 (9%) high risk by RS ODX increased the number of patients classified as low risk by 12% No CTX to CTX: 2/16 CTX to No CTX: 13/42	No CTX: 16 (28%) low risk by National Comprehensive Cancer Network CTX: 42 (72%) high risk by National Comprehensive Cancer Network
Schneider, 2012[146] Cohort 89 Oncotype DX (breast) Medium	Overall change: 45% CTX to HT: 39% HT to CTX: 4.5%	CTX: 61% HT: 39%

Table 38. Impact of test results on treatment decisions (continued)

Author, Year, Design, N, Test (Cancer), Risk of Bias	Decisions Based on Molecular Pathology Test Results Combined With Standard Prognostic Markers	Decisions Based on Standard Prognostic Markers Alone
Yamauchi, 2013[147] Uncontrolled trial 124 patients 17 physicians Oncotype DX (breast) Medium	Change in treatment recommendations among all patients: 47/124 patients (38%) Change in treatment recommendations for N0 patients: 34/104 patients (33%) Change in treatment recommendations for N+ patients: 13/20 patients (65%) CHT to HT: 40/63 patients (63%) CHT to HT in N0 patients: 27/48 patients (56%) CHT to HT in N+ patients: 13/15 patients (87%) HT to CHT: 7/61 patients (11%) HT to CHT in N0 patients: 7/56 patients (13%) HT to CHT in N+ patients: 0 patients (0%)	CHT: 63/124 patients (51%) HT: 61/124 patients (49%)

Abbreviations: CI = confidence interval; CHT = chemohormonal therapy; CTX = chemotherapy; HER2/neu = Human Epidermal Growth Factor Receptor 2, also known as Neu; HT = hormonal therapy; HTX = hormone treatment; N = number; NR = not reported; ODX = original diagnosis; RS= risk score; TAILORx = Tailored treatment; vs. = versus.

Characteristics of Included Studies Assessing MammaPrint and Treatment Decisions

We included one cohort study regarding decisionmaking (Table 39). This study was performed entirely in the Netherlands and included 427 women with a median age of 48 years and with node-negative invasive breast cancers. None of the patients were over 65 years of age. In the cohort study, physicians' treatment recommendations based on Dutch clinical guidelines were compared with recommendations based on the combination of clinical guidelines and the MammaPrint result.[148]

Table 39. Characteristics of included studies: MammaPrint and treatment decisions

Author, Year Test (Cancer Type) Risk of Bias	N Study Type	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Overall Age (years) % Female
Bueno-de-Mesquita, 2007[148] MammaPrint (breast) Low	427 Pre-post-test design an uncontrolled trial	The Netherlands	T I–IIII Node negative	Mean (SD): 48 (7); Median (range) 49 (27 to 60) 100%

Abbreviations: N = number; SD: standard deviation.

Results: MammaPrint and Treatment Decisions

The addition of the MammaPrint test result increased the percentage of patients recommended to receive some form of adjuvant therapy (either hormonal therapy, chemotherapy, or both) from 48 to 62 percent. However, the greatest increase was among women moving from no therapy to hormonal therapy. One significant limitation of this study for application to the U.S. population is that Dutch treatment guidelines during the study period were unusually restrictive in terms of indications for breast cancer adjuvant therapy compared with more widely accepted guidelines in the United States; specifically, women with hormone-receptor positive breast cancers would be uniformly recommended to receive hormonal therapy under U.S. guidelines.

Characteristics of Included Studies Assessing Oncotype DX Colon and Treatment Decisions

We identified three eligible studies (Table 40). Two were conducted in the United States and one in Israel. One cross-sectional survey assessed the treatment decisions of 116 physicians;[149] one uncontrolled trial assessed treatment decisions of 105 oncologists;[150] and one cohort study examined treatment recommendations before assay results compared with actual treatment received after assay results for 269 patients.[151] One study was rated as high RoB and two were rated as unclear RoB. Appendix C provides additional rationale for RoB ratings.

Table 40. Characteristics of included studies: Oncotype DX Colon and treatment decisions

Author, Year Test (Cancer Type) Risk of Bias	N Study Type	Country(ies)	Disease Stage(s) Other Tumor/Disease Characteristics	Overall Age (years) % Female
Brenner, 2013[151] Oncotype DX (colorectal) High	269 patients Cohort	Israel	II	NR (but reported 48% were at least 70 years old) NR
Cartwright, 2012[149] Oncotype DX (colorectal) High	116 physicians 92 patients Cross-sectional survey	United States	II	Mean (SD): 61.3 (11.8) NR
Srivastava, 2013[150] Oncotype DX (colorectal) Unclear	105 oncologists 141 patients Uncontrolled trial	United States	II	NR NR

Abbreviations: N = number; NR = not reported; SD = standard deviation.

Results: Oncotype DX Colon and Treatment Decisions

With no studies rated as low or medium RoB, we found no reliable data to inform conclusions about the impact of the Oncotype DX Colon test on treatment decisions.

Key Question 4b: Clinical Utility—Evidence that Modified Decisions Lead to Improved Outcomes

We found no eligible studies that addressed this question.

Key Question 5. Harms Associated with Treatment Decisions that are Informed by Molecular Pathology Tests

We found no eligible studies that addressed this question.

Discussion

Below, we summarize the main findings and strength of evidence (SOE). We then discuss implications for decision-making, limitations, future research, and conclusions.

Key Findings and Strength of Evidence

We found no studies that directly addressed our overarching question (Key Question [KQ] 1) (i.e., no studies directly assessed the impact of a test of interest on physician decision-making and subsequent health outcomes). Further, no studies directly addressed whether modified decisions lead to improved health outcomes (KQ 4b, clinical utility), and none reported harms associated with treatment decisions that are informed by molecular pathology tests (KQ 5).

The majority of studies meeting inclusion criteria for our review focused on clinical validity (KQ 3), and they evaluated associations between prognostic tests and outcomes. For two tests, MammaPrint and Oncotype DX Breast, we found studies rated as low or medium risk of bias (RoB) addressing whether prognostic information provided by the molecular pathology tests modified treatment decisions (KQ 4a).

Analytic Validity

Of the six molecular pathology tests with studies of low or medium RoB, the *EGFR* and *KRAS* mutation tests for lung cancer, the MammaPrint test for breast cancer, and the *KRAS* mutation test for colorectal cancer (CRC) were determined to have an overall moderate or high SOE for analytic validity (Table 41). Studies of these tests used fairly large panels of samples, were all low RoB, were directly measuring the test, and were consistent in their measurements.

Table 41. Summary of findings and strength of evidence for analytic validity

Test	Parameter	N studies; N subjects	Value	Strength of Evidence
MammaPrint: Breast	SS	2;399	NR	Moderate
	CV	1;100	NA	Low
EGFR: Lung:	SS	5; 898	Sensitivity range: 68–100%; Specificity range: 79–100%	Moderate
	CV	5;636	Kappa score range: 0.20–0.73	High
KRAS: Lung:	SS	2;188	NR	Moderate
	CV	4;686	Kappa score range: 0.15–0.66	High
BRAF: Colorectal	SS	1;117	NR	Insufficient
	CV	3;533	NA	High
KRAS: Colorectal	SS	5;576	Sensitivity range: 69–98%; Specificity range: 90–100%	High
	CV	5;747	Reproducibility: 98%	High
Oncotype DX: Colorectal:	SS	1; NR	NR	Insufficient [
	CV	NA	NA	NA

Abbreviations: *BRAF* = gene name; CV = cross-lab validity; *EGFR* = gene name; *KRAS* = gene name; N = number; NA = not applicable; NR = not reported; SS = sensitivity and specificity.

The SOE for the overall analytic validities of the *BRAF* mutation test for CRC and the Oncotype DX Colon multigene assay for CRC were determined to be low. There were only one or two studies to consider, RoB was generally higher, and consistency was often

unknown (when there was a single study). However, the *BRAF* mutation test for CRC, due to the CAP report data, showed a high SOE for cross-lab validity.

In general, we found limited evidence in the published literature assessing analytic validity of the included tests. For most of these tests, we found evidence from fewer than 200 subjects; just 2 of the tests (*EGFR*/Lung and *KRAS*/CRC) had more than 200 samples contributing to our SOE grade (Table 41).

However, the best data on reproducibility we found is from the College of American Pathologists' report data for *EGFR* mutation testing, *KRAS* mutation testing, *BRAF* mutation testing, microsatellite instability for colorectal cancer, and UroVysion cytogenetics for urinary bladder cancer, which support the analytic validity of these tests.

In general, in most of the studies, tests were developed in the laboratories and compared with the gold standard of direct Sanger sequencing of the gene in formalin-fixed, paraffin-embedded tissue samples. We had very few studies directly assessing the analytic validity (test performance) using direct sequencing of frozen tissue (the current gold standard), which would be of great importance in determining the true analytic validity of the tests.

In general, the studies showed that their tests in comparison with the gold standard were of comparable marker sensitivity/specificity and had similar proportions of valid results (level of detection). Because the analytic validity, sensitivity, and specificity of direct sequencing of frozen tissue have not been established against their "true" tumor diagnosis, the false-positive rate cannot truly be determined for the other tests used in these studies. In Beau-Faller et al.,[35] the false positive rate for direct sequencing of *EGFR* and *KRAS* was stated to be about 10 to 15 percent. Therefore, any numbers we have given for marker sensitivity/specificity and predictive value are estimates, assuming accurate direct sequencing.

Clinical Validity

Included studies provided some evidence on clinical validity for 9 of the included tests, adjusted for known prognostic factors (Table 42). Evidence from multiple studies supports prognostic value , beyond known prognostic factors, for MammaPrint, Oncotype DX Breast, *KRAS* mutation testing for lung cancer, *BRAF* mutation testing for CRC, *KRAS* mutation testing for CRC, and microsatellite instability (MSI) for CRC for at least one of our included outcomes (i.e., risk of recurrence [RR], cancer-specific survival [CSS], or overall survival [OS]). For UroVysion, limited evidence from 2 small studies (total N=168) rated as low or medium RoB supported prognostic value for RR. None of the studies we evaluated suggested that *EGFR* added prognostic value to the traditional factors used to determine prognosis for lung cancer. For CRC, evidence did not adequately support added prognostic value for Oncotype DX Colon.

Table 42. Summary of findings on clinical validity (i.e., Association between Test Result and Prognosis)

Test: Cancer	Outcome	N studies; N subjects	Results Effect Size (95% CI)	Evidence from Multiple Studies Supports Association between Test Result and Prognosis?
MammaPrint: Breast	RR	6; 1,913	HR: 2.84 (2.11 to 3.89) for poor prognosis vs. good prognosis	Yes
	CSS	5; 1,615	HR: 3.3 (2.22 to 4.9) for poor prognosis vs. good prognosis	Yes
	OS	1; 144	HR: 1.67 (0.73 to 3.82) for poor prognosis vs. good prognosis	No
Oncotype DX Breast	RR	6; 3,222	HR: 2.97 (2.19 to 4.02) for high risk vs. low risk	Yes
	CSS	2; 1,234	HR: 2.02 (1.35 to 3.00) for high risk vs. low risk	Yes
	OS	1; 668	HR: 1.65 (1.24 to 2.19) for high risk vs. low risk	No, single study
EGFR: Lung	RR	6; 1,870	HR: 0.87 (0.65 to 1.15); No association	No
	CSS	0; 0	NA	No
	OS	6; 1,820	HR: 0.76 (0.50 to 1.19); No association	No
KRAS: Lung	RR	4; 611	2.84 (1.14, 7.1) *KRAS* mutation associated with greater RR	Yes
	CSS	0; 0	NA	No
	OS	2; 253	2.69 (1.91,3.8); 3.33 (1.03, 10.82)	Yes[a]
BRAF: CRC	RR	5; 4,106	HR 1.07 (0.76 to 1.52) for wild-type vs. mutation	No
	CSS	7; 5,409	HR 1.50 (1.26 to 1.77) for wild-type vs. mutation	Yes
	OS	11;7,610	HR 1.45 (1.29 to 1.62) for wild-type vs. mutation	Yes
KRAS: CRC	RR	5; 4,085	HR 1.02 (0.91 to 1.14) for wild-type vs. mutation	No
	CSS	2; 1,174	HR 1.30 (1.02 to 1.66) for wild-type vs. mutation	Yes
	OS	10; 5,328	HR 1.22 (0.93 to 1.60) for wild-type vs. mutation	No
MSI: CRC	RR	10; 7,130	HR 0.60 (0.50 to 0.72) for MSI-H vs. MSS	Yes
	CSS	6; 3,439	HR 0.65 (0.51 to 0.82) for MSI-H vs. MSS	Yes
	OS	12; 8,839	HR 0.57 (0.43 to 0.77) for MSI-H vs. MSS	Yes
Oncotype DX: CRC	RR	1; 690	HR 1.68 (1.18 to 2.38)	No, single study
	CSS	0; 0	NA	No
	OS	0; 0	NA	No
UroVysion: Bladder	RR	2; 168	Association between mutation and RR in 2 small studies	Yes, but limited to 168 subjects
	CSS	0; 0	NA	No
	OS	0; 0	NA	No

[a] Data synthesiswas done only when we had 3 or more HRs to combine to create a summary.

Notes: Table includes results of our main analyses that were based on studies rated as low or medium RoB. HRs reported are results of our meta-analyses of studies rated as low or medium RoB reporting adjusted associations for clinical validity (KQ 3).

Abbreviations: *BRAF* = gene name; CRC = colorectal cancer; CSS = cancer-specific survival; *EGFR* = gene name; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; MSI-H = microsatellite instability high; MSS = microsatellite stability: N = number; NA = not applicable; OS = overall survival; RR = risk of recurrence; vs. = versus.

Breast Cancer: MammaPrint

Our meta-analysis suggests that patients classified as having a poor prognosis by MammaPrint consistently do worse than those with a good prognosis signature with respect to distant metastatis-free survival and CSS. Studies included in the search varied somewhat in the subpopulations they tested; the fact that the signature added prognostic

value across these various subpopulations suggests that it has broad applicability. Because no studies looked at the value of MammaPrint with respect to loco-regional recurrence (LRR) and only one considered overall survival, there is scant or no evidence regarding the clinical validity of the MammaPrint signature in terms of these outcomes.

Breast Cancer: Oncotype DX

Some evidence indicates that the risk score (RS) estimated by Oncotype DX adds prognostic value in predicting distant recurrence and CSS. The two included studies that used LRR as an outcome reported that the RS does not add value in determining prognosis (with respect to LRR). Thus, evidence suggests that the Oncotype DX score adds value in determining prognosis for distant metastasis and CSS but not for LRR.

Lung Cancer: KRAS mutation

Some evidence suggest that testing for KRAS mutations has added prognostic value in lung cancer for both RR and OS. Our meta-analysis resulted in significant HRs for both RR and OS. There were no studies that looked at CSS. The results seem to hold for all stages. However, because the studies did not examine differences by race, age, or gender, it is not known if the prognostic value of the mutation varies by any of these factors.

Colorectal Cancer: *BRAF* Mutation

Evidence suggests that *BRAF* mutation does not have added prognostic value for RR. But our meta-analysis found that *BRAF* mutation testing has prognostic value for CSS and OS. The populations studied included proximal and distal colon cancers as well as rectal cancers. The studies did not examine if the risk varied by race, age, or gender. The results seem to hold for proximal, distal, and rectal cancers. However, because the studies did not examine differences by race, age, or gender, it is not known if the prognostic value of the mutation varies by any of these factors.

Colorectal Cancer: *KRAS* Mutation

Evidence suggests that *KRAS* mutation does not have added prognostic value for RR or OS. But our meta-analysis found that *KRAS* mutation testing has prognostic value for CSS. The populations studied included proximal and distal colon cancers as well as rectal cancers. The studies did not examine if the risk varied by race, age, or gender. The results seem to hold for proximal, distal, and rectal cancers. However, because the studies did not examine differences by race, age, or gender, it is not known if the prognostic value of the mutation varies by any of these factors.

Colorectal Cancer: MSI

Our meta-analyses found that testing for MSI adds prognostic value with respect to RR, CSS, and OS in CRC patients. Patients with MSI-H tumors have a lower risk of recurrence, death due to CRC, and death due to any cause compared with patients with MSS tumors.

The studies included looked at proximal, distal, and rectal cancers but did not look for differences by race, age, or gender. Thus, although it is possible to say that in general

MSI-H has a protective effect for CRC patients, evidence was lacking to allow determination of whether protection varies by race, age, or gender.

Clinical Utility and the Overarching Question

We found no studies that directly addressed our overarching question (KQ 1) (i.e., no studies directly assessed the impact of test use on downstream health outcomes). We attempted to construct an indirect chain of evidence to answer the overarching question, but no studies addressed whether modified decisions lead to improved health outcomes (KQ 4b, clinical utility), so we were unable to do so. The furthest downstream evidence that we found addressed whether prognostic information provided by the molecular pathology tests modifies treatment decisions (KQ 4a); we found such evidence for three tests, MammaPrint, Oncotype DX Breast, and Oncotype DX Colon (but the three studies identified for Oncotype DX Colon were rated as high or unclear risk of bias).

For impact on treatment decisions, we found moderate SOE that one test, Oncotype DX Breast, leads to changes in decisions. Although the decision changes were observed in both directions for individual patients, studies consistently showed an overall shift to less-intensive treatment recommendations as a result of using Oncotype DX Breast, with fewer recommendations for chemotherapy (and therefore less exposure to potential harms of chemotherapy; but studies did not follow patients to actually report on harms or to assess the overall balance of clinical benefits and harms).We found just one study of low or medium RoB for the impact of MammaPrint on treatment decisions, and we concluded that evidence was insufficient to determine the impact of MammaPrint on treatment decisions, primarily because of unknown consistency and imprecision.

Table 43 summarizes the evidence on the overarching question, clinical utility, and the impact of test use on treatment decisions. Ultimately, for the reasons described above, we found insufficient SOE to answer the overarching question for most tests. Even in the cases where the tests seemed to add value in determining prognosis (i.e., evidence of clinical validity), we found no direct evidence that using the test was related to improved outcomes for patients. For a few tests, we found low SOE, suggesting that using the test would not improve outcomes for patients—for these tests we found evidence that did not support clinical validity (because with evidence suggesting lack of clinical validity, it is unlikely that the tests will be found to have clinical utility).

Table 43. Summary of findings and strength of evidence for impact on treatment decisions, clinical utility, and our overarching question

Test: Cancer	Outcome	N studies; N subjects	Conclusions	Strength of Evidence
MammaPrint: Breast	RR	6; 1,913	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	5; 1,615	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	1; 144	Study assessed clinical validity; no evidence that test use leads to improved morality	Insufficient
	Decisions about Rx	1; 427	Adjuvant therapy was used less if the prognosis signature is used	Insufficient
Oncotype DX Breast	RR	6; 3,222	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	2; 1,234	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	1; 668	All studies assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	16; 2,251	~30% of treatment decisions changed by the test	Moderate
EGFR: Lung	RR	6; 1,870	All studies assessed clinical validity and found no prognostic value; test is unlikely to improve outcomes	Low
	CSS	0; 0	NA	Insufficient
	OS	6;1,820	No prognostic value; none of the reported HRs were statistically significant; test unlikely to improve mortality	Low
	Decisions about Rx	0; 0	NA	Insufficient
KRAS: Lung	RR	4; 611	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	0; 0	NA	Insufficient
	OS	2; 253	Studies assessed clinical validity; no prognostic value; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient
BRAF: CRC	RR	5; 4,106	All studies assessed clinical validity; no prognostic value; test is unlikely to improve outcomes	Low
	CSS	7; 5,409	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	10; 7,610	All studies assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient
KRAS: CRC	RR	5; 4,085	All studies assessed clinical validity; no prognostic value; test is unlikely to improve outcomes	Low
	CSS	2; 1,174	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	OS	10; 5,328	All studies assessed clinical validity; no prognostic value; test is unlikely to improve mortality	Low
	Decisions about Rx	0; 0	NA	Insufficient
MSI: CRC	RR	10; 7,130	All studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	6; 3,439	All studies assessed clinical validity; no evidence that test use leads to improved CSS	Insufficient
	OS	12; 8,839	All studies assessed clinical validity; no evidence that test use leads to improved mortality	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient

Table 43. Summary of findings and strength of evidence for impact on treatment decisions, clinical utility, and our overarching question (continued)

Test: Cancer	Outcome	N studies; N subjects	Conclusions	Strength of Evidence
Oncotype DX: CRC	RR	1; 690	Study assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	0; 0	NA	Insufficient
	OS	0; 0	NA	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient
UroVysion: Bladder	RR	2; 168	Both studies assessed clinical validity; no evidence that test use leads to improved outcomes	Insufficient
	CSS	0; 0	NA	Insufficient
	OS	0; 0	NA	Insufficient
	Decisions about Rx	0; 0	NA	Insufficient

Notes: Table included results of our main analyses that were based on studies rated as low or medium RoB. HRs reported are results of our meta-analyses of studies rated as low or medium RoB reporting adjusted associations for clinical validity (KQ 3).

Strength of evidence grades are for the overarching question not for clinical validity (i.e., not for prognostic value or accuracy).

Abbreviations: *BRAF* = gene name; CRC = colorectal cancer; CSS = cancer-specific survival; *EGFR* = gene name; HR = hazard ratio; *KRAS* = gene name; MSI = microsatellite instability; N = number; NA = not applicable; OS = overall survival; RR = risk of recurrence; Rx = treatment.

Implications for Clinical and Policy Decisionmaking

Our review demonstrated that the weight of published research to date in the area of molecular pathology tests for improving estimates of cancer prognosis has focused on the clinical validity of the tests of interest in giving information about prognosis and little emphasis on how these tests can be integrated into the overall care of cancer patients in terms of measuring changes in management decisions or the effect of those altered decisions on downstream outcomes of value to patients. Such changes in management may be occurring and may be of benefit, or possibly of harm, to patients but have not been measured and studied, with the notable exception of the Oncotype DX assay in breast cancer, which does have a sizeable body of evidence to suggest an effect on treatment decisions (resulting in fewer recommendations for chemotherapy), though not yet a clear effect on downstream outcomes.

At this point, physicians can, in many cases, rely on the prognostic value of molecular pathology tests, can share test results with patients, and discuss whether the test result indicates a better or worse than average prognosis. However, in most cases, physicians cannot be sure whether they should direct management plans based on the results. Although having accurate information about prognosis may be valued by patients independent of its effects on treatment planning or on survival and recurrence outcomes, this has yet to be demonstrated for the tests of interest.

Policymakers may request information and research that not only demonstrate the prognostic value of a test for cancer recurrence, but also evaluate the broader context of how the test fits into the overall plan of care and how information provided by the test changes a patient's long-term outcomes. Because molecular pathology tests for risk of cancer recurrence are relatively recent innovations in cancer care, it is reasonable for

policymakers to expect that their value may be better demonstrated over the next decade as the research base matures.

Limitations of the Comparative Effectiveness Review Process

We did not include studies focused on populations with advanced/metastatic cancer. The focus of this report is on whether the included tests are useful for prognosis as related to the risk of cancer recurrence. People with advanced/metastatic cancer would first have to achieve remission before recurrence is a possibility. Studies of people with advanced/metastatic cancer tend to focus on overall survival and typically do not provide information about the risk of cancer recurrence.

Also publication bias and selective reporting are potential limitations. Although we searched for unpublished studies and unpublished outcomes and did not find direct evidence of either of these biases, many of the included studies were published prior to the availability of registries that would allow for greater certainty in determining the potential for either type of bias.

Finally, although the r eview was focused om the Medicare population we did not find evidence specific to thulation. Many studies included participants from this age group and we did not find any evidence suggesting that the prognostic value of the tests varies with age.

Limitations of the Evidence Base

The evidence base was inadequate to draw conclusions for some of our questions or subquestions of interest. In particular, as described above, we found no direct evidence addressing our overarching question and insufficient evidence to determine whether modified decisions that result from prognostic testing lead to improved health outcomes. Further, we found scant evidence meeting our inclusion criteria regarding ALK translocation testing.

Although we found insufficient evidence to determine whether modified decisions that result from prognostic testing lead to improved health outcomes, studies of the use of Oncotype DX Breast reveal a pattern of less aggressive treatment recommendations as a result of using test information to inform decision-making (i.e., more recommendations for patients to have hormonal therapy alone than hormonal plus chemotherapy). We would expect this to improve quality of life and to decrease the costs of treatment, but we did not find empiric evidence to confirm this, and it might only hold true if recurrence and survival were either unchanged or were improved.

Many of the included studies had methodological limitations introducing some RoB. For example, most of them were observational studies assessing associations between test results and outcomes and are susceptible to potential confounding. To limit such bias, we only included studies for KQ 3 that adjusted for most or all standard prognostic factors. Also, we assessed potential selection bias and confounding in our RoB assessments—limiting our main data syntheses to studies with low or medium RoB.

We were also not able to address the prognostic value of the tests for the Medicare population due to a lack of data that was specific to that age group.

Future Research

We found no direct evidence of the impact of the information provided by these tests on downstream health outcomes such as patients' quality of life or survival. Thus, future research should focus on quality of life, survival, and other health outcomes. There is no information on the differential effects of the test by race or cancer subtype (e.g., ductal versus lobular in breast cancer) or location (e.g., proximal versus distal in CRC). As described in the results, the subpopulations that were represented in the studies varied in terms of stage, tumor type, and location in the case of CRC. Race and location have both been shown to be important predictors of the prognostic value of genetic markers in CRC.[152,153] Similar differences in prognostic value by subpopulations could be a factor in terms of *EGFR* and *KRAS* in the lung. There is thus a need to create an evidence base that replicates results in the same subpopulations, particularly in CRC and lung cancer.

As in many studies in the oncology field, the published literature uses a variety of specific definitions for outcomes such as recurrence and CSS, making comparison of effects across studies more difficult. Future research should take into account careful selection of the most appropriate endpoints, both in the context of the existing body of literature and the endpoints of most clinical relevance to doctors and patients.

Research on the prognostic value of molecular pathology tests, whether existing or novel, is limited by the lack of availability of biologic samples or detailed molecular pathology test result information from large population-based cohorts making assessment and generalizability of genetic prognostic tests difficult. Because many published studies used subsets of the same clinical trial populations, there is overlap in the sample of patients studied (e.g., three of the studies included for Oncotype DX in breast cancer report on the same 668 patients). There is a need to expand the research into independent populations and to incorporate collection of molecular pathology test results and/or biologic samples into the design of registry and prospective cohort studies. Although concerns may arise regarding privacy issues, these may be allayed by understanding that genetic alterations in the carcinomas, rather than those in germline DNA, are the focus of the research, and well-established ethical guidelines exist for handling and storing patient biospecimens.

Conclusions

We found modest evidence supporting added prognostic value (i.e., clinical validity), beyond traditional prognostic factors, for MammaPrint, Oncotype DX Breast, *KRAS* mutation testing for lung, *BRAF* mutation testing for CRC, *KRAS* mutation testing for CRC, and MSI for CRC for RR, CSS, and/or OS. For UroVysion, which is marketed as a diagnostic (not prognostic) test, limited evidence supported an association between test result and prognosis for RR. For the other included tests, evidence either did not support added prognostic value or we found no studies with sufficiently low RoB to support a conclusion about prognostic value.

We found no studies that directly addressed our overarching question (i.e., no studies directly assessed the impact of test use on downstream health outcomes to establish clinical utility). We attempted to construct an indirect chain of evidence to answer the overarching question, but evidence was generally insufficient to do so. Even for the tests

with good evidence supporting clinical validity, we found no evidence that using the test was related to improved outcomes for patients.

For impact of test use on treatment decisions, we found moderate SOE that Oncotype DX Breast leads to changes in decisions. One study of low or medium RoB found a significant impact of MammaPrint on treatment decisions, but evidence was insufficient to draw any firm conclusions, primarily because of unknown consistency and imprecision.

Many of the included tests are currently used to predict response to specific treatments, an aspect that was not evaluated in this report. A determination of whether the tests have clinical utility for predicting therapeutic response is beyond the scope of this review.

References

1. Howlader N, Noone AM, Krapcho M, et al., eds. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). Bethesda, MD: National Cancer Institute; 2012.
2. Webster's Encyclopedia Unabridged Dictionary of the English Language. New York: Portland House; 2001.
3. Rector TS, Taylor BC, Wilt TJ. Systematic review of prognostic tests. Chapter 12 of Methods Guide for Medical Test Reviews. AHRQ Publication No. 12-EHC017. Rockville, MD: Agency for Healthcare Research and Quality; June 2012. www.effectivehealthcare.ahrq.gov/reports/final.cfm. Also published in a special supplement to the Journal of General Internal Medicine, July 2012.
4. National Cancer Institute at the National Institutes of Health. Cancer Staging. Bethesda, MD: National Cancer Institute www.cancer.gov/cancertopics/factsheet/detection/staging.
5. American Joint Committee on Cancer. Cancer Staging Manual. 7th ed., New York: Springer; 2010.
6. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006 Jun 7;295(21):2492-502. PMID: 16757721.
7. Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000 Jul;124(7):979-94. PMID: 10888773.
8. Sun LC, Chu KS, Cheng SC, et al. Preoperative serum carcinoembryonic antigen, albumin and age are supplementary to UICC staging systems in predicting survival for colorectal cancer patients undergoing surgical treatment. BMC Cancer. 2009;9:288. PMID: 19691850.
9. Fitzgibbons PL, Page DL, Weaver D, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000 Jul;124(7):966-78. PMID: 10888772.
10. Rakha EA, El-Sayed ME, Lee AH, et al. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol. 2008 Jul 1;26(19):3153-8. PMID: 18490649.
11. Groome PA, Bolejack V, Crowley JJ, et al. The IASLC Lung Cancer Staging Project: validation of the proposals for revision of the T, N, and M descriptors and consequent stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol. 2007 Aug;2(8):694-705. PMID: 17762335.
12. Sculier JP, Chansky K, Crowley JJ, et al. The impact of additional prognostic factors on survival and their relationship with the anatomical extent of disease expressed by the 6th edition of the TNM Classification of Malignant Tumors and the proposals for the 7th edition. J Thorac Oncol. 2008 May;3(5):457-66. PMID: 18448996.
13. International Bladder Cancer Nomogram Consortium, Bochner BH, Kattan MW, et al. Postoperative nomogram predicting risk of recurrence after radical cystectomy for bladder cancer. J Clin Oncol. 2006 Aug 20;24(24):3967-72. PMID: 16864855.
14. Shariat SF, Karakiewicz PI, Palapattu GS, et al. Nomograms provide improved accuracy for predicting survival after radical cystectomy. Clin Cancer Res. 2006 Nov 15;12(22):6663-76. PMID: 17121885.
15. Iasonos A, Schrag D, Raj GV, et al. How to build and interpret a nomogram for cancer prognosis. J Clin Oncol. 2008 Mar 10;26(8):1364-70. PMID: 18323559.

16. Newman TB, Kohn MA. Evidence-Based Diagnosis. New York: Cambridge University Press; 2009.

17. Lloyd-Jones DM. Cardiovascular risk prediction: basic concepts, current status, and future directions. Circulation. 2010 Apr 20;121(15):1768-77. PMID: 20404268.

18. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., et al. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008 Jan 30;27(2):157-72; discussion 207-12. PMID: 17569110.

19. So HC, Sham PC. A unifying framework for evaluating the predictive power of genetic variants based on the level of heritability explained. PLoS Genet. 2010;6(12):e1001230. PMID: 21151957.

20. Methods Guide for Medical Test Reviews. Agency for Healthcare Research and Quality. AHRQ Publication No. 12-EC017. Rockville, MD: June 2012. www.effectivehealthcare.ahrq.gov/reports/final.cfm.

21. Smetana GW, Umscheid CA, Chang S, et al. Methods guide for authors of systematic reviews of medical tests: A collaboration between the Agency for Healthcare Research and Quality (AHRQ) and the Journal of General Internal Medicine. J Gen Intern Med. 2012 Jun;27 Suppl 1:S1-3. PMID: 22648668.

22. Jonas DE, Wilt TJ, Taylor BC, et al. Chapter 11: Challenges in and principles for conducting systematic reviews of genetic tests used as predictive indicators. J Gen Intern Med. 2012 Jun;27(Suppl 1):S83-S93. PMID: ISI:000304661300012.

23. Jonas DE, Wilt TJ, Taylor BC, et al. Challenges in and principles for conducting systematic reviews of genetic tests used as predictive indicators. Agency for Healthcare Research and Quality AHRQ Publication No. 12-EHC083-EF. Rockville, MD: June 2012. www.effectivehealthcare.ahrq.gov/reports/final.cfm.

24. Whiting PF, Rutjes AW, Sterne J, et al. A quality assessment tool for diagnostic accuracy studies. Bristol, UK: University of Bristol http://www.bris.ac.uk/quadas/resources/quadas2.pdf. Accessed September 12, 2013.

25. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011 Oct 18;155(8):529-36. PMID: 22007046.

26. Viswanathan M, Berkman ND. Development of the RTI item bank on risk of bias and precision of observational studies. J Clin Epidemiol. 2012 Feb;65(2):163-78. PMID: 21959223.

27. Hedges LV, Vevea JL. Fixed- and random-effects models in meta-analysis. Psychological Methods. 1998;3(4):486-504.

28. Atkins D, Chang SM, Gartlehner G, et al. Assessing applicability when comparing medical interventions: AHRQ and the Effective Health Care Program. J Clin Epidemiol. 2011 Nov;64(11):1198-207. PMID: 21463926.

29. Owens DK, Lohr KN, Atkins D, et al. AHRQ series paper 5: Grading the strength of a body of evidence when comparing medical interventions—Agency for Healthcare Research and Quality and the Effective Health-Care Program. J Clin Epidemiol. 2010 May;63(5):513-23. PMID: 19595577.

30. Norris S, Atkins D, Bruening W, et al. Selecting Observational Studies for Comparing Medical Interventions. Methods Guide for Effectiveness and Comparative Effectiveness Reviews. Rockville, MD: Agency for Healthcare Research and Quality; 2010.

31. Atkins D, Chang S, Gartlehner G, et al. Assessing the Applicability of Studies When Comparing Medical Interventions. Agency for Healthcare Research and Quality, Methods Guide for Comparative Effectiveness Reviews. AHRQ Publication No. 11-EHC019-EF. Rockville, MD: January 2011. http://effectivehealthcare.ahrq.gov/.

32. Abbott Molecular Division. Vysis ALK Break Apart FISH Probe Kit Information Package. March 2013.

33. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004 May 20;350(21):2129-39. PMID: 15118073.

34. Bando I, Cillero L, Sanz-Ortega J, et al. Study of KRAS new predictive marker in a clinical laboratory. Clin Transl Oncol. 2012 Dec;14(12):937-42. PMID: 22865324.

35. Beau-Faller M, Degeorges A, Rolland E, et al. Cross-validation study for epidermal growth factor receptor and KRAS mutation detection in 74 blinded non-small cell lung carcinoma samples: a total of 5550 exons sequenced by 15 molecular French laboratories (evaluation of the EGFR mutation status for the administration of EGFR-TKIs in non-small cell lung carcinoma [ERMETIC] project--part 1). J Thorac Oncol. 2011 Jun;6(6):1006-15. PMID: 21532509.

36. Beau-Faller M, Legrain M, Voegeli AC, et al. Detection of K-Ras mutations in tumour samples of patients with non-small cell lung cancer using PNA-mediated PCR clamping. Br J Cancer. 2009 Mar 24;100(6):985-92. PMID: 19293811.

37. Clark-Langone KM, Sangli C, Krishnakumar J, et al. Translating tumor biology into personalized treatment planning: analytical performance characteristics of the Oncotype DX Colon Cancer Assay. BMC Cancer. 2010;10:691. PMID: 21176237.

38. Cronin M, Sangli C, Liu ML, et al. Analytical validation of the Oncotype DX genomic diagnostic test for recurrence prognosis and therapeutic response prediction in node-negative, estrogen receptor-positive breast cancer. Clin Chem. 2007 Jun;53(6):1084-91. PMID: 17463177.

39. Delahaye LJM, Wehkamp D, Floore AN, et al. Performance characteristics of the MammaPrint®breast cancer diagnostic gene signature. Personalized Medicine. 2013;10(8):801-11.

40. Feigelson HS, Goddard KA, Johnson MA, et al. Reliability of KRAS mutation testing in metastatic colorectal cancer patients across five laboratories. BMC Res Notes. 2012;5:196. PMID: 22534075.

41. Gao J, Li YY, Sun PN, et al. Comparative analysis of dideoxy sequencing, the KRAS StripAssay and pyrosequencing for detection of KRAS mutation. World J Gastroenterol. 2010 Oct 14;16(38):4858-64. PMID: 20939116.

42. Gonzalez de Castro D, Angulo B, Gomez B, et al. A comparison of three methods for detecting KRAS mutations in formalin-fixed colorectal cancer specimens. Br J Cancer. 2012 Jul 10;107(2):345-51. PMID: 22713664.

43. Hancer VS, Buyukdogan M, Turkmen I, et al. Comparison of KRAS mutation tests in colorectal cancer patients. Genet Test Mol Biomarkers. 2011 Nov;15(11):831-4. PMID: 21699410.

44. Jancik S, Drabek J, Berkovcova J, et al. A comparison of Direct sequencing, Pyrosequencing, High resolution melting analysis, TheraScreen DxS, and the K-ras StripAssay for detecting KRAS mutations in non small cell lung carcinomas. J Exp Clin Cancer Res. 2012;31:79. PMID: 22995035.

45. Kobunai T, Watanabe T, Yamamoto Y, et al. The frequency of KRAS mutation detection in human colon carcinoma is influenced by the sensitivity of assay methodology: a comparison between direct sequencing and real-time PCR. Biochem Biophys Res Commun. 2010 Apr 23;395(1):158-62. PMID: 20361930.

46. Lopez-Rios F, Angulo B, Gomez B, et al. Comparison of molecular testing methods for the detection of EGFR mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer. J Clin Pathol. 2013 May;66(5):381-5. PMID: 23386666.

47. Mancini I, Santucci C, Sestini R, et al. The use of COLD-PCR and high-resolution melting analysis improves the limit of detection of KRAS and BRAF mutations in colorectal cancer. J Molec Diagnost. 2010;12(5):705-11.

48. Pang NK, Nga ME, Chin SY, et al. KRAS and BRAF mutation analysis can be reliably performed on aspirated cytological specimens of metastatic colorectal carcinoma. Cytopathology. 2011 Dec;22(6):358-64. PMID: 21029218.

49. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002 Jan 31;415(6871):530-6. PMID: 11823860.

50. Angulo B, Conde E, Suarez-Gauthier A, et al. A comparison of EGFR mutation testing methods in lung carcinoma: direct sequencing, real-time PCR and immunohistochemistry. PLoS One. 2012;7(8):e43842. PMID: 22952784.

51. Naoki K, Soejima K, Okamoto H, et al. The PCR-invader method (structure-specific 5' nuclease-based method), a sensitive method for detecting EGFR gene mutations in lung cancer specimens; comparison with direct sequencing. Int J Clin Oncol. 2011 Aug;16(4):335-44. PMID: 21311943.

52. Poulet B, Jamshidian F, Butler S, et al. Risk Classification of Early Stage Breast Cancer as Assessed by MammaPrint and Oncotype DX Genomic Assays. SABCS; 2012.

53. Sriram KB, Tan ME, Savarimuthu SM, et al. Screening for activating EGFR mutations in surgically resected nonsmall cell lung cancer. Eur Respir J. 2011 Oct;38(4):903-10. PMID: 21349912.

54. CAP Molecular Oncology Committee. EGFR Educational Challenge Participant Summary Report. Surveys 2012 EGFR-B, 2013 EGFR-A, 2013 EGFR-B. . Northfield, IL: College of American Pathologists; 2012, 2013.

55. CAP Molecular Oncology Committee. KRAS Educational Challenge Participant Summary Report. Surveys 2012 KRAS-B, 2013 KRAS-A, 2013 KRAS-B. . Northfield, IL: College of American Pathologists; 2012, 2013.

56. CAP Molecular Oncology Committee. BRAF Educational Challenge Participant Summary Report. Surveys 2012 BRAF-B, 2013 BRAF-A, 2013 BRAF-B. Northfield, IL: College of American Pathologists; 2012, 2013.

57. CAP Molecular Oncology Committee. MSI Educational Challenge Participant Summary Report. Surveys 2012 MSI-B, 2013 MSI-A, 2013 MSI-B. Northfield, IL: College of American Pathologists; 2012, 2013.

58. CAP/ACMG Cytogenetics Resource Committee. FISH for Urothelial Carcinoma Educational Challenge Participant Summary Report. Surveys 2013 CYI-A, CYI-B, CYI-C. . Northfield, IL: College of American Pathologists; 2013.

59. Bueno-de-Mesquita JM, Linn SC, Keijzer R, et al. Validation of 70-gene prognosis signature in node-negative breast cancer. Breast Cancer Res Treat. 2009 Oct;117(3):483-95. PMID: 18819002.

60. Buyse M, Loi S, van't Veer L, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. J Natl Cancer Inst. 2006 Sep 6;98(17):1183-92. PMID: 16954471.

61. Iwamoto T, Lee JS, Bianchini G, et al. First generation prognostic gene signatures for breast cancer predict both survival and chemotherapy sensitivity and identify overlapping patient populations. Breast Cancer Res Treat. 2011 Nov;130(1):155-64. PMID: 21833625.

62. Knauer M, Cardoso F, Wesseling J, et al. Identification of a low-risk subgroup of HER-2-positive breast cancer by the 70-gene prognosis signature. Br J Cancer. 2010 Dec 7;103(12):1788-93. PMID: 21081926.

63. Mook S, Schmidt MK, Viale G, et al. The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. Breast Cancer Res Treat. 2009 Jul;116(2):295-302. PMID: 18661261.

64. Mook S, Knauer M, Bueno-De-Mesquita JM, et al. Metastatic potential of T1 breast cancer can be predicted by the 70-gene MammaPrint signature. Ann Surg Oncol. 2010;17(5):1406-13.

65. Mook S, Schmidt MK, Weigelt B, et al. The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age. Ann Oncol. 2010 Apr;21(4):717-22. PMID: 19825882.

66. Nuyten DSA, Hastie T, Chi JTA, et al. Combining biological gene expression signatures in predicting outcome in breast cancer: An alternative to supervised classification. Eur J Cancer. 2008;44(15):2319-29.

67. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002 Dec 19;347(25):1999-2009. PMID: 12490681.

68. Saghatchian M, Mook S, Pruneri G, et al. Additional prognostic value of the 70-gene signature (MammaPrint((R))) among breast cancer patients with 4-9 positive lymph nodes. Breast. 2013 Oct;22(5):682-90. PMID: 23347730.

69. Olivotto IA, Bajdik CD, Ravdin PM, et al. Population-based validation of the prognostic model ADJUVANT! for early breast cancer. J Clin Oncol. 2005 Apr 20;23(12):2716-25. PMID: 15837986.

70. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. J Clin Oncol. 2010 Apr 10;28(11):1829-34. PMID: 20212256.

71. Habel LA, Shak S, Jacobs MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. Breast Cancer Res. 2006;8(3):R25. PMID: 16737553.

72. Mamounas EP, Tang G, Fisher B, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. J Clin Oncol. 2010 Apr 1;28(10):1677-83. PMID: 20065188.

73. Mamounas EP, Tang G, Paik S, et al. Abstract #1: Prognostic impact of the 21-gene recurrence score (RS) on disease-free and overall survival of node-positive, ER-positive breast cancer patients (pts) treated with adjuvant chemotherapy: Results from NSABP B-28. ASCO; 2012.

74. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004 Dec 30;351(27):2817-26. PMID: 15591335.

75. Solin LJ, Gray R, Goldstein LJ, et al. Prognostic value of biologic subtype and the 21-gene recurrence score relative to local recurrence after breast conservation treatment with radiation for early stage breast carcinoma: results from the Eastern Cooperative Oncology Group E2197 study. Breast Cancer Res Treat. 2012 Jul;134(2):683-92. PMID: 22547108.

76. Tang G, Shak S, Paik S, et al. Comparison of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. Breast Cancer Res Treat. 2011 May;127(1):133-42. PMID: 21221771.

77. Yorozuya K, Takeuchi T, Yoshida M, et al. Evaluation of Oncotype DX Recurrence Score as a prognostic factor in Japanese women with estrogen receptor-positive, node-negative primary Stage I or IIA breast cancer. J Cancer Res Clin Oncol. 2010 Jun;136(6):939-44. PMID: 19946706.

78. An SJ, Chen ZH, Su J, et al. Identification of enriched driver gene alterations in subgroups of non-small cell lung cancer patients based on histology and smoking status. PLoS One. 2012;7(6).

79. Hiramatsu M, Ninomiya H, Inamura K, et al. Activation status of receptor tyrosine kinase downstream pathways in primary lung adenocarcinoma with reference of KRAS and EGFR mutations. Lung Cancer. 2010 Oct;70(1):94-102. PMID: 20117855.

80. Kim YT, Kim TY, Lee DS, et al. Molecular changes of epidermal growth factor receptor (EGFR) and KRAS and their impact on the clinical outcomes in surgically resected adenocarcinoma of the lung. Lung Cancer. 2008 Jan;59(1):111-8. PMID: 17904685.

81. Kobayashi N, Toyooka S, Ichimura K, et al. Non-BAC component but not epidermal growth factor receptor gene mutation is associated with poor outcomes in small adenocarcinoma of the lung. J Thorac Oncol. 2008 Jul;3(7):704-10. PMID: 18594314.

82. Koh Y, Jang B, Han SW, et al. Expression of class III beta-tubulin correlates with unfavorable survival outcome in patients with resected non-small cell lung cancer. J Thorac Oncol. 2010 Mar;5(3):320-5. PMID: 20087230.

83. Liu HP, Isaac Wu HD, Chang JW, et al. Prognostic implications of epidermal growth factor receptor and KRAS gene mutations and epidermal growth factor receptor gene copy numbers in patients with surgically resectable non-small cell lung cancer in Taiwan. J Thorac Oncol. 2010 Aug;5(8):1175-84. PMID: 20559151.

84. Mak RH, Doran E, Muzikansky A, et al. Outcomes after combined modality therapy for EGFR-mutant and wild-type locally advanced NSCLC. Oncologist. 2011;16(6):886-95. PMID: 21632451.

85. Matsumoto S, Iwakawa R, Kohno T, et al. Frequent EGFR mutations in noninvasive bronchioloalveolar carcinoma. Int J Cancer. 2006 May 15;118(10):2498-504. PMID: 16353158.

86. Rouquette I, Lauwers-Cances V, Allera C, et al. Characteristics of lung cancer in women: Importance of hormonal and growth factors. Lung Cancer. 2012;76(3):280-5.

87. Scoccianti C, Vesin A, Martel G, et al. Prognostic value of TP53, KRAS and EGFR mutations in nonsmall cell lung cancer: The EUELC cohort. Eur Respir J. 2012;40(1):177-84.

88. Kim YT, Seong YW, Jung YJ, et al. The presence of mutations in epidermal growth factor receptor gene is not a prognostic factor for long-term outcome after surgical resection of non-small-cell lung cancer. J Thorac Oncol. 2013 Feb;8(2):171-8. PMID: 23287850.

89. Tsao MS, Sakurada A, Ding K, et al. Prognostic and predictive value of epidermal growth factor receptor tyrosine kinase domain mutation status and gene copy number for adjuvant chemotherapy in non-small cell lung cancer. J Thorac Oncol. 2011;6(1):139-47.

90. Woo T, Okudela K, Yazawa T, et al. Prognostic value of KRAS mutations and Ki-67 expression in stage I lung adenocarcinomas. Lung Cancer. 2009 Sep;65(3):355-62. PMID: 19162366.

91. Guan JL, Zhong WZ, An SJ, et al. KRAS mutation in patients with lung cancer: a predictor for poor prognosis but not for EGFR-TKIs or chemotherapy. Ann Surg Oncol. 2013 Apr;20(4):1381-8. PMID: 23208128.

92. Farina-Sarasqueta A, van Lijnschoten G, Moerland E, et al. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol. 2010 Dec;21(12):2396-402. PMID: 20501503.

93. Kakar S, Deng G, Sahai V, et al. Clinicopathologic characteristics, CpG island methylator phenotype, and BRAF mutations in microsatellite-stable colorectal cancers without chromosomal instability. Arch Pathol Lab Med. 2008 Jun;132(6):958-64. PMID: 18517279.

94. Kalady MF, Dejulius KL, Sanchez JA, et al. BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. Dis Colon Rectum. 2012 Feb;55(2):128-33. PMID: 22228154.

95. Liou JM, Wu MS, Shun CT, et al. Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population. Int J Colorectal Dis. 2011 Nov;26(11):1387-95. PMID: 21553007.

96. Maestro ML, Vidaurreta M, Sanz-Casla MT, et al. Role of the BRAF mutations in the microsatellite instability genetic pathway in sporadic colorectal cancer. Ann Surg Oncol. 2007 Mar;14(3):1229-36. PMID: 17195912.

97. Ogino S, Shima K, Meyerhardt JA, et al. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: results from intergroup trial CALGB 89803. Clin Cancer Res. 2012 Feb 1;18(3):890-900. PMID: 22147942.

98. Pai RK, Jayachandran P, Koong AC, et al. BRAF-mutated, microsatellite-stable adenocarcinoma of the proximal colon: an aggressive adenocarcinoma with poor survival, mucinous differentiation, and adverse morphologic features. Am J Surg Pathol. 2012 May;36(5):744-52. PMID: 22314188.

99. Phipps AI, Buchanan DD, Makar KW, et al. BRAF mutation status and survival after colorectal cancer diagnosis according to patient and tumor characteristics. Cancer Epidemiol Biomarkers Prev. 2012 Oct;21(10):1792-8. PMID: 22899730.

100. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010 Jan 20;28(3):466-74. PMID: 20008640.

101. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. 2005 Jul 15;65(14):6063-9. PMID: 16024606.

102. Shaukat A, Arain M, Thaygarajan B, et al. Is BRAF mutation associated with interval colorectal cancers? Dig Dis Sci. 2010 Aug;55(8):2352-6. PMID: 20300843.

103. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. Int J Cancer. 2011 May 1;128(9):2075-84. PMID: 20635392.

104. Zlobec I, Bihl MP, Schwarb H, et al. Clinicopathological and protein characterization of BRAF- and K-RAS-mutated colorectal cancer and implications for prognosis. Int J Cancer. 2010 Jul 15;127(2):367-80. PMID: 19908233.

105. Lochhead P, Kuchiba A, Imamura Y, et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst. 2013 Aug 7;105(15):1151-6. PMID: 23878352.

106. Samadder NJ, Vierkant RA, Tillmans LS, et al. Associations between colorectal cancer molecular markers and pathways with clinicopathologic features in older women. Gastroenterology. 2013 Aug;145(2):348-56 e1-2. PMID: 23665275.

107. Roth AD, Delorenzi M, Tejpar S, et al. Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. J Natl Cancer Inst. 2012 Nov 7;104(21):1635-46. PMID: 23104212.

108. Eklof V, Wikberg ML, Edin S, et al. The prognostic role of KRAS, BRAF, PIK3CA and PTEN in colorectal cancer. Br J Cancer. 2013 May 28;108(10):2153-63. PMID: 23660947.

109. Bazan V, Agnese V, Corsale S, et al. Specific TP53 and/or Ki-ras mutations as independent predictors of clinical outcome in sporadic colorectal adenocarcinomas: results of a 5-year Gruppo Oncologico dell'Italia Meridionale (GOIM) prospective study. Ann Oncol. 2005 May;16 Suppl 4:iv50-5. PMID: 15923430.

110. Deschoolmeester V, Boeckx C, Baay M, et al. KRAS mutation detection and prognostic potential in sporadic colorectal cancer using high-resolution melting analysis. Br J Cancer. 2010;103(10):1627-36.

111. Geido E, Sciutto A, Rubagotti A, et al. Combined DNA flow cytometry and sorting with k-ras2 mutation spectrum analysis and the prognosis of human sporadic colorectal cancer. Cytometry. 2002 Aug 15;50(4):216-24. PMID: 12210601.

112. Imamura Y, Morikawa T, Liao X, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res. 2012;18(17):4753-63.

113. Ogino S, Meyerhardt JA, Irahara N, et al. KRAS mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803. Clin Cancer Res. 2009 Dec 1;15(23):7322-9. PMID: 19934290.

114. Li Z, Chen Y, Wang D, et al. Detection of KRAS mutations and their associations with clinicopathological features and survival in Chinese colorectal cancer patients. J Int Med Res. 2012;40(4):1589-98. PMID: 22971512.

115. Chang SC, Lin JK, Yang SH, et al. Relationship between genetic alterations and prognosis in sporadic colorectal cancer. Int J Cancer. 2006 Apr 1;118(7):1721-7. PMID: 16231316.

116. Donada M, Bonin S, Nardon E, et al. Thymidilate synthase expression predicts longer survival in patients with stage II colon cancer treated with 5-flurouracil independently of microsatellite instability. J Cancer Res Clin Oncol. 2011 Feb;137(2):201-10. PMID: 20387074.

117. Guidoboni M, Gafa R, Viel A, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. Am J Pathol. 2001 Jul;159(1):297-304. PMID: 11438476.

118. Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000 Jan 13;342(2):69-77. PMID: 10631274.

119. Hong SP, Min BS, Kim TI, et al. The differential impact of microsatellite instability as a marker of prognosis and tumour response between colon cancer and rectal cancer. Eur J Cancer. 2012 May;48(8):1235-43. PMID: 22071131.

120. Jensen SA, Vainer B, Kruhoffer M, et al. Microsatellite instability in colorectal cancer and association with thymidylate synthase and dihydropyrimidine dehydrogenase expression. BMC Cancer. 2009;9:25. PMID: 19154585.

121. Kim GP, Colangelo LH, Wieand HS, et al. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J Clin Oncol. 2007 Mar 1;25(7):767-72. PMID: 17228023.

122. Lin CC, Lai YL, Lin TC, et al. Clinicopathologic features and prognostic analysis of MSI-high colon cancer. Int J Colorectal Dis. 2012 Mar;27(3):277-86. PMID: 22076610.

123. Shia J, Klimstra DS, Nitzkorski JR, et al. Immunohistochemical expression of folate receptor alpha in colorectal carcinoma: patterns and biological significance. Hum Pathol. 2008 Apr;39(4):498-505. PMID: 18342661.

124. Soreide K, Slewa A, Stokkeland PJ, et al. Microsatellite instability and DNA ploidy in colorectal cancer: potential implications for patients undergoing systematic surveillance after resection. Cancer. 2009 Jan 15;115(2):271-82. PMID: 19109816.

125. Yoon YS, Yu CS, Kim TW, et al. Mismatch repair status in sporadic colorectal cancer: immunohistochemistry and microsatellite instability analyses. J Gastroenterol Hepatol. 2011 Dec;26(12):1733-9. PMID: 21615788.

126. Venook AP, Niedzwiecki D, Lopatin M, et al. Biologic Determinants of Tumor Recurrence in Stage II Colon Cancer: Validation Study of the 12-Gene Recurrence Score in Cancer and Leukemia Group B (CALGB) 9581. J Clin Oncol. 2013 Mar 25PMID: 23530100.

127. Kamat AM, Dickstein RJ, Messetti F, et al. Use of fluorescence in situ hybridization to predict response to bacillus Calmette-Guerin therapy for bladder cancer: results of a prospective trial. J Urol. 2012 Mar;187(3):862-7. PMID: 22245325.

128. Whitson J, Berry A, Carroll P, et al. A multicolour fluorescence in situ hybridization test predicts recurrence in patients with high-risk superficial bladder tumours undergoing intravesical therapy. BJU Int. 2009 Aug;104(3):336-9. PMID: 19220253.

129. Zellweger T, Benz G, Cathomas G, et al. Multi-target fluorescence in situ hybridization in bladder washings for prediction of recurrent bladder cancer. Int J Cancer. 2006 Oct 1;119(7):1660-5. PMID: 16646074.

130. Gligorov J, Pivot XB, Naman HL, et al. Prospective study of the impact of using the 21-gene recurrence score assay on clinical decision making in women with estrogen receptor-positive, HER2-negative, early-stage breast cancer in France. ASCO; 2012.

131. Davidson JA, Croomwell I, Ellard S, et al. A prospective clinical utility study of the impact of the 21-gene recurrence score assay (Oncotype DX) in estrogen receptor positive (ER+) node negative (pN0) breast cancer in academic Canadian centers. ASCO; 2012.

132. Ademuyiwa FO, Miller A, O'Connor T, et al. The effects of oncotype DX recurrence scores on chemotherapy utilization in a multi-institutional breast cancer cohort. Breast Cancer Res Treat. 2011 Apr;126(3):797-802. PMID: 21197567.

133. Albanell J, Gonzalez A, Ruiz-Borrego M, et al. Prospective transGEICAM study of the impact of the 21-gene Recurrence Score assay and traditional clinicopathological factors on adjuvant clinical decision making in women with estrogen receptor-positive (ER+) node-negative breast cancer. Ann Oncol. 2012 Mar;23(3):625-31. PMID: 21652577.

134. Bargallo JER, Lara F, Shaw Dulin RJ, et al. A study of the impact of the 21-gene breast cancer assay on the use of adjuvant chemotherapy in women with breast cancer in a Mexican public hospital. European Society for Medical Oncology Congress; 2012 September; Vienna, Austria.

135. Davidson JA, Cromwell I, Ellard SL, et al. A prospective clinical utility and pharmacoeconomic study of the impact of the 21-gene Recurrence Score(R) assay in oestrogen receptor positive node negative breast cancer. Eur J Cancer. 2013 Apr 20PMID: 23611660.

136. de Boer RH, Baker C, Speakman D, et al. The impact of a genomic assay (Oncotype DX) on adjuvant treatment recommendations in early breast cancer. Med J Aust. 2013 Aug 5;199(3):205-8. PMID: 23909545.

137. Eiermann W, Rezai M, Kummel S, et al. The 21-gene recurrence score assay impacts adjuvant therapy recommendations for ER-positive, node-negative and node-positive early breast cancer resulting in a risk-adapted change in chemotherapy use. Ann Oncol. 2013 Mar;24(3):618-24. PMID: 23136233.

138. Geffen DB, Abu-Ghanem S, Sion-Vardy N, et al. The impact of the 21-gene recurrence score assay on decision making about adjuvant chemotherapy in early-stage estrogen-receptor-positive breast cancer in an oncology practice with a unified treatment policy. Ann Oncol. 2011 Nov;22(11):2381-6. PMID: 21363879.

139. Henry LR, Stojadinovic A, Swain SM, et al. The influence of a gene expression profile on breast cancer decisions. J Surg Oncol. 2009 May 1;99(6):319-23. PMID: 19204954.

140. Holt S, Bertelli G, Humphreys I, et al. A decision impact, decision conflict and economic assessment of routine Oncotype DX testing of 146 women with node-negative or pNImi, ER-positive breast cancer in the U.K. Br J Cancer. 2013 Jun 11;108(11):2250-8. PMID: 23695023.

141. Joh JE, Esposito NN, Kiluk JV, et al. The effect of Oncotype DX recurrence score on treatment recommendations for patients with estrogen receptor-positive early stage breast cancer and correlation with estimation of recurrence risk by breast cancer specialists. Oncologist. 2011;16(11):1520-6.

142. Kamal AH, Loprinzi CL, Reynolds C, et al. Breast medical oncologists' use of standard prognostic factors to predict a 21-gene recurrence score. Oncologist. 2011;16(10):1359-66. PMID: 21934103.

143. Klang SH, Hammerman A, Liebermann N, et al. Economic implications of 21-gene breast cancer risk assay from the perspective of an Israeli-managed health-care organization. Value Health. 2010 Jun-Jul;13(4):381-7. PMID: 20412544.

144. Lo SS, Mumby PB, Norton J, et al. Prospective multicenter study of the impact of the 21-gene recurrence score assay on medical oncologist and patient adjuvant breast cancer treatment selection. J Clin Oncol. 2010 Apr 1;28(10):1671-6. PMID: 20065191.

145. Rayhanabad JA, Difronzo LA, Haigh PI, et al. Changing paradigms in breast cancer management: introducing molecular genetics into the treatment algorithm. Am Surg. 2008 Oct;74(10):887-90. PMID: 18942607.

146. Schneider JG, Khalil DN. Why does Oncotype DX recurrence score reduce adjuvant chemotherapy use? Breast Cancer Res Treat. 2012 Aug;134(3):1125-32. PMID: 22723033.

147. Yamauchi H, Nakagawa C, Takei H, et al. Prospective Study of the Effect of the 21-Gene Assay on Adjuvant Clinical Decision-Making in Japanese Women With Estrogen Receptor-Positive, Node-Negative, and Node-Positive Breast Cancer. Clin Breast Cancer. 2013 Oct 26PMID: 24321102.

148. Bueno-de-Mesquita JM, van Harten WH, Retel VP, et al. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). Lancet Oncol. 2007 Dec;8(12):1079-87. PMID: 18042430.

149. Cartwright T, Chao C, Lee M, et al. Effect of the 12-gene colon cancer assay results on adjuvant treatment recommendations in patients with stage II colon cancer. Curr Med Res Opin. 2013 Nov 7PMID: 24127781.

150. Srivastava G, Renfro LA, Behrens RJ, et al. Prospective evaluation of a 12-gene assay on treatment recommendations in patients with stage II colon cancer. ASCO Gastrointestinal Symposium; 2013 January; San Francisco, CA.

151. Brenner B, Lopatin M, Lee M, et al. Impact of the 12-gene colon cancer recurrence score assay on clinical decision-making for adjuvant therapy in stage II colon cancer patients in Israel. European Cancer Congress; 2013 September; Amsterdam, Netherlands.

152. Katkoori VR, Jia X, Shanmugam C, et al. Prognostic significance of p53 codon 72 polymorphism differs with race in colorectal adenocarcinoma. Clin Cancer Res. 2009 Apr 1;15(7):2406-16. PMID: 19339276.

153. Manne U, Weiss HL, Myers RB, et al. Nuclear accumulation of p53 in colorectal adenocarcinoma: prognostic importance differs with race and location of the tumor. Cancer. 1998 Dec 15;83(12):2456-67. PMID: 9874449.

Appendix A. Search Strategies

We conducted searches for relevant scientific literature using the following search strategies on 10/29/2012 and found 3,340 articles. We conducted an update literature using the same search terms and databases on 11/12/2013 with the following date limits: 10/29/2011 through 11/12/2013 to find more recently published articles. The update searches yielded a total of 1,233 citations, and after removing duplicates we added 468 new articles. The total number of unique sources found from the original and update searches and other sources such as hand searching and peer and public review comments was 3,850.

Table A1. PubMed update search strategies 11-11-2013

Search	Queries	Number of Citations
Urinary Bladder Cancer Search		
#1	Search "Urinary Bladder Neoplasms"[Mesh]	42283
#2	Search UroVysion	148
#3	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	6
#4	Search #1 AND (#2 OR #3)	104
#5	Search "Biological Assay"[Mesh]	32692
#6	Search "Predictive Value of Tests"[Mesh]	133463
#7	Search "Proportional Hazards Models"[Mesh]	40213
#8	Search "Outcome and Process Assessment (Health Care)"[Mesh]	670462
#9	Search assay OR assays OR test OR tests	7676643
#10	Search #5 OR #6 OR #7 OR #8 OR #9	8058148
#11	Search #4 AND #10	101
#12	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	257185
#13	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	16217
#14	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	71690
#15	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1194419
#16	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	96343
#17	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1212754
#18	Search #12 OR #13 OR #14 OR #15 OR #16 OR #17	2407314
#19	Search #4 AND (#10 OR #18)	103
#20	Search #4 AND (#10 OR #18) Humans	103
#21	Search #4 AND (#10 OR #18) Humans; English	97
#22	Search #4 AND (#10 OR #18) Humans; English; Adult: 19+ years	54
#23	Search #4 AND (#10 OR #18) Humans; English; Adult: 19+ years; Publication date from 1980/01/01	54
#24	Search #4 AND (#10 OR #18) Humans; English; Adult: 19+ years; Publication date from 2011/10/29	7
Breast Cancer Search		
#1	Search "Breast Neoplasms"[Mesh]	207319
#2	Search "oncotype DX" OR "21 gene recurrence score" OR "21 gene recurrence"	210

Search	Queries	Number of Citations
#3	Search “Mammaprint” OR “70 gene signature”	130
#4	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	6
#5	Search #2 OR #3 OR #4	298
#6	Search #1 AND #5	213
#7	Search "Biological Assay"[Mesh]	32692
#8	Search "Predictive Value of Tests"[Mesh]	133463
#9	Search "Proportional Hazards Models"[Mesh]	40213
#10	Search "Outcome and Process Assessment (Health Care)"[Mesh]	670462
#11	Search assay OR assays OR test OR tests	7676643
#12	Search #7 OR #8 OR #9 OR #10 OR #11	8058148
#13	Search #6 AND #12	194
#14	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	257185
#15	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	16217
#16	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	71690
#17	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1194419
#18	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	96343
#19	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH Terms] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1212754
#20	Search #14 OR #15 OR #16 OR #17 OR #18 OR #19	2407314
#21	Search #6 AND (#12 OR #20)	202
#22	Search #6 AND (#12 OR #20) Humans	202
#23	Search #6 AND (#12 OR #20) Humans; English	195
#24	Search #6 AND (#12 OR #20) Humans; English; Adult: 19+ years	97
#25	Search #6 AND (#12 OR #20) Humans; English; Adult: 19+ years; Publication date from 1980/01/01	97
#26	Search #6 AND (#12 OR #20) Humans; English; Adult: 19+ years; Publication date from 2011/10/29	33
Colorectal Cancer Search		
#1	Search "Colorectal Neoplasms"[Mesh]	140842
#2	Search "Microsatellite Instability"[Mesh] OR "Microsatellite Instability"	5399
#3	Search MLH1 promoter methylation	627
#4	Search "KRAS protein, human" [Supplementary Concept] OR KRAS	8312
#5	Search "BRAF protein, human" [Supplementary Concept] OR BRAF	4522
#6	Search "Oncotype DX Colon" OR "21 gene recurrence score" OR "21 gene recurrence"	58
#7	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	6
#8	Search #2 OR #3 OR #4 OR #5 OR #6 OR #7	16721
#9	Search #1 AND #8	4188
#10	Search "Biological Assay"[Mesh]	32692
#11	Search "Predictive Value of Tests"[Mesh]	133463
#12	Search "Proportional Hazards Models"[Mesh]	40213
#13	Search "Outcome and Process Assessment (Health Care)"[Mesh]	670462
#14	Search assay OR assays OR test OR tests	7676643
#15	Search #10 OR #11 OR #12 OR #13 OR #14	8058148
#16	Search #9 AND #15	2770
#17	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	257185
#18	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	16217

Search	Queries	Number of Citations
#19	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	71690
#20	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1194419
#21	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	96343
#22	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH Terms] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1212754
#23	Search #17 OR #18 OR #19 OR #20 OR #21 OR #22	2407314
#24	Search #9 AND (#15 OR #23)	2993
#25	Search #9 AND (#15 OR #23) Filters: Humans	2962
#26	Search #9 AND (#15 OR #23) Filters: Humans; English	2797
#27	Search #9 AND (#15 OR #23) Filters: Humans; English; Adult: 19+ years	1563
#28	Search #9 AND (#15 OR #23) Filters: Humans; English; Adult: 19+ years; Publication date from 1980/01/01	1563
#29	Search #9 AND (#15 OR #23) Filters: Humans; English; Adult: 19+ years; Publication date from 2011/10/29	345
Lung Cancer Search		
#1	Search "Lung Neoplasms"[Mesh]	166161
#2	Search "Carcinoma, Bronchogenic"[Mesh]	38075
#3	Search #1 OR #2	166161
#4	Search EML4-ALK fusion protein, human[Supplementary Concept]	143
#5	Search ALK	3768
#6	Search "EGFR protein, human"[Supplementary Concept] OR EGFR	26495
#7	Search "KRAS protein, human"[Supplementary Concept] OR KRAS	8312
#8	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	6
#9	Search #4 OR #5 OR #6 OR #7 OR #8	36797
#10	Search #3 AND #9	4329
#11	Search "Biological Assay"[Mesh]	32692
#12	Search "Predictive Value of Tests"[Mesh]	133463
#13	Search "Proportional Hazards Models"[Mesh]	40213
#14	Search "Outcome and Process Assessment (Health Care)"[Mesh]	670462
#15	Search assay OR assays OR test OR tests	7676643
#16	Search #11 OR #12 OR #13 OR #14 OR #15	8058148
#17	Search #10 AND #16	2682
#18	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	257185
#19	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	16217
#20	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	71690
#21	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1194419
#22	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	96343
#23	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH Terms] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1212754

Search	Queries	Number of Citations
#24	Search #18 OR #19 OR #20 OR #21 OR #22 OR #23	2407314
#25	Search #10 AND (#16 OR #24)	2992
#26	Search #10 AND (#16 OR #24) Filters: Humans	2912
#27	Search #10 AND (#16 OR #24) Humans; English	2696
#28	Search #10 AND (#16 OR #24) Humans; English; Adult: 19+ years	1292
#29	Search #10 AND (#16 OR #24) Humans; English; Adult: 19+ years; Publication date from 1980/01/01	1292
#30	Search #10 AND (#16 OR #24) Humans; English; Adult: 19+ years; Publication date from 2011/10/29	423

Table A2. Embase search strategies update 11-12-2013

Search	Queries	Number of Citations
Urinary Bladder Cancer Search		
#1	'bladder tumor'/exp AND ([embase]/lim OR [embase classic]/lim)	46,402
#2	urovysion AND ([embase]/lim OR [embase classic]/lim)	257
#3	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	13
#4	#1 AND (#2 OR #3)	170
#5	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,120
#6	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	36,618
#7	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	31,586
#8	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	770,815
#9	'assay'/de OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,791,862
#10	#5 OR #6 OR #7 OR #8 OR #9	3,455,670
#11	#4 AND #10	124
#12	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	58,613
#13	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	21,815
#14	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	42,593
#15	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	553,407
#16	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,266,288
#17	'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	284,237
#18	#12 OR #13 OR #14 OR #15 OR #16 OR #17	1,942,881
#19	#4 AND (#10 OR #18)	158
#20	#19 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	33
#21	#19 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py AND [29-10-2011]/sd	5
Breast Cancer Search		
#1	'breast cancer'/exp AND ([embase]/lim OR [embase classic]/lim)	269,829
#2	'oncotype dx' OR '21 gene recurrence score' OR '21 gene recurrence' AND	606

Search	Queries	Number of Citations
	([embase]/lim OR [embase classic]/lim)	
#3	'mammaprint' OR '70 gene signature' AND ([embase]/lim OR [embase classic]/lim)	341
#4	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	13
#5	#2 OR #3 OR #4	825
#6	#1 AND #5	762
#7	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,120
#8	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	36,618
#9	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	31,586
#10	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	770,815
#11	'assay' OR 'assay'/exp OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,791,862
#12	#7 OR #8 OR #9 OR #10 OR #11	3,455,570
#13	#6 AND #12	551
#14	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	58,613
#15	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	21,815
#16	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	42,593
#17	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	563,407
#18	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,266,228
#19	'probability' OR 'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	284,237
#20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	1,942,881
#21	#6 AND (#12 OR #20)	610
#22	#21 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	68
#23	#21 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py AND [29-10-2011]/sd	22
Colorectal Cancer Search		
#1	'colorectal cancer'/exp AND ([embase]/lim OR [embase classic]/lim)	75,215
#2	'microsatellite instability'/exp OR 'microsatellite instability' AND ([embase]/lim OR [embase classic]/lim)	6,746
#3	mlh1 AND ('promoter'/exp OR promoter) AND ('methylation'/exp OR methylation) AND ([embase]/lim OR [embase classic]/lim)	275
#4	kras AND ([embase]/lim OR [embase classic]/lim)	7,176
#5	braf AND ([embase]/lim OR [embase classic]/lim)	6,862
#6	'oncotype dx colon' OR '21 gene recurrence score' OR '21 gene recurrence' AND ([embase]/lim OR [embase classic]/lim)	135
#7	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	13
#8	#2 OR #3 OR #4 OR #5 OR #6 OR #7	18,195
#9	#1 AND #8	4,955
#10	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,120
#11	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	36,618

Search	Queries	Number of Citations
#12	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	31,586
#13	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	770,815
#14	'assay' OR 'assay'/exp OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,791,862
#15	#10 OR #11 OR #12 OR #13 OR #14	3,455,670
#16	#9 AND #15	1,455
#17	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	58,613
#18	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	21,815
#19	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	42,593
#20	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	553,407
#21	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,266,228
#22	'probability' OR 'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	284,237
#23	#17 OR #18 OR #19 OR #20 OR #21 OR #22	1,942,881
#24	#9 AND (#15 OR #23)	2,108
#25	#24 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	446
#26	#24 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py AND [29-10-2011]/sd	100
Lung Cancer Search		
#1	'lung cancer'/exp AND ([embase]/lim OR [embase classic]/lim)	185,331
#2	'lung carcinoma'/exp AND ([embase]/lim OR [embase classic]/lim)	95,316
#3	#1 OR #2	185,331
#4	'eml4-alk' AND ([embase]/lim OR [embase classic]/lim)	592
#5	alk AND ([embase]/lim OR [embase classic]/lim)	6,136
#6	egfr AND ([embase]/lim OR [embase classic]/lim)	37,705
#7	kras AND ([embase]/lim OR [embase classic]/lim)	7,176
#8	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	13
#9	#4 OR #5 OR #6 OR #7 OR #8	47,551
#10	#3 AND #9	8,953
#11	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,120
#12	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	36,618
#13	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	31,586
#14	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	770,815
#15	'assay' OR 'assay'/exp OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,791,862
#16	#11 OR #12 OR #13 OR #14 OR #15	3,455,670
#17	#10 AND #16	2,799
#18	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	58,613
#19	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	21,815

Search	Queries	Number of Citations
#20	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	42,593
#21	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	553,407
#22	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,266,228
#23	'probability' OR 'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	284,237
#24	#18 OR #19 OR #20 OR #21 OR #22 OR #23	1,942,881
#25	#10 AND (#16 OR #24)	4,029
#26	#25 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	756
#27	#25 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py AND [29-10-2011]/sd	241

Table A3. Cochrane search strategies update 11-12-2013

Search	Queries	Number of Citations
Urinary Bladder Cancer Search		
#1	MeSH descriptor: [Urinary Bladder Neoplasms] explode all trees	988
#2	urovysion	2
#3	ivdmia	1
#4	"in vitro multivariate index assay"	0
#5	#1 and (#2 or #3 or #4)	2
#6	MeSH descriptor: [Biological Assay] explode all trees	153
#7	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#8	MeSH descriptor: [Proportional Hazards Models] explode all trees	3086
#9	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	89878
#10	assay	11865
#11	assays	11865
#12	test	149897
#13	tests	149897
#14	#6 or #7 or #8 or #9 or #10 or #11 or #12 or #13	225622
#15	MeSH descriptor: [Reproducibility of Results] explode all trees	8399
#16	"reproducibility of results"	8720
#17	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	4
#18	"signal-to-noise ratio"	154
#19	MeSH descriptor: [ROC Curve] explode all trees	939
#20	"ROC Curve"	1224
#21	"receiver operating characteristic"	1253
#22	MeSH descriptor: [Observer Variation] explode all trees	1601
#23	“observer variation”	1796
#24	MeSH descriptor: [Sensitivity and Specificity] explode all trees	14453
#25	sensitivity:ti (Word variations have been searched)	2853
#26	sensitivity:ab (Word variations have been searched)	15946
#27	"sensitivity and specificity"	11631

Search	Queries	Number of Citations
#28	“analytic validity”	9
#29	MeSH descriptor: [Diagnostic Errors] explode all trees	2444
#30	diagnos*:ti,ab	35756
#31	MeSH descriptor: [Diagnosis] this term only	232499
#32	MeSH descriptor: [Diagnosis, Differential] this term only	1401
#33	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	35316
#34	accuracy	10492
#35	MeSH descriptor: [Diagnosis] explode all trees	232499
#36	diagnostic accuracy	4494
#37	precision	3052
#38	"diagnostic error*"	312
#39	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#40	MeSH descriptor: [Probability] explode all trees	34522
#41	probability	12210
#42	likelihood	6636
#43	MeSH descriptor: [False Negative Reactions] explode all trees	323
#44	MeSH descriptor: [False Positive Reactions] explode all trees	490
#45	"predictive value of test*"	5548
#46	forecasting	624
#47	MeSH descriptor: [Forecasting] explode all trees	456
#48	#15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47	289183
#49	#5 and (#14 or #48)	2
#50	#5 and (#14 or #48) from 1980	2
#51	#5 and (#14 or #48) from 2011	0
Breast Cancer Search		
#1	MeSH descriptor: [Breast Neoplasms] explode all trees	8030
#2	"oncotype DX"	13
#3	"21 gene recurrence score"	11
#4	"21 gene recurrence"	11
#5	Mammaprint	10
#6	"70 gene signature"	3
#7	ivdmia	1
#8	"in vitro multivariate index assay"	0
#9	#1 AND (#2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8)	30
#10	MeSH descriptor: [Biological Assay] explode all trees	153
#11	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#12	MeSH descriptor: [Proportional Hazards Models] explode all trees	3086
#13	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	89878
#14	assay	11865
#15	assays	11865
#16	test	149897
#17	tests	149897
#18	#10 or #11 or #12 or #13 or #14 or #15 or #16 or #17	225622
#19	MeSH descriptor: [Reproducibility of Results] explode all trees	8399
#20	"reproducibility of results"	8720
#21	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	4
#22	"signal-to-noise ratio"	154
#23	MeSH descriptor: [ROC Curve] explode all trees	939
#24	"ROC Curve"	1224
#25	"receiver operating characteristic"	1253
#26	MeSH descriptor: [Observer Variation] explode all trees	1601

Search	Queries	Number of Citations
#27	“observer variation”	1796
#28	MeSH descriptor: [Sensitivity and Specificity] explode all trees	14453
#29	sensitivity:ti (Word variations have been searched)	2853
#30	sensitivity:ab (Word variations have been searched)	15946
#31	"sensitivity and specificity"	11631
#32	“analytic validity”	9
#33	MeSH descriptor: [Diagnostic Errors] explode all trees	2444
#34	diagnos*:ti,ab	35756
#35	MeSH descriptor: [Diagnosis] this term only	232499
#36	MeSH descriptor: [Diagnosis, Differential] this term only	1401
#37	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	35316
#38	accuracy	10492
#39	MeSH descriptor: [Diagnosis] explode all trees	232499
#40	diagnostic accuracy	4494
#41	precision	3052
#42	"diagnostic error*"	312
#43	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#44	MeSH descriptor: [Probability] explode all trees	34522
#45	probability	12210
#46	likelihood	6636
#47	MeSH descriptor: [False Negative Reactions] explode all trees	323
#48	MeSH descriptor: [False Positive Reactions] explode all trees	490
#49	"predictive value of test*"	5548
#50	forecasting	624
#51	MeSH descriptor: [Forecasting] explode all trees	456
#52	#19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51	289183
#53	#9 and (#18 or #52)	29
#54	#9 and (#18 or #52) from 1980	29
#55	#9 and (#18 or #52) from 2011	16
Colorectal Cancer Search		
#1	MeSH descriptor: [Colorectal Neoplasms] explode all trees	8030
#2	MeSH descriptor: [Microsatellite Instability] explode all trees	25
#3	"Microsatellite Instability"	59
#4	MLH1 promoter methylation	3
#5	KRAS protein, human	90
#6	KRAS	140
#7	BRAF protein, human	41
#8	"Oncotype DX Colon"	0
#9	"21 gene recurrence score"	11
#10	"21 gene recurrence"	11
#11	ivdmia	1
#12	"in vitro multivariate index assay"	0
#13	#1 and (#2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12)	14
#14	MeSH descriptor: [Biological Assay] explode all trees	153
#15	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#16	MeSH descriptor: [Proportional Hazards Models] explode all trees	3086
#17	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	89878
#18	assay	11865
#19	assays	11865
#20	test	149897
#21	tests	149897

Search	Queries	Number of Citations
#22	#14 or #15 or #16 or #17 or #18 or #19 or #20 or #21	225622
#23	MeSH descriptor: [Reproducibility of Results] explode all trees	8399
#24	"reproducibility of results"	8720
#25	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	4
#26	"signal-to-noise ratio"	154
#27	MeSH descriptor: [ROC Curve] explode all trees	939
#28	"ROC Curve"	1224
#29	"receiver operating characteristic"	1253
#30	MeSH descriptor: [Observer Variation] explode all trees	1601
#31	“observer variation”	1796
#32	MeSH descriptor: [Sensitivity and Specificity] explode all trees	14453
#33	sensitivity:ti (Word variations have been searched)	2853
#34	sensitivity:ab (Word variations have been searched)	15946
#35	"sensitivity and specificity"	11631
#36	“analytic validity”	9
#37	MeSH descriptor: [Diagnostic Errors] explode all trees	2444
#38	diagnos*:ti,ab	35756
#39	MeSH descriptor: [Diagnosis] this term only	232499
#40	MeSH descriptor: [Diagnosis, Differential] this term only	1401
#41	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	35316
#42	accuracy	10492
#43	MeSH descriptor: [Diagnosis] explode all trees	232499
#44	diagnostic accuracy	4494
#45	precision	3052
#46	"diagnostic error*"	312
#47	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#48	MeSH descriptor: [Probability] explode all trees	34522
#49	probability	12210
#50	likelihood	6636
#51	MeSH descriptor: [False Negative Reactions] explode all trees	323
#52	MeSH descriptor: [False Positive Reactions] explode all trees	490
#53	"predictive value of test*"	5548
#54	forecasting	624
#55	MeSH descriptor: [Forecasting] explode all trees	456
#56	#23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54 or #55	289183
#57	#13 and (#22 or #56)	14
#58	#13 and (#22 or #56) from 1980	14
#59	#13 and (#22 or #56) from 2011	7
Lung Cancer Search		
#1	MeSH descriptor: [Lung Neoplasms] explode all trees	4548
#2	MeSH descriptor: [Carcinoma, Bronchogenic] explode all trees	2388
#3	#1 or #2	4548
#4	EML4-ALK fusion protein, human	0
#5	ALK	125
#6	EGFR protein, human	221
#7	EGFR	655
#8	KRAS protein, human	90
#9	KRAS	140
#10	ivdmia	1
#11	"in vitro multivariate index assay"	0
#12	#3 and (#4 or #5 or #6 or #8 or #9 or #10 or #11)	75
#13	MeSH descriptor: [Biological Assay] explode all trees	153

Search	Queries	Number of Citations
#14	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#15	MeSH descriptor: [Proportional Hazards Models] explode all trees	3086
#16	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	89878
#17	assay	11865
#18	assays	11865
#19	test	149897
#20	tests	149897
#21	#13 or #14 or #15 or #16 or #17 or #18 or #19 or #20	225622
#22	MeSH descriptor: [Reproducibility of Results] explode all trees	8399
#23	"reproducibility of results"	8720
#24	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	4
#25	"signal-to-noise ratio"	154
#26	MeSH descriptor: [ROC Curve] explode all trees	939
#27	"ROC Curve"	1224
#28	"receiver operating characteristic"	1253
#29	MeSH descriptor: [Observer Variation] explode all trees	1601
#30	“observer variation”	1796
#31	MeSH descriptor: [Sensitivity and Specificity] explode all trees	14453
#32	sensitivity:ti (Word variations have been searched)	2853
#33	sensitivity:ab (Word variations have been searched)	15946
#34	"sensitivity and specificity"	11631
#35	“analytic validity”	9
#36	MeSH descriptor: [Diagnostic Errors] explode all trees	2444
#37	diagnos*:ti,ab	35756
#38	MeSH descriptor: [Diagnosis] this term only	232499
#39	MeSH descriptor: [Diagnosis, Differential] this term only	1401
#40	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	35316
#41	accuracy	10492
#42	MeSH descriptor: [Diagnosis] explode all trees	232499
#43	diagnostic accuracy	4494
#44	precision	3052
#45	"diagnostic error*"	312
#46	MeSH descriptor: [Predictive Value of Tests] explode all trees	5420
#47	MeSH descriptor: [Probability] explode all trees	34522
#48	probability	12210
#49	likelihood	6636
#50	MeSH descriptor: [False Negative Reactions] explode all trees	323
#51	MeSH descriptor: [False Positive Reactions] explode all trees	490
#52	"predictive value of test*"	5548
#53	forecasting	624
#54	MeSH descriptor: [Forecasting] explode all trees	456
#55	#22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54	289183
#56	#12 and (#21 or #55)	60
#57	#12 and (#21 or #55) from 1980	60
#58	#12 and (#21 or #55) from 2011	34

Table A4. PubMed search strategies 10-29-2012

Search	Queries	Number of Citations
Urinary Bladder Cancer Search		
#1	Search "Urinary Bladder Neoplasms"[Mesh]	40721
#2	Search UroVysion	127
#3	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	138
#4	Search #1 AND (#2 OR #3)	98
#5	Search "Biological Assay"[Mesh]	31814
#6	Search "Predictive Value of Tests"[Mesh]	123188
#7	Search "Proportional Hazards Models"[Mesh]	35131
#8	Search "Outcome and Process Assessment (Health Care)"[Mesh]	616326
#9	Search assay OR assays OR test OR tests	7254565
#10	Search #5 OR #6 OR #7 OR #8 OR #9	7608298
#11	Search #4 AND #10	95
#12	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	237807
#13	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	14372
#14	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	63708
#15	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1119176
#16	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	87894
#17	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1129894
#18	Search #12 OR #13 OR #14 OR #15 OR #16 OR #17	2249689
#19	Search #4 AND (#10 OR #18)	97
#20	Search #4 AND (#10 OR #18) Humans	97
#21	Search #4 AND (#10 OR #18) Humans; English	92
#22	Search #4 AND (#10 OR #18) Humans; English; Adult: 19+ years	51
#23	Search #4 AND (#10 OR #18) Humans; English; Adult: 19+ years; Publication date from 1980/01/01	51
Breast Cancer Search		
#1	Search "Breast Neoplasms"[Mesh]	196562
#2	Search "oncotype DX" OR "21 gene recurrence score" OR "21 gene recurrence"	158
#3	Search “Mammaprint” OR “70 gene signature”	101
#4	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	138
#5	Search #2 OR #3 OR #4	358
#6	Search #1 AND #5	178
#7	Search "Biological Assay"[Mesh]	31814
#8	Search "Predictive Value of Tests"[Mesh]	123188
#9	Search "Proportional Hazards Models"[Mesh]	35131
#10	Search "Outcome and Process Assessment (Health Care)"[Mesh]	616326
#11	Search assay OR assays OR test OR tests	7254565
#12	Search #7 OR #8 OR #9 OR #10 OR #11	7588075
#13	Search #6 AND #12	164
#14	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	237807
#15	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	14372
#16	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR	63708

Search	Queries	Number of Citations
	“observer variation”[all fields]	
#17	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1119176
#18	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	87894
#19	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH Terms] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1129894
#20	Search #14 OR #15 OR #16 OR #17 OR #18 OR #19	2249689
#21	Search #6 AND (#12 OR #20)	170
#22	Search #6 AND (#12 OR #20) Humans	170
#23	Search #6 AND (#12 OR #20) Humans; English	166
#24	Search #6 AND (#12 OR #20) Humans; English; Adult: 19+ years	84
#25	Search #6 AND (#12 OR #20) Humans; English; Adult: 19+ years; Publication date from 1980/01/01	84
Colorectal Cancer Search		
#1	Search "Colorectal Neoplasms"[Mesh]	133525
#2	Search "Microsatellite Instability"[Mesh] OR "Microsatellite Instability"	5029
#3	Search MLH1 promoter methylation	581
#4	Search "KRAS protein, human" [Supplementary Concept] OR KRAS	3263
#5	Search "BRAF protein, human" [Supplementary Concept] OR BRAF	3374
#6	Search "Oncotype DX Colon" OR "21 gene recurrence score" OR "21 gene recurrence"	46
#7	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	138
#8	Search #2 OR #3 OR #4 OR #5 OR #6 OR #7	10733
#9	Search #1 AND #8	3532
#10	Search "Biological Assay"[Mesh]	31814
#11	Search "Predictive Value of Tests"[Mesh]	123188
#12	Search "Proportional Hazards Models"[Mesh]	35131
#13	Search "Outcome and Process Assessment (Health Care)"[Mesh]	616326
#14	Search assay OR assays OR test OR tests	7254565
#15	Search #10 OR #11 OR #12 OR #13 OR #14	7608298
#16	Search #9 AND #15	2315
#17	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	237807
#18	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	14372
#19	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	63708
#20	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1119176
#21	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	87894
#22	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH Terms] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1129894
#23	Search #17 OR #18 OR #19 OR #20 OR #21 OR #22	2249689
#24	Search #9 AND (#15 OR #23)	2513
#25	Search #9 AND (#15 OR #23) Filters: Humans	2492
#26	Search #9 AND (#15 OR #23) Filters: Humans; English	2363

Search	Queries	Number of Citations
#27	Search #9 AND (#15 OR #23) Filters: Humans; English; Adult: 19+ years	1338
#28	Search #9 AND (#15 OR #23) Filters: Humans; English; Adult: 19+ years; Publication date from 1980/01/01	1338
Lung Cancer Search		
#1	Search "Lung Neoplasms"[Mesh]	158827
#2	Search "Carcinoma, Bronchogenic"[Mesh]	35282
#3	Search #1 OR #2	158827
#4	Search EML4-ALK fusion protein, human[Supplementary Concept]	90
#5	Search ALK	3208
#6	Search "EGFR protein, human"[Supplementary Concept] OR EGFR	22440
#7	Search "KRAS protein, human"[Supplementary Concept] OR KRAS	3263
#8	Search IVDMIA OR "in vitro diagnostic multivariate index assay"	138
#9	Search #4 OR #5 OR #6 OR #7 OR #8	27905
#10	Search #3 AND #9	3380
#11	Search "Biological Assay"[Mesh]	31814
#12	Search "Predictive Value of Tests"[Mesh]	123188
#13	Search "Proportional Hazards Models"[Mesh]	35131
#14	Search "Outcome and Process Assessment (Health Care)"[Mesh]	616326
#15	Search assay OR assays OR test OR tests	7254565
#16	Search #11 OR #12 OR #13 OR #14 OR #15	7608298
#17	Search #10 AND #16	2081
#18	Search "Reproducibility of Results"[Mesh] OR “reproducibility of results”[all fields]	237807
#19	Search “Signal-to-Noise Ratio”[MeSH] OR “signal-to-noise ratio”[all fields]	14372
#20	Search “ROC Curve”[Mesh] OR "ROC curve"[All Fields] OR "receiver operating characteristic"[All Fields] OR “observer variation”[MeSH] OR “observer variation”[all fields]	63708
#21	Search “Sensitivity and Specificity”[Mesh] OR sensitiv*[Title/Abstract] OR "sensitivity and specificity"[All Fields] OR “analytic validity”[all fields]	1119176
#22	Search “Diagnostic Errors”[Mesh] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp] OR “accuracy”[All Fields] OR "diagnosis"[MeSH] "diagnostic accuracy”[All Fields] OR “precision”[All Fields] OR "diagnostic error*"[All Fields]	87894
#23	Search “Predictive Value of Tests”[Mesh] OR "probability"[MeSH Terms] OR "probability"[All Fields] OR "likelihood"[All Fields] OR “false negative reactions”[MeSH] OR “false positive reactions”[MeSH] OR "predictive value of tests"[All Fields] OR "forecasting"[All Fields] OR "forecasting"[MeSH]	1129894
#24	Search #18 OR #19 OR #20 OR #21 OR #22 OR #23	2249689
#25	Search #10 AND (#16 OR #24)	2338
#26	Search #10 AND (#16 OR #24) Filters: Humans	2294
#27	Search #10 AND (#16 OR #24) Humans; English	2122
#28	Search #10 AND (#16 OR #24) Humans; English; Adult: 19+ years	1005
#29	Search #10 AND (#16 OR #24) Humans; English; Adult: 19+ years; Publication date from 1980/01/01	1005

Table A5. Embase search strategies 10-26-2012

Search	Queries	Number of Citations
Urinary Bladder Cancer Search		
#1	'bladder tumor'/exp AND ([embase]/lim OR [embase classic]/lim)	44,524
#2	urovysion AND ([embase]/lim OR [embase classic]/lim)	221
#3	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	11
#4	#1 AND (#2 OR #3)	147
#5	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,952
#6	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	20,862
#7	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	24,303
#8	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	700,975
#9	'assay'/de OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,963,585
#10	#5 OR #6 OR #7 OR #8 OR #9	3,557,123
#11	#4 AND #10	114
#12	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	55,781
#13	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	22,964
#14	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	45,019
#15	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	532,369
#16	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,314,969
#17	'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	809,717
#18	#12 OR #13 OR #14 OR #15 OR #16 OR #17	2,381,955
#19	#4 AND (#10 OR #18)	142
#20	#19 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	32
Breast Cancer Search		
#1	'breast cancer'/exp AND ([embase]/lim OR [embase classic]/lim)	250,650
#2	'oncotype dx' OR '21 gene recurrence score' OR '21 gene recurrence' AND ([embase]/lim OR [embase classic]/lim)	404
#3	'mammaprint' OR '70 gene signature' AND ([embase]/lim OR [embase classic]/lim)	251
#4	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	11
#5	#2 OR #3 OR #4	562
#6	#1 AND #5	520
#7	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,957
#8	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	20,904
#9	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	24,333
#10	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	701,175
#11	'assay' OR 'assay'/exp OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,964,565
#12	#7 OR #8 OR #9 OR #10 OR #11	3,558,292
#13	#6 AND #12	386
#14	'reproducibility of results'/exp OR 'reproducibility of results' AND	55,807

Search	Queries	Number of Citations
	([embase]/lim OR [embase classic]/lim)	
#15	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	22,980
#16	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	45,053
#17	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	532,590
#18	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,315,423
#19	'probability' OR 'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	810,061
#20	#14 OR #15 OR #16 OR #17 OR #18 OR #19	2,382,853
#21	#6 AND (#12 OR #20)	480
#22	#21 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	82
Colorectal Cancer Search		
#1	'colorectal cancer'/exp AND ([embase]/lim OR [embase classic]/lim)	64,587
#2	'microsatellite instability'/exp OR 'microsatellite instability' AND ([embase]/lim OR [embase classic]/lim)	7,045
#3	mlh1 AND ('promoter'/exp OR promoter) AND ('methylation'/exp OR methylation) AND ([embase]/lim OR [embase classic]/lim)	703
#4	kras AND ([embase]/lim OR [embase classic]/lim)	4,869
#5	braf AND ([embase]/lim OR [embase classic]/lim)	4,411
#6	'oncotype dx colon' OR '21 gene recurrence score' OR '21 gene recurrence' AND ([embase]/lim OR [embase classic]/lim)	86
#7	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	11
#8	#2 OR #3 OR #4 OR #5 OR #6 OR #7	14,748
#9	#1 AND #8	4,149
#10	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,957
#11	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	20,904
#12	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	24,333
#13	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	701,175
#14	'assay' OR 'assay'/exp OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,964,565
#15	#10 OR #11 OR #12 OR #13 OR #14	3,558,292
#16	#9 AND #15	1,190
#17	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	55,807
#18	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	22,980
#19	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	45,053
#20	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	532,590
#21	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase	1,315,423

Search	Queries	Number of Citations
	classic]/lim)	
#22	'probability' OR 'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	810,061
#23	#17 OR #18 OR #19 OR #20 OR #21 OR #22	2,382,853
#24	#9 AND (#15 OR #23)	2,184
#25	#24 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	605
Lung Cancer Search		
#1	'lung cancer'/exp AND ([embase]/lim OR [embase classic]/lim)	176,206
#2	'lung carcinoma'/exp AND ([embase]/lim OR [embase classic]/lim)	90,226
#3	#1 OR #2	176,206
#4	'eml4-alk' AND ([embase]/lim OR [embase classic]/lim)	337
#5	alk AND ([embase]/lim OR [embase classic]/lim)	4,992
#6	egfr AND ([embase]/lim OR [embase classic]/lim)	30,017
#7	kras AND ([embase]/lim OR [embase classic]/lim)	4,869
#8	ivdmia OR 'in vitro diagnostic multivariate index assay' AND ([embase]/lim OR [embase classic]/lim)	11
#9	#4 OR #5 OR #6 OR #7 OR #8	37,784
#10	#3 AND #9	6,492
#11	'biological assay'/exp AND ([embase]/lim OR [embase classic]/lim)	27,957
#12	'predictive value of tests'/exp AND ([embase]/lim OR [embase classic]/lim)	20,904
#13	'proportional hazards models'/exp AND ([embase]/lim OR [embase classic]/lim)	24,333
#14	'treatment outcome'/exp AND ([embase]/lim OR [embase classic]/lim)	701,175
#15	'assay' OR 'assay'/exp OR assay OR assays OR test OR tests AND ([embase]/lim OR [embase classic]/lim)	2,964,565
#16	#11 OR #12 OR #13 OR #14 OR #15	3,558,292
#17	#10 AND #16	2,150
#18	'reproducibility of results'/exp OR 'reproducibility of results' AND ([embase]/lim OR [embase classic]/lim)	55,807
#19	'signal-to-noise ratio'/exp OR 'signal-to-noise ratio' AND ([embase]/lim OR [embase classic]/lim)	22,980
#20	'roc curve'/exp OR 'roc curve' OR 'receiver operating characteristic'/exp OR 'receiver operating characteristic' OR 'observer variation'/exp OR 'observer variation' AND ([embase]/lim OR [embase classic]/lim)	45,053
#21	'sensitivity and specificity'/exp OR 'sensitivity and specificity' OR 'sensitivity':ti OR 'sensitivity':ab OR 'analytic validity' AND ([embase]/lim OR [embase classic]/lim)	532,590
#22	'diagnostic errors'/exp OR 'diagnostic errors' OR 'diagnosis':ti OR 'diagnosis':ab OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy' OR 'precision'/exp OR 'precision' AND ([embase]/lim OR [embase classic]/lim)	1,315,423
#23	'probability' OR 'probability'/exp OR probability OR likelihood OR 'false negative reactions'/exp OR 'false negative reactions' OR 'false positive reactions'/exp OR 'false positive reactions' OR 'predictive value of tests'/exp OR 'predictive value of tests' OR 'forecasting' OR 'forecasting'/exp OR forecasting AND ([embase]/lim OR [embase classic]/lim)	810,061
#24	#18 OR #19 OR #20 OR #21 OR #22 OR #23	2,382,853
#25	#10 AND (#16 OR #24)	3,437
#26	#25 AND ([adult]/lim OR [aged]/lim) AND [humans]/lim AND [english]/lim AND ([embase]/lim OR [embase classic]/lim) AND [1980-2012]/py	878

Table A6. Cochrane search strategies 10-29-2012

Search	Queries	Number of Citations
Urinary Bladder Cancer Search		
#1	MeSH descriptor: [Urinary Bladder Neoplasms] explode all trees	919
#2	urovysion	2
#3	ivdmia	1
#4	"in vitro multivariate index assay"	0
#5	#1 and (#2 or #3 or #4)	2
#6	MeSH descriptor: [Biological Assay] explode all trees	152
#7	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#8	MeSH descriptor: [Proportional Hazards Models] explode all trees	2827
#9	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	83811
#10	assay	11447
#11	assays	11447
#12	test	144378
#13	tests	144378
#14	#6 or #7 or #8 or #9 or #10 or #11 or #12 or #13	215226
#15	MeSH descriptor: [Reproducibility of Results] explode all trees	8077
#16	"reproducibility of results"	8375
#17	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	0
#18	"signal-to-noise ratio"	145
#19	MeSH descriptor: [ROC Curve] explode all trees	851
#20	"ROC Curve"	1113
#21	"receiver operating characteristic"	1107
#22	MeSH descriptor: [Observer Variation] explode all trees	1556
#23	“observer variation”	2444
#24	MeSH descriptor: [Sensitivity and Specificity] explode all trees	13656
#25	sensitivity:ti (Word variations have been searched)	2714
#26	sensitivity:ab (Word variations have been searched)	15456
#27	"sensitivity and specificity"	11058
#28	“analytic validity”	1481
#29	MeSH descriptor: [Diagnostic Errors] explode all trees	2362
#30	diagnos*:ti,ab	34133
#31	MeSH descriptor: [Diagnosis] this term only	79
#32	MeSH descriptor: [Diagnosis, Differential] this term only	1338
#33	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	33493
#34	accuracy	9811
#35	MeSH descriptor: [Diagnosis] explode all trees	221504
#36	diagnostic accuracy	4126
#37	precision	2746
#38	"diagnostic error*"	288
#39	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#40	MeSH descriptor: [Probability] explode all trees	31596
#41	probability	11646
#42	likelihood	6058
#43	MeSH descriptor: [False Negative Reactions] explode all trees	314
#44	MeSH descriptor: [False Positive Reactions] explode all trees	470
#45	"predictive value of test*"	5202
#46	forecasting	588
#47	MeSH descriptor: [Forecasting] explode all trees	423
#48	#15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47	275562
#49	#5 and (#14 or #48)	2
#50	#5 and (#14 or #48) from 1980	2
Breast Cancer Search		

Search	Queries	Number of Citations
#1	MeSH descriptor: [Breast Neoplasms] explode all trees	7522
#2	"oncotype DX"	10
#3	"21 gene recurrence score"	6
#4	"21 gene recurrence"	6
#5	Mammaprint	8
#6	"70 gene signature"	3
#7	ivdmia	1
#8	"in vitro multivariate index assay"	0
#9	#1 AND (#2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8)	21
#10	MeSH descriptor: [Biological Assay] explode all trees	152
#11	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#12	MeSH descriptor: [Proportional Hazards Models] explode all trees	2827
#13	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	83811
#14	assay	11447
#15	assays	11447
#16	test	144378
#17	tests	144378
#18	#10 or #11 or #12 or #13 or #14 or #15 or #16 or #17	215226
#19	MeSH descriptor: [Reproducibility of Results] explode all trees	8077
#20	"reproducibility of results"	8375
#21	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	0
#22	"signal-to-noise ratio"	145
#23	MeSH descriptor: [ROC Curve] explode all trees	851
#24	"ROC Curve"	1113
#25	"receiver operating characteristic"	1107
#26	MeSH descriptor: [Observer Variation] explode all trees	1556
#27	“observer variation”	2444
#28	MeSH descriptor: [Sensitivity and Specificity] explode all trees	13656
#29	sensitivity:ti (Word variations have been searched)	2714
#30	sensitivity:ab (Word variations have been searched)	15456
#31	"sensitivity and specificity"	11058
#32	“analytic validity”	1481
#33	MeSH descriptor: [Diagnostic Errors] explode all trees	2362
#34	diagnos*:ti,ab	34133
#35	MeSH descriptor: [Diagnosis] this term only	79
#36	MeSH descriptor: [Diagnosis, Differential] this term only	1338
#37	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	33493
#38	accuracy	9811
#39	MeSH descriptor: [Diagnosis] explode all trees	221504
#40	diagnostic accuracy	4126
#41	precision	2746
#42	"diagnostic error*"	288
#43	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#44	MeSH descriptor: [Probability] explode all trees	31596
#45	probability	11646
#46	likelihood	6058
#47	MeSH descriptor: [False Negative Reactions] explode all trees	314
#48	MeSH descriptor: [False Positive Reactions] explode all trees	470
#49	"predictive value of test*"	5202
#50	forecasting	588
#51	MeSH descriptor: [Forecasting] explode all trees	423
#52	#19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51	275562
#53	#9 and (#18 or #52)	20

Search	Queries	Number of Citations
#54	#9 and (#18 or #52) from 1980	20
Colorectal Cancer Search		
#1	MeSH descriptor: [Colorectal Neoplasms] explode all trees	4390
#2	MeSH descriptor: [Microsatellite Instability] explode all trees	21
#3	"Microsatellite Instability"	54
#4	MLH1 promoter methylation	2
#5	KRAS protein, human	58
#6	KRAS	93
#7	BRAF protein, human	27
#8	"Oncotype DX Colon"	0
#9	"21 gene recurrence score"	6
#10	"21 gene recurrence"	6
#11	ivdmia	1
#12	"in vitro multivariate index assay"	0
#13	#1 and (#2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12)	87
#14	MeSH descriptor: [Biological Assay] explode all trees	152
#15	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#16	MeSH descriptor: [Proportional Hazards Models] explode all trees	2827
#17	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	83811
#18	assay	11447
#19	assays	11447
#20	test	144378
#21	tests	144378
#22	#14 or #15 or #16 or #17 or #18 or #19 or #20 or #21	215226
#23	MeSH descriptor: [Reproducibility of Results] explode all trees	8077
#24	"reproducibility of results"	8375
#25	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	0
#26	"signal-to-noise ratio"	145
#27	MeSH descriptor: [ROC Curve] explode all trees	851
#28	"ROC Curve"	1113
#29	"receiver operating characteristic"	1107
#30	MeSH descriptor: [Observer Variation] explode all trees	1556
#31	“observer variation”	2444
#32	MeSH descriptor: [Sensitivity and Specificity] explode all trees	13656
#33	sensitivity:ti (Word variations have been searched)	2714
#34	sensitivity:ab (Word variations have been searched)	15456
#35	"sensitivity and specificity"	11058
#36	“analytic validity”	1481
#37	MeSH descriptor: [Diagnostic Errors] explode all trees	2362
#38	diagnos*:ti,ab	34133
#39	MeSH descriptor: [Diagnosis] this term only	79
#40	MeSH descriptor: [Diagnosis, Differential] this term only	1338
#41	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	33493
#42	accuracy	9811
#43	MeSH descriptor: [Diagnosis] explode all trees	221504
#44	diagnostic accuracy	4126
#45	precision	2746
#46	"diagnostic error*"	288
#47	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#48	MeSH descriptor: [Probability] explode all trees	31596
#49	probability	11646
#50	likelihood	6058
#51	MeSH descriptor: [False Negative Reactions] explode all trees	314
#52	MeSH descriptor: [False Positive Reactions] explode all trees	470
#53	"predictive value of test*"	5202
#54	forecasting	588

Search	Queries	Number of Citations
#55	MeSH descriptor: [Forecasting] explode all trees	423
#56	#23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54 or #55	275562
#57	#13 and (#22 or #56)	71
#58	#13 and (#22 or #56) from 1980	71
Lung Cancer Search		
#1	MeSH descriptor: [Lung Neoplasms] explode all trees	4189
#2	MeSH descriptor: [Carcinoma, Bronchogenic] explode all trees	2159
#3	#1 or #2	4189
#4	EML4-ALK fusion protein, human	0
#5	ALK	115
#6	EGFR protein, human	177
#7	EGFR	526
#8	KRAS protein, human	58
#9	KRAS	93
#10	ivdmia	1
#11	"in vitro multivariate index assay"	0
#12	#3 and (#4 or #5 or #6 or #8 or #9 or #10 or #11)	56
#13	MeSH descriptor: [Biological Assay] explode all trees	152
#14	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#15	MeSH descriptor: [Proportional Hazards Models] explode all trees	2827
#16	MeSH descriptor: [Outcome and Process Assessment (Health Care)] explode all trees	83811
#17	assay	11447
#18	assays	11447
#19	test	144378
#20	tests	144378
#21	#13 or #14 or #15 or #16 or #17 or #18 or #19 or #20	215226
#22	MeSH descriptor: [Reproducibility of Results] explode all trees	8077
#23	"reproducibility of results"	8375
#24	MeSH descriptor: [Signal-To-Noise Ratio] explode all trees	0
#25	"signal-to-noise ratio"	145
#26	MeSH descriptor: [ROC Curve] explode all trees	851
#27	"ROC Curve"	1113
#28	"receiver operating characteristic"	1107
#29	MeSH descriptor: [Observer Variation] explode all trees	1556
#30	“observer variation”	2444
#31	MeSH descriptor: [Sensitivity and Specificity] explode all trees	13656
#32	sensitivity:ti (Word variations have been searched)	2714
#33	sensitivity:ab (Word variations have been searched)	15456
#34	"sensitivity and specificity"	11058
#35	“analytic validity”	1481
#36	MeSH descriptor: [Diagnostic Errors] explode all trees	2362
#37	diagnos*:ti,ab	34133
#38	MeSH descriptor: [Diagnosis] this term only	79
#39	MeSH descriptor: [Diagnosis, Differential] this term only	1338
#40	Any MeSH descriptor with qualifier(s): [Diagnosis - DI]	33493
#41	accuracy	9811
#42	MeSH descriptor: [Diagnosis] explode all trees	221504
#43	diagnostic accuracy	4126
#44	precision	2746
#45	"diagnostic error*"	288
#46	MeSH descriptor: [Predictive Value of Tests] explode all trees	5083
#47	MeSH descriptor: [Probability] explode all trees	31596
#48	probability	11646

Search	Queries	Number of Citations
#49	likelihood	6058
#50	MeSH descriptor: [False Negative Reactions] explode all trees	314
#51	MeSH descriptor: [False Positive Reactions] explode all trees	470
#52	"predictive value of test*"	5202
#53	forecasting	588
#54	MeSH descriptor: [Forecasting] explode all trees	423
#55	#22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54	275562
#56	#12 and (#21 or #55)	44
#57	#12 and (#21 or #55) from 1980	44

Appendix B. Exclusions

Exclusion Code Key:

- EXC1: Systematic Review
- EXC2: Wrong publication type
- EXC3: Wrong population
- EXC4: Wrong intervention
- EXC5: Wrong or no comparator
- EXC6: Wrong or no outcome
- EXC7: Wrong study design
- EXC8: Does not apply to a key question
- EXC9: Irretrievable

1. Bladder cancer surveillance (Structured abstract). Health Technology Assessment Database. 2007(3)PMID: HTA-32010001141. EXC1
2. Epidermal growth factor receptor mutations and tyrosine kinase inhibitor therapy in advanced non-small-cell lung cancer (Structured abstract). Health Technology Assessment Database. 2007(3)PMID: HTA-32010000201. EXC2
3. CYP2D6 pharmacogenomics of tamoxifen treatment (Structured abstract). Health Technology Assessment Database. 2008(3)PMID: HTA-32010000213. EXC2
4. Oncotype DX prognostic and predictive test for early breast cancer (Structured abstract). Health Technology Assessment Database. 2008(3)PMID: HTA-32010000622. EXC2
5. KRAS mutations and epidermal growth factor receptor inhibitor therapy in metastatic colorectal cancer (Structured abstract). Health Technology Assessment Database. 2008(3)PMID: HTA-32010000218. EXC2
6. MammaPrint (gene test) for breast cancer - prognostic test: horizon scanning technology briefing (Project record). Health Technology Assessment Database. 2009(3)PMID: HTA-32008100372. EXC2
7. Gene expression profiling in women with lymph-node-positive breast cancer to select adjuvant chemotherapy treatment (Structured abstract). Health Technology Assessment Database. 2010(3)PMID: HTA-32011000017. EXC2
8. KRAS testing for anti-EGFR therapy in advanced colorectal cancer: an evidence-based and economic analysis (Provisional abstract). Database of Abstracts of Reviews of Effects. 2010(3):1. PMID: DARE-12011004254. EXC2
9. Epidermal growth factor receptor mutation (EGFR) testing for prediction of response to EGFR-targeting tyrosine kinase inhibitor (TKI) drugs in patients with advanced non-small-cell lung cancer: an evidence-based analysis (Provisional abstract). Database of Abstracts of Reviews of Effects. 2010(3):1. PMID: DARE-12011004253. EXC1
10. Aaltonen L, Johns L, Jarvinen H, et al. Explaining the familial colorectal cancer risk associated with mismatch repair (MMR)-deficient and MMR-stable tumors. Clin Cancer Res. 2007 Jan 1;13(1):356-61. PMID: 17200375. EXC3
11. Abbott. Diagnosis and Management of Bladder Cancer Testing Technology to Identify New and Recurrent Bladder Cancer Evidence to support UroVysion Bladder Cancer Kit. 2013. EXC2
12. Abbott Molecular. UroVysion Bladder Cancer Kit, Package Insert. 30-608385/R4. . 2012. EXC6
13. Abd El Kader Y, Emera G, Safwat E, et al. The KRAS StripAssay for detection of KRAS mutation in Egyptian patients with colorectal cancer (CRC): a pilot study. J Egypt Natl Canc Inst. 2013 Mar;25(1):37-41. PMID: 23499205. EXC5

14. Abdel Salam I, Gaballa HE, Abdel Wahab N. Serum levels of epidermal growth factor and HER-2 neu in non small-cell lung cancer: prognostic correlation. Med Oncol. 2009;26(2):161-6. PMID: 19093231. EXC4

15. Abe Y, Masuda H, Okubo R. Microsatellite instability of each tumor in sporadic synchronous multiple colorectal cancers. Oncol Rep. 2001 Mar-Apr;8(2):299-304. PMID: 11182044. EXC4

16. Abedi-Ardekani B, Dar NA, Mir MM, et al. Epidermal growth factor receptor (EGFR) mutations and expression in squamous cell carcinoma of the esophagus in central Asia. BMC Cancer. 2012;12(602)PMID: 2013044056 MEDLINE PMID 23244191 (http://www.ncbi.nlm.nih.gov/pubmed/23244191) FULL TEXT LINK http://dx.doi.org/10.1186/1471-2407-12-602. EXC3

17. Abubaker J, Bavi P, Al-Haqawi W, et al. Prognostic significance of alterations in KRAS isoforms KRAS-4A/4B and KRAS mutations in colorectal carcinoma. J Pathol. 2009 Dec;219(4):435-45. PMID: 19824059. EXC5

18. Ach RA, Floore A, Curry B, et al. Robust interlaboratory reproducibility of a gene expression signature measurement consistent with the needs of a new generation of diagnostic tools. BMC Genomics. 2007;8:148. PMID: 17553173. EXC5

19. Achille A, Biasi MO, Zamboni G, et al. Cancers of the papilla of vater: mutator phenotype is associated with good prognosis. Clin Cancer Res. 1997 Oct;3(10):1841-7. PMID: 9815572. EXC4

20. Acs G, Esposito NN, Kiluk J, et al. A mitotically active, cellular tumor stroma and/or inflammatory cells associated with tumor cells may contribute to intermediate or high Oncotype DX Recurrence Scores in low-grade invasive breast carcinomas. Mod Pathol. 2012 Apr;25(4):556-66. PMID: 22173289. EXC5

21. Addison CL, Ding K, Zhao H, et al. Plasma transforming growth factor alpha and amphiregulin protein levels in NCIC Clinical Trials Group BR.21. J Clin Oncol. 2010 Dec 20;28(36):5247-56. PMID: 21079146. EXC7

22. Ademuyiwa FO, Thorat MA, Jain RK, et al. Expression of Forkhead-box protein A1, a marker of luminal A type breast cancer, parallels low Oncotype DX 21-gene recurrence scores. Mod Pathol. 2010 Feb;23(2):270-5. PMID: 19946260. EXC6

23. Agostini M, Enzo MV, Morandi L, et al. A ten markers panel provides a more accurate and complete microsatellite instability analysis in mismatch repair-deficient colorectal tumors. Cancer Biomarkers. 2009;6(1):49-61. EXC4

24. Ahlquist DA, Zou H, Domanico M, et al. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. Gastroenterology. 2012 Feb;142(2):248-56; quiz e25-6. PMID: 22062357. EXC5

25. Ahn HK, Choi YL, Han JH, et al. Epidermal growth factor receptor mutation and treatment outcome of mediastinoscopic N2 positive non-small cell lung cancer patients treated with neoadjuvant chemoradiotherapy followed by surgery. Lung Cancer. 2013 Mar;79(3):300-6. PMID: 23261144. EXC3

26. Ahn JB, Chung WB, Maeda O, et al. DNA methylation predicts recurrence from resected stage III proximal colon cancer. Cancer. 2011 May 1;117(9):1847-54. PMID: 21509761. EXC6

27. Ahn JH, Kim SW, Hong SM, et al. Epidermal growth factor receptor (EGFR) expression in operable non-small cell lung carcinoma. J Korean Med Sci. 2004 Aug;19(4):529-35. PMID: 15308843. EXC4

28. Ahn MJ, Park BB, Ahn JS, et al. Are there any ethnic differences in molecular predictors of erlotinib efficacy in advanced non-small cell lung cancer? Clin Cancer Res. 2008 Jun 15;14(12):3860-6. PMID: 18559606. EXC3

29. Akashi-Tanaka S, Shimizu C, Ando M, et al. 21-Gene expression profile assay on core needle biopsies predicts responses to neoadjuvant endocrine therapy in breast cancer patients. Breast. 2009 Jun;18(3):171-4. PMID: 19410462. EXC5

30. Akca H, Demiray A, Yaren A, et al. Utility of serum DNA and pyrosequencing for the detection of EGFR mutations in non-small cell lung cancer. Cancer Genet. 2013 Mar;206(3):73-80. PMID: 23491080. EXC6

31. Al Zobair AA, Al Obeidy BF, Yang L, et al. Concomitant overexpression of EGFR and CXCR4 is associated with worse prognosis in a new molecular subtype of non-small cell lung cancer. Oncol Rep. 2013 Apr;29(4):1524-32. PMID: 23443279. EXC4

32. Albain K, Barlow W, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal, node-positive, ER-positive breast cancer (S8814, INT0100). Breast Cancer Res Treat. 2008 Jun;109(3):585-. PMID: ISI:000255851900021. Exc6

33. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol. 2010 Jan;11(1):55-65. PMID: 20005174. EXC4

34. Alberts SR, Sargent DJ, Nair S, et al. Effect of oxaliplatin, fluorouracil, and leucovorin with or without cetuximab on survival among patients with resected stage III colon cancer: a randomized trial. JAMA. 2012 Apr 4;307(13):1383-93. PMID: 22474202. EXC6

35. Alemayehu A, Tomkova K, Zavodna K, et al. The role of clinical criteria, genetic and epigenetic alterations in Lynch-syndrome diagnosis. Neoplasma. 2007;54(5):391-401. PMID: 17688369. EXC6

36. Algars A, Lintunen M, Carpen O, et al. EGFR gene copy number assessment from areas with highest EGFR expression predicts response to anti-EGFR therapy in colorectal cancer. Br J Cancer. 2011 Jul 12;105(2):255-62. PMID: 21694725. EXC3

37. Alldinger I, Schaefer KL, Goedde D, et al. Microsatellite instability in Ewing tumor is not associated with loss of mismatch repair protein expression. J Cancer Res Clin Oncol. 2007;133(10):749-59. EXC3

38. Allegrini S, Antona J, Mezzapelle R, et al. Epidermal growth factor receptor gene analysis with a highly sensitive molecular assay in routine cytologic specimens of lung adenocarcinoma. Am J Clin Pathol. 2012 Sep;138(3):377-81. PMID: 22912354. EXC3

39. Allison KH, Kandalaft PL, Sitlani CM, et al. Routine pathologic parameters can predict Oncotype DX recurrence scores in subsets of ER positive patients: who does not always need testing? Breast Cancer Res Treat. 2012 Jan;131(2):413-24. PMID: 21369717. EXC6

40. Aloulou N, Bastuji-Garin S, Le Gouvello S, et al. Involvement of the leptin receptor in the immune response in intestinal cancer. Cancer Res. 2008;68(22):9413-22. EXC4

41. Al-Saad S, Al-Shibli K, Donnem T, et al. Clinical significance of epidermal growth factor receptors in non-small cell lung cancer and a prognostic role for HER2 gene copy number in female patients. J Thorac Oncol. 2010 Oct;5(10):1536-43. PMID: 20802349. EXC4

42. Altomare DF, Guanti G, Hoch J, et al. Noncolonic cancer stem cells in bone marrow of colorectal cancer patients. Colorectal Dis. 2010 Mar;12(3):206-12. PMID: 19183332. EXC5

43. Alvarez K, Hurtado C, Hevia MA, et al. Spectrum of MLH1 and MSH2 mutations in Chilean families with suspected Lynch syndrome. Dis Colon Rectum. 2010 Apr;53(4):450-9. PMID: 20305446. EXC6

44. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008(10):1626-34. PMID: CN-00630611. EXC7

45. Amann JM, Lee JW, Roder H, et al. Genetic and proteomic features associated with survival after treatment with erlotinib in first-line therapy of non-small cell lung cancer in Eastern Cooperative Oncology Group 3503. J Thorac Oncol. 2010 Feb;5(2):169-78. PMID: 20035238. EXC5

46. Ambrosini-Spaltro A, Campanini N, Bortesi B, et al. EGFR mutation-specific antibodies in pulmonary adenocarcinoma: a comparison with DNA direct sequencing. Appl Immunohistochem Mol Morphol. 2012 Jul;20(4):356-62. PMID: 22710815. EXC4

47. American Cancer Society. Cancer Facts & Figures 2011. Atlanta, GA: 2011. www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-029771.pdf EXC8

48. American Joint Committee on Cancer. Cancer Staging Manual. 7th ed. New York: Springer; 2010. EXC2

49. Amira N, Rivet J, Soliman H, et al. Microsatellite instability in urothelial carcinoma of the upper urinary tract. J Urol. 2003;170(4 I):1151-4. EXC6

50. An CH, Kim SS, Kang MR, et al. Frameshift mutations of ATBF1, WNT9A, CYLD and PARK2 in gastric and colorectal carcinomas with high microsatellite instability. Pathology (Phila). 2010;42(6):583-5. PMID: 20854080. EXC4

51. Andersen CL, Lamy P, Thorsen K, et al. Frequent genomic loss at chr16p13.2 is associated with poor prognosis in colorectal cancer. Int J Cancer. 2011;129(8):1848-58. EXC4

52. Anderson JC, Rangasamy P, Rustagi T, et al. Risk factors for sessile serrated adenomas. J Clin Gastroenterol. 2011 Sep;45(8):694-9. PMID: 21325950. EXC3

53. Andreason M, Zhang C, Onitilo AA, et al. Rural versus urban differences among patients (pts) with hormone-receptor positive (HR+) breast cancer (BC) and a 21-gene assay recurrence score (RS). ASCO; 2012. EXC6

54. Angulo B, Garcia-Garcia E, Martinez R, et al. A commercial real-time PCR kit provides greater sensitivity than direct sequencing to detect KRAS mutations: a morphology-based approach in colorectal carcinoma. J Mol Diagn. 2010 May;12(3):292-9. PMID: 20203003. EXC3

55. Ann MJ, Park BB, Ann JS, et al. Are there any ethnic differences in molecular predictors of erlotinib efficacy in advanced non-small cell lung cancer? Clin Cancer Res. 2008;14(12):3860-6. EXC3

56. Ansen S, Bangard C, Querings S, et al. Osteoblastic response in patients with non-small cell lung cancer with activating EGFR mutations and bone metastases during treatment with EGFR kinase inhibitors. J Thorac Oncol. 2010;5(3):407-9. EXC7

57. Antelo M, Balaguer F, Shia J, et al. A High Degree of LINE-1 Hypomethylation Is a Unique Feature of Early-Onset Colorectal Cancer. PLoS One. 2012;7(9). EXC4

58. Aoyagi H, Iida S, Uetake H, et al. Effect of classification based on combination of mutation and methylation in colorectal cancer prognosis. Oncol Rep. 2011 Mar;25(3):789-94. PMID: 21174064. EXC6

59. Aparicio T, Schischmanoff O, Poupardin C, et al. Deficient mismatch repair phenotype is a prognostic factor for colorectal cancer in elderly patients. Dig Liver Dis. 2013 Mar;45(3):245-50. PMID: 23102497. EXC7

60. Arain MA, Sawhney M, Sheikh S, et al. CIMP status of interval colon cancers: another piece to the puzzle. Am J Gastroenterol. 2010 May;105(5):1189-95. PMID: 20010923. EXC4

61. Araki K, Hashimoto K, Ardyanto TD, et al. Co-expression of Cox-2 and EGFR in stage I human bronchial adenocarcinomas. Lung Cancer. 2004 Aug;45(2):161-9. PMID: 15246187. EXC4

62. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res. 2011 Mar 1;17(5):1169-80. PMID: 21248300. EXC4

63. Ardavanis A, Koumna S, Fragos I, et al. Erlotinib monotherapy in patients with advanced non-small cell lung cancer: an effective approach with low toxicity. Anticancer Res. 2008 Jul-Aug;28(4C):2409-15. PMID: 18751427. EXC4

64. Argiris A, Hensing T, Yeldandi A, et al. Combined analysis of molecular and clinical predictors of gefitinib activity in advanced non-small cell lung cancer: epidermal growth factor receptor mutations do not tell the whole story. J Thorac Oncol. 2006 Jan;1(1):52-60. PMID: 17409827. EXC6

65. Arnold CN, Nagasaka T, Goel A, et al. Molecular characteristics and predictors of survival in patients with malignant neuroendocrine tumors. Int J Cancer. 2008 Oct 1;123(7):1556-64. PMID: 18646189. EXC5

66. Arrieta O, Campos-Parra AD, Zuloaga C, et al. Clinical and pathological characteristics, outcome and mutational profiles regarding non-small-cell lung cancer related to wood-smoke exposure. J Thorac Oncol. 2012;7(8):1228-34. EXC6

67. Arrieta O, Saavedra-Perez D, Kuri R, et al. Brain metastasis development and poor survival associated with carcinoembryonic antigen (CEA) level in advanced non-small cell lung cancer: a prospective analysis. BMC Cancer. 2009;9:119. PMID: 19386089. EXC4

68. Artac M, Pehlivan S, Akcan S, et al. The role of microsatellite instability to predict clinical benefit from irinotecan-based regimens in metastatic colorectal cancer. Turkish Journal of Cancer. 2008;38(2):49-56. EXC3

69. Artale S, Sartore-Bianchi A, Veronese SM, et al. Mutations of KRAS and BRAF in primary and matched metastatic sites of colorectal cancer. J Clin Oncol. 2008 Sep 1;26(25):4217-9. PMID: 18757341. EXC2

70. Artinyan A, Essani R, Lake J, et al. Molecular predictors of lymph node metastasis in colon cancer: increased risk with decreased thymidylate synthase expression. J Gastrointest Surg. 2005 Dec;9(9):1216-21; discussion 21. PMID: 16332476. EXC5

71. Asad J, Jacobson AF, Estabrook A, et al. Does oncotype DX recurrence score affect the management of patients with early-stage breast cancer? Am J Surg. 2008 Oct;196(4):527-9. PMID: 18809056. EXC5

72. Ashktorab H, Brim H, Al-Riyami M, et al. Sporadic colon cancer: mismatch repair immunohistochemistry and microsatellite instability in Omani subjects. Dig Dis Sci. 2008 Oct;53(10):2723-31. PMID: 18299982. EXC4

73. Ashktorab H, Smoot DT, Carethers JM, et al. High incidence of microsatellite instability in colorectal cancer from African Americans. Clin Cancer Res. 2003 Mar;9(3):1112-7. PMID: 12631615. EXC6

74. Asseburg C, Frank M, Kohne CH, et al. Cost-effectiveness of targeted therapy with cetuximab in patients with K-ras wild-type colorectal cancer presenting with initially unresectable metastases limited to the liver in a German setting (Structured abstract). Clin Ther. 2011(4):482-97. PMID: NHSEED-22011000996. EXC7

75. Au HJ, Karapetis CS, O'Callaghan CJ, et al. Health-related quality of life in patients with advanced colorectal cancer treated with cetuximab: overall and KRAS-specific results of the NCIC CTG and AGITG CO.17 Trial. J Clin Oncol. 2009 Apr 10;27(11):1822-8. PMID: 19273701. EXC3

76. Azzoni C, Bottarelli L, Cecchini S, et al. Sporadic colorectal carcinomas with low-level microsatellite instability: A distinct subgroup with specific clinicopathological and molecular features. Int J Colorectal Dis. 2011;26(4):445-53. EXC4

77. Baba T, Shiota H, Kuroda K, et al. Cancer/testis antigen expression as a predictor for epidermal growth factor receptor mutation and prognosis in lung adenocarcinoma. Eur J Cardiothorac Surg. 2013 Apr;43(4):759-64. PMID: 22826471. EXC7

78. Baba Y, Huttenhower C, Nosho K, et al. Epigenomic diversity of colorectal cancer indicated by LINE-1 methylation in a database of 869 tumors. Molecular Cancer. 2010;9. EXC4

79. Baba Y, Nosho K, Shima K, et al. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. Clin Cancer Res. 2009 Jul 15;15(14):4665-73. PMID: 19584150. EXC6

80. Baba Y, Nosho K, Shima K, et al. PTGER2 overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype. Cancer Epidemiology Biomarkers and Prevention. 2010;19(3):822-31. EXC4

81. Baba Y, Nosho K, Shima K, et al. Phosphorylated AKT expression is associated with PIK3CA mutation, low stage, and favorable outcome in 717 colorectal cancers. Cancer. 2011 Apr 1;117(7):1399-408. PMID: 21425139. EXC6

82. Baba Y, Nosho K, Shima K, et al. Hypomethylation of the IGF2 DMR in colorectal tumors, detected by bisulfite pyrosequencing, is associated with poor prognosis. Gastroenterology. 2010 Dec;139(6):1855-64. PMID: 20682317. EXC4

83. Baba Y, Nosho K, Shima K, et al. HIF1A overexpression is associated with poor prognosis in a cohort of 731 colorectal cancers. Am J Pathol. 2010 May;176(5):2292-301. PMID: 20363910. EXC4

84. Badalian G, Barbai T, Raso E, et al. Phenotype of bone metastases of non-small cell lung cancer: epidermal growth factor receptor expression and K-RAS mutational status. Pathol Oncol Res. 2007;13(2):99-104. PMID: 17607370. EXC4

85. Bae JM, Cho NY, Kim TY, et al. Clinicopathologic and molecular characteristics of synchronous colorectal cancers: heterogeneity of clinical outcome depending on microsatellite instability status of individual tumors. Dis Colon Rectum. 2012 Feb;55(2):181-90. PMID: 22228162. EXC6

86. Bae JM, Kim MJ, Kim JH, et al. Differential clinicopathological features in microsatellite instability-positive colorectal cancers depending on CIMP status. Virchows Arch. 2011 Jul;459(1):55-63. PMID: 21494758. EXC7

87. Bae NC, Chae MH, Lee MH, et al. EGFR, ERBB2, and KRAS mutations in Korean non-small cell lung cancer patients. Cancer Genet Cytogenet. 2007 Mar;173(2):107-13. PMID: 17321325. EXC6

88. Baehner FL, Achacoso N, Maddala T, et al. Human epidermal growth factor receptor 2 assessment in a case-control study: comparison of fluorescence in situ hybridization and quantitative reverse transcription polymerase chain reaction performed by central laboratories. J Clin Oncol. 2010 Oct 1;28(28):4300-6. PMID: 20697093. EXC7

89. Baehner FL, Yoshizawa CN, Butler SM, et al. The Development of the DCIS Score: Scaling and Normalization in the Marin Medical Laboratories Cohort: Marin Medical Laboratories; 2012. EXC7

90. Bago-Horvath Z, Sieghart W, Grusch M, et al. Synergistic effects of erlotinib and everolimus on bronchial carcinoids and large-cell neuroendocrine carcinomas with activated EGFR/AKT/mTOR pathway. Neuroendocrinology. 2012;96(3):228-37. PMID: 22378048. EXC4

91. Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. J Clin Oncol. 2009 Jun 1;27(16):2653-9. PMID: 19414683. EXC5

92. Bai H, Wang Z, Wang Y, et al. Detection and clinical significance of intratumoral EGFR mutational heterogeneity in Chinese patients with advanced non-small cell lung cancer. PLoS One. 2013;8(2):e54170. PMID: 23418425. EXC3

93. Balleste B, Bessa X, Pinol V, et al. Detection of metachronous neoplasms in colorectal cancer patients: identification of risk factors. Dis Colon Rectum. 2007 Jul;50(7):971-80. PMID: 17468913. EXC6

94. Bando H, Yoshino T, Tsuchihara K, et al. KRAS mutations detected by the amplification refractory mutation system-Scorpion assays strongly correlate with therapeutic effect of cetuximab. Br J Cancer. 2011 Jul 26;105(3):403-6. PMID: 21730978. EXC6

95. Banerjea A, Feakins RM, Nickols CD, et al. Immunogenic hsp-70 is overexpressed in colorectal cancers with high-degree microsatellite instability. Dis Colon Rectum. 2005 Dec;48(12):2322-8. PMID: 16258706. EXC6

96. Banerjea A, Hands RE, Powar MP, et al. Microsatellite and chromosomal stable colorectal cancers demonstrate poor immunogenicity and early disease recurrence. Colorectal Dis. 2009 Jul;11(6):601-8. PMID: 18637931. EXC5

97. Barault L, Charon-Barra C, Jooste V, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. Cancer Res. 2008 Oct 15;68(20):8541-6. PMID: 18922929. EXC6

98. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer. 2008 May 15;122(10):2255-9. PMID: 18224685. EXC6

99. Barratt PL, Seymour MT, Stenning SP, et al. DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: a molecular study. Lancet. 2002 Nov 2;360(9343):1381-91. PMID: 12423985. EXC4

100. Bartley AN, Luthra R, Saraiya DS, et al. Identification of cancer patients with Lynch syndrome: clinically significant discordances and problems in tissue-based mismatch repair testing. Cancer Prev Res (Phila). 2012 Feb;5(2):320-7. PMID: 22086678. EXC3

101. Bast RC, Jr., Hortobagyi GN. Individualized care for patients with cancer - a work in progress. N Engl J Med. 2004 Dec 30;351(27):2865-7. PMID: 15591336. EXC2

102. Becouarn Y, Rullier A, Gorry P, et al. Value of microsatellite instability typing in detecting hereditary non-polyposis colorectal cancer. A prospective multicentric study by the Association Aquitaine Gastro. Gastroenterol Clin Biol. 2005 Jun-Jul;29(6-7):667-75. PMID: 16142001. EXC6

103. Bedwell C, Rowe D, Moulton D, et al. Cytogenetically complex SEC31A-ALK fusions are recurrent in ALK-positive large B-cell lymphomas. Haematologica. 2011 Feb;96(2):343-6. PMID: 21109691. EXC3

104. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol. 2005 Nov 1;23(31):8081-92. PMID: 16204011. EXC3

105. Belt EJ, Fijneman RJ, van den Berg EG, et al. Loss of lamin A/C expression in stage II and III colon cancer is associated with disease recurrence. Eur J Cancer. 2011 Aug;47(12):1837-45. PMID: 21621406. EXC7

106. Belt EJ, te Velde EA, Krijgsman O, et al. High lymph node yield is related to microsatellite instability in colon cancer. Ann Surg Oncol. 2012 Apr;19(4):1222-30. PMID: 21989661. EXC7

107. Benatti P, Gafa R, Barana D, et al. Microsatellite instability and colorectal cancer prognosis. Clin Cancer Res. 2005;11(23):8332-40. EXC4

108. Benes C. Discovery of candidate biomarkers of anticancer drug sensitivity by high-throughput cell line screening. Molecul Cancer Therapeut. 2011;10(11). EXC2

109. Benmoussa A, Badre W, Pedroni M, et al. Clinical and molecular characterization of colorectal cancer in young Moroccan patients. Turk J Gastroenterol. 2012 2012;23(6):686-90. PMID: 2013417989 FULL TEXT LINK http://dx.doi.org/10.4318/tjg.2012.0474. EXC3

110. Bennett L, Zhao Z, Barber B, et al. Health-related quality of life in patients with metastatic colorectal cancer treated with panitumumab in first- or second-line treatment. Br J Cancer. 2011 Nov 8;105(10):1495-502. PMID: 21989186. EXC6

111. Bentivegna A, Conconi D, Panzeri E, et al. Biological heterogeneity of putative bladder cancer stem-like cell populations from human bladder transitional cell carcinoma samples. Cancer Sci. 2010 Feb;101(2):416-24. PMID: 19961489. EXC4

112. Berardi R, Santinelli A, Brunelli A, et al. Epidermal growth factor receptor status in stages of resected non-small cell lung cancer: implications for treatment with epidermal growth factor receptor inhibitors. Anal Quant Cytol Histol. 2011 Aug;33(4):196-204. PMID: 21980623. EXC4

113. Berg M, Danielsen SA, Ahlquist T, et al. DNA sequence profiles of the colorectal cancer critical gene set KRAS-BRAF-PIK3CA-PTEN-TP53 related to age at disease onset. PLoS One. 2010;5(11):e13978. PMID: 21103049. EXC5

114. Berghmans T, Mascaux C, Haller A, et al. EGFR, TTF-1 and Mdm2 expression in stage III non-small cell lung cancer: a positive association. Lung Cancer. 2008 Oct;62(1):35-44. PMID: 18355939. EXC4

115. Bergman J, Reznichek RC, Rajfer J. Surveillance of patients with bladder carcinoma using fluorescent in-situ hybridization on bladder washings. BJU Int. 2008 Jan;101(1):26-9. PMID: 17850364. EXC6

116. Berney CR, Fisher RJ, Yang J, et al. Genomic alterations (LOH, MI) on chromosome 17q21-23 and prognosis of sporadic colorectal cancer. Int J Cancer. 2000 Jan 20;89(1):1-7. PMID: 10719723. EXC4

117. Berney CR, Fisher RJ, Yang JL, et al. Genomic alterations (LOH, MI) on chromosome 17q21-23 and prognosis of sporadic colorectal cancer. Int J Cancer. 2000;89(1):1-7. EXC6

118. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. J Clin Oncol. 2009 Apr 10;27(11):1814-21. PMID: 19273709. EXC6

119. Bertagnolli MM, Redston M, Compton CC, et al. Microsatellite instability and loss of heterozygosity at chromosomal location 18q: prospective evaluation of biomarkers for stages II and III colon cancer--a study of CALGB 9581 and 89803. J Clin Oncol. 2011(23):3153-62. PMID: CN-00798827. EXC4

120. Bertagnolli MM, Warren RS, Niedzwiecki D, et al. p27Kip1 in stage III colon cancer: implications for outcome following adjuvant chemotherapy in cancer and leukemia group B protocol 89803. Clin Cancer Res. 2009 Mar 15;15(6):2116-22. PMID: 19276255. EXC5

121. Bettstetter M, Dechant S, Ruemmele P, et al. Distinction of hereditary nonpolyposis colorectal cancer and sporadic microsatellite-unstable colorectal cancer through quantification of MLH1 methylation by real-time PCR. Clin Cancer Res. 2007 Jun 1;13(11):3221-8. PMID: 17545526. EXC6

122. Bettstetter M, Dechant S, Ruemmele P, et al. MethyQESD, a robust and fast method for quantitative methylation analyses in HNPCC diagnostics using formalin-fixed and paraffin-embedded tissue samples. Lab Invest. 2008;88(12):1367-75. EXC3

123. Betz BL, Roh MH, Weigelin HC, et al. The application of molecular diagnostic studies interrogating EGFR and KRAS mutations to stained cytologic smears of lung carcinoma. Am J Clin Pathol. 2011;136(4):564-71. EXC6

124. Bianchini G, Zantheni M, Mariani P, et al. Recurrence score by oncotype DX evaluated on the primary breast tumor predicts the 2-year survival after first relapse. Breast Cancer Res Treat. 2007 Dec;106(suppl 1):S55-S. PMID: ISI:000251398500152. EXC2

125. Bibeau F, Lopez-Crapez E, Di Fiore F, et al. Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol. 2009 Mar 1;27(7):1122-9. PMID: 19164213. EXC3

126. Bighin C, Del Mastro L, Canavese G, et al. Use in current clinical practice of 70-gene signature in early breast cancer. Int J Cancer. 2010;127(11):2736-7. EXC2

127. Billah S, Stewart J, Staerkel G, et al. EGFR and KRAS mutations in lung carcinoma: molecular testing by using cytology specimens. Cancer Cytopathol. 2011 Apr 25;119(2):111-7. PMID: 21400670. EXC5

128. Bleeker WA, Hayes VM, Karrenbeld A, et al. Impact of KRAS and TP53 mutations on survival in patients with left- and right-sided Dukes' C colon cancer. Am J Gastroenterol. 2000 Oct;95(10):2953-7. PMID: 11051374. EXC5

129. Blohmer JU, Rezai M, Kummel S, et al. Using the 21-gene assay to guide adjuvant chemotherapy decision-making in early-stage breast cancer: a cost-effectiveness evaluation in the German setting. J Med Econ. 2013;16(1):30-40. PMID: 22966753. EXC 7

130. Blons H, Cote JF, Le Corre D, et al. Epidermal growth factor receptor mutation in lung cancer are linked to bronchioloalveolar differentiation. Am J Surg Pathol. 2006;30(10):1309-15. EXC6

131. Boissiere-Michot F, Lopez-Crapez E, Frugier H, et al. KRAS genotyping in rectal adenocarcinoma specimens with low tumor cellularity after neoadjuvant treatment. Mod Pathol. 2012 May;25(5):731-9. PMID: 22282307. EXC3

132. Bokemeyer C, Bondarenko I, Hartmann JT, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol. 2011 Jul;22(7):1535-46. PMID: 21228335. EXC6

133. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol. 2009;27(5):663-71. EXC7

134. Bokemeyer C, Cutsem EV, Rougier P, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer. 2012 Jul;48(10):1466-75. PMID: 22446022. EXC5

135. Boland JM, Erdogan S, Vasmatzis G, et al. Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. Hum Pathol. 2009 Aug;40(8):1152-8. PMID: 19386350. EXC4

136. Boldrini L, Ali G, Gisfredi S, et al. Epidermal growth factor receptor and K-RAS mutations in 411 lung adenocarcinoma: a population-based prospective study. Oncol Rep. 2009 Oct;22(4):683-91. PMID: 19724844. EXC6

137. Boldrini L, Gisfredi S, Ursino S, et al. Mutational analysis in cytological specimens of advanced lung adenocarcinoma: a sensitive method for molecular diagnosis. J Thorac Oncol. 2007 Dec;2(12):1086-90. PMID: 18090579. EXC6

138. Bollmann D, Bollmann M, Bankfalvi A, et al. Quantitative molecular grading of bladder tumours: a tool for objective assessment of the biological potential of urothelial neoplasias. Oncol Rep. 2009 Jan;21(1):39-47. PMID: 19082441. EXC6

139. Bollmann M, Heller H, Bankfalvi A, et al. Quantitative molecular urinary cytology by fluorescence in situ hybridization: a tool for tailoring surveillance of patients with superficial bladder cancer? BJU Int. 2005 Jun;95(9):1219-25. PMID: 15892805. EXC7

140. Bonanno L, Schiavon M, Nardo G, et al. Prognostic and predictive implications of EGFR mutations, EGFR copy number and KRAS mutations in advanced stage lung adenocarcinoma. Anticancer Res. 2010 Dec;30(12):5121-8. PMID: 21187500. EXC6

141. Bond CE, Umapathy A, Ramsnes I, et al. p53 mutation is common in microsatellite stable, BRAF mutant colorectal cancers. Int J Cancer. 2012 Apr 1;130(7):1567-76. PMID: 21557216. EXC6

142. Bonis PA, Trikalinos TA, Chung M, et al. Hereditary nonpolyposis colorectal cancer: diagnostic strategies and their implications (Structured abstract). Database of Abstracts of Reviews of Effects. 2007(3):180. PMID: DARE-12007008450. EXC3

143. Bonnet D, Selves J, Toulas C, et al. Simplified identification of Lynch syndrome: a prospective, multicenter study. Dig Liver Dis. 2012 Jun;44(6):515-22. PMID: 22480969. EXC3

144. . Genomic comparison of paired primary breast carcinomas and macrometastatic lymph node metastases using quantitative RT-PCR by Oncotype DX: Assessment of the Recurrence Score and quantitative single genes. SABCS; 2011. EXC6

145. Bosman FT, Yan P, Tejpar S, et al. Tissue biomarker development in a multicentre trial context: a feasibility study on the PETACC3 stage II and III colon cancer adjuvant treatment trial. Clin Cancer Res. 2009(17):5528-33. PMID: CN-00734350. EXC7

146. Bossuyt V, Fadare O, Martel M, et al. Remarkably high frequency of EGFR expression in breast carcinomas with squamous differentiation. Int J Surg Pathol. 2005 Oct;13(4):319-27. PMID: 16273187. EXC4

147. Bouzourene H, Taminelli L, Chaubert P, et al. A cost-effective algorithm for hereditary nonpolyposis colorectal cancer detection. Am J Clin Pathol. 2006 Jun;125(6):823-31. PMID: 16690480. EXC6

148. . Evaluation of variables that may impact chemotherapy (CT) administration after determination of Oncotype DX (ODX) recurrence score (RS). ASCO; 2012. EXC6

149. Bowles DW, Rabinovitch R, Borges V, et al. A young woman with a small ER-positive breast cancer, a micrometastatic axillary lymph node, and an intermediate oncotype DX recurrence score. Oncology (Williston Park). 2007 Sep;21(10):1212-7. PMID: 17926799. EXC2

150. Bozzetti C, Tiseo M, Lagrasta C, et al. Comparison between epidermal growth factor receptor (EGFR) gene expression in primary non-small cell lung cancer (NSCLC) and in fine-needle aspirates from distant metastatic sites. J Thorac Oncol. 2008 Jan;3(1):18-22. PMID: 18166836. EXC6

151. Brabender J, Danenberg KD, Metzger R, et al. Epidermal growth factor receptor and HER2-neu mRNA expression in non-small cell lung cancer Is correlated with survival. Clin Cancer Res. 2001 Jul;7(7):1850-5. PMID: 11448895. EXC4

152. Brassett C, Joyce JA, Froggatt NJ, et al. Microsatellite instability in early onset and familial colorectal cancer. J Med Genet. 1996;33(12):981-5. EXC4

153. Brattstrom D, Wester K, Bergqvist M, et al. HER-2, EGFR, COX-2 expression status correlated to microvessel density and survival in resected non-small cell lung cancer. Acta Oncol. 2004;43(1):80-6. PMID: 15068324. EXC4

154. Brazowski E, Rozen P, Pel S, et al. Can a gastrointestinal pathologist identify microsatellite instability in colorectal cancer with reproducibility and a high degree of specificity? Familial Cancer. 2012;11(2):249-57. EXC5

155. Brenner BM, Swede H, Jones BA, et al. Genomic instability measured by inter-(simple sequence repeat) PCR and high-resolution microsatellite instability are prognostic of colorectal carcinoma survival after surgical resection. Ann Surg Oncol. 2012 Jan;19(1):344-50. PMID: 21487966. EXC4

156. Brosens RP, Belt EJ, Haan JC, et al. Deletion of chromosome 4q predicts outcome in stage II colon cancer patients. Cell Oncol (Dordr). 2011 Jun;34(3):215-23. PMID: 21717218. EXC7

157. Brotto K, Malisic E, Cavic M, et al. The Usability of Allele-Specific PCR and Reverse-Hybridization Assays for KRAS Genotyping in Serbian Colorectal Cancer Patients. Dig Dis Sci. 2013 Apr;58(4):998-1003. PMID: 23108567. EXC3

158. Brueckl WM, Moesch C, Brabletz T, et al. Relationship between microsatellite instability, response and survival in palliative patients with colorectal cancer undergoing first-line chemotherapy. Anticancer Res. 2003 Mar-Apr;23(2C):1773-7. PMID: 12820457. EXC3

159. Brugger W, Triller N, Blasinska-Morawiec M, et al. Prospective molecular marker analyses of EGFR and KRAS from a randomized, placebo-controlled study of erlotinib maintenance therapy in advanced non-small-cell lung cancer. J Clin Oncol. 2011 Nov 1;29(31):4113-20. PMID: 21969500. EXC4

160. Bruijn MT, Raats DA, Tol J, et al. Combined KRAS and TP53 mutation status is not predictive in CAPOX-treated metastatic colorectal cancer. Anticancer Res. 2011(4):1379-85. PMID: CN-00788818. EXC8

161. Bruin SC, He Y, Mikolajewska-Hanclich I, et al. Molecular alterations associated with liver metastases development in colorectal cancer patients. Br J Cancer. 2011 Jul 12;105(2):281-7. PMID: 21673680. EXC6

162. Bruno P, Mariotta S, Ricci A, et al. Reliability of direct sequencing of EGFR: comparison between cytological and histological samples from the same patient. Anticancer Res. 2011 Dec;31(12):4207-10. PMID: 22199282. EXC6

163. Bubendorf L, Grilli B, Sauter G, et al. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. Am J Clin Pathol. 2001 Jul;116(1):79-86. PMID: 11447756. EXC3

164. Buckowitz A, Knaebel HP, Benner A, et al. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. Br J Cancer. 2005 May 9;92(9):1746-53. PMID: 15856045. EXC3

165. Burger M, Denzinger S, Hammerschmied CG, et al. Elevated microsatellite alterations at selected tetranucleotides (EMAST) and mismatch repair gene expression in prostate cancer. J Mol Med. 2006;84(10):833-41. EXC3

166. Burke E, Baehner F, Yoshizawa C, et al. The 21-gene Breast Cancer Assay: Summary of Clinical Evidence. Redwood City, CA: Genomic Health, Inc.; 2013. EXC6

167. Busby K, Morris A. Detection of BRAF mutations in colorectal tumours and peritoneal washings using a mismatch ligation assay. J Clin Pathol. 2005 Apr;58(4):372-5. PMID: 15790700. EXC4

168. Buxhofer-Ausch V, Ausch C, Zeillinger R, et al. Duplex reverse-hybridization assay for the simultaneous detection of KRAS/BRAF mutations in FFPE-extracted genomic DNA from colorectal cancer specimens. Dis Markers. 2013;34(3):171-7. PMID: 23324583. EXC4

169. Byrne BJ, Garst J. Epidermal growth factor receptor inhibitors and their role in non-small-cell lung cancer. Curr Oncol Rep. 2005 Jul;7(4):241-7. PMID: 15946581. EXC4

170. Cadranel J, Mauguen A, Faller M, et al. Impact of systematic EGFR and KRAS mutation evaluation on progression-free survival and overall survival in patients with advanced non-small-cell lung cancer treated by erlotinib in a French prospective cohort (ERMETIC project-Part 2). J Thorac Oncol. 2012;7(10):1490-502. EXC3

171. Calistri D, Presciuttini S, Buonsanti G, et al. Microsatellite instability in colorectal-cancer patients with suspected genetic predisposition. Int J Cancer. 2000 Jan 20;89(1):87-91. PMID: 10719736. EXC3

172. Calistri D, Rengucci C, Bocchini R, et al. Fecal multiple molecular tests to detect colorectal cancer in stool. Clin Gastroenterol Hepatol. 2003 Sep;1(5):377-83. PMID: 15017656. EXC6

173. Camidge DR, Kono SA, Lu X, et al. Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. J Thorac Oncol. 2011 Apr;6(4):774-80. PMID: 21336183. EXC6

174. Camidge DR, Theodoro M, Maxson DA, et al. Correlations between the percentage of tumor cells showing an anaplastic lymphoma kinase (ALK) gene rearrangement, ALK signal copy number, and response to crizotinib therapy in ALK fluorescence in situ hybridization-positive nonsmall cell lung cancer. Cancer. 2012 Sep 15;118(18):4486-94. PMID: 22282074. EXC5

175. Campanella C, Mottolese M, Cianciulli A, et al. Epidermal growth factor receptor gene copy number in 101 advanced colorectal cancer patients treated with chemotherapy plus cetuximab. J Transl Med. 2010;8:36. PMID: 20398370. EXC6

176. Camps C, Jantus-Lewintre E, Cabrera A, et al. The identification of KRAS mutations at codon 12 in plasma DNA is not a prognostic factor in advanced non-small cell lung cancer patients. Lung Cancer. 2011 Jun;72(3):365-9. PMID: 21074889. EXC4

177. Capper D, Berghoff AS, Magerle M, et al. Immunohistochemical testing of BRAF V600E status in 1,120 tumor tissue samples of patients with brain metastases. Acta Neuropathol (Berl). 2012;123(2):223-33. EXC3

178. Capper D, Voigt A, Bozukova G, et al. BRAF V600E-specific immunohistochemistry for the exclusion of Lynch syndrome in MSI-H colorectal cancer. Int J Cancer. 2013 Oct 1;133(7):1624-30. PMID: 23553055. EXC6

179. Cappuzzo F, Ciuleanu T, Stelmakh L, et al. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. Lancet Oncol. 2010(6):521-9. PMID: CN-00749543. EXC5

180. Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. J Clin Oncol. 2003 Jul 15;21(14):2658-63. PMID: 12860941. EXC4

181. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst. 2005 May 4;97(9):643-55. PMID: 15870435. EXC3

182. Cappuzzo F, Ligorio C, Janne PA, et al. Prospective study of gefitinib in epidermal growth factor receptor fluorescence in situ hybridization-positive/phospho-Akt-positive or never smoker patients with advanced non-small-cell lung cancer: the ONCOBELL trial. J Clin Oncol. 2007 Jun 1;25(16):2248-55. PMID: 17538169. EXC6

183. Cappuzzo F, Ligorio C, Toschi L, et al. EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). J Thorac Oncol. 2007 May;2(5):423-9. PMID: 17473658. EXC4

184. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol. 2009 Apr 1;27(10):1667-74. PMID: 19255323. EXC4

185. Cappuzzo F, Varella-Garcia M, Finocchiaro G, et al. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. Br J Cancer. 2008 Jul 8;99(1):83-9. PMID: 18577988. EXC3

186. Caraway NP, Khanna A, Fernandez RL, et al. Fluorescence in situ hybridization for detecting urothelial carcinoma: a clinicopathologic study. Cancer Cytopathol. 2010 Oct 25;118(5):259-68. PMID: 20665656. EXC6

187. Cardoso F, Van't Veer L, Rutgers E, et al. Clinical application of the 70-gene profile: the MINDACT trial. J Clin Oncol. 2008 Feb 10;26(5):729-35. PMID: 18258980. EXC2

188. Carethers JM, Smith EJ, Behling CA, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. Gastroenterology. 2004 Feb;126(2):394-401. PMID: 14762775. EXC3

189. Carlson JJ, Garrison LP, Ramsey SD, et al. The potential clinical and economic outcomes of pharmacogenomic approaches to EGFR-tyrosine kinase inhibitor therapy in non-small-cell lung cancer (Structured abstract). Value in Health. 2009(1):20-7. PMID: NHSEED-22009100664. EXC7

190. Carneiro BA, Ramanathan RK, Fakih MG, et al. Phase II study of irinotecan and cetuximab given every 2 weeks as second-line therapy for advanced colorectal cancer. Clin Colorectal Cancer. 2012 Mar;11(1):53-9. PMID: 21813336. EXC3

191. Carotenuto P, Roma C, Rachiglio AM, et al. Detection of KRAS mutations in colorectal carcinoma patients with an integrated PCR/sequencing and real-time PCR approach. Pharmacogenomics. 2010 Aug;11(8):1169-79. PMID: 20712532. EXC3

192. Carrillo EF, Arias YR, Perdomo SJ, et al. Oncogene amplification as tumor marker in a group of Colombian lung cancer patients. Colombia Medica. 2009;40(2):148-57. EXC7

193. Cartwright T, Chao C, Lee M, et al. Effect of the 12-gene colon cancer assay results on adjuvant treatment recommendations in patients with stage II colon cancer. Curr Med Res Opin. 2013 Nov 7PMID: 24127781.

194. Casali C, Rossi G, Marchioni A, et al. A single institution-based retrospective study of surgically treated bronchioloalveolar adenocarcinoma of the lung: clinicopathologic analysis, molecular features, and possible pitfalls in routine practice. J Thorac Oncol. 2010 Jun;5(6):830-6. PMID: 20521350. EXC6

195. Castro AS, Parente B, Goncalves I, et al. Epidermal growth factor receptor mutation study for 5 years, in a population of patients with non-small cell lung cancer. Rev Port Pneumol. 2013 Jan-Feb;19(1):7-12. PMID: 23265235. EXC6

196. Catalano V, Loupakis F, Graziano F, et al. Mucinous histology predicts for poor response rate and overall survival of patients with colorectal cancer and treated with first-line oxaliplatin- and/or irinotecan-based chemotherapy. Br J Cancer. 2009 Mar 24;100(6):881-7. PMID: 19259089. EXC4

197. Cavallini A, Valentini AM, Lippolis C, et al. KRAS genotyping as biomarker in colorectal cancer: a comparison of three commercial kits on histologic material. Anticancer Res. 2010 Dec;30(12):5251-6. PMID: 21187522. EXC3

198. Cawkwell L, Gray S, Murgatroyd H, et al. Choice of management strategy for colorectal cancer based on a diagnostic immunohistochemical test for defective mismatch repair. Gut. 1999;45(3):409-15. EXC4

199. Cawkwell L, Li D, Lewis FA, et al. Microsatellite instability in colorectal cancer: improved assessment using fluorescent polymerase chain reaction. Gastroenterology. 1995 Aug;109(2):465-71. PMID: 7615195. EXC4

200. Cejas P, Lopez-Gomez M, Aguayo C, et al. KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. PLoS One. 2009;4(12):e8199. PMID: 20020061. EXC6

201. Cejas P, Lopez-Gomez M, Aguayo C, et al. Analysis of the concordance in the EGFR pathway status between primary tumors and related metastases of colorectal cancer patients:implications for cancer therapy. Curr Cancer Drug Targets. 2012 Feb;12(2):124-31. PMID: 22229245. EXC6

202. Ceppi P, Volante M, Novello S, et al. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. Ann Oncol. 2006 Dec;17(12):1818-25. PMID: 16980606. EXC6

203. Chagpar A, Magliocco A, Kerviche A, et al. The Replication Error Phenotype Is Associated with the Development of Distant Metastases in Hormonally Treated Patients with Breast Carcinoma. Cancer. 2004;100(5):913-9. EXC4

204. Chai SM, Zeps N, Shearwood AM, et al. Screening for defective DNA mismatch repair in stage II and III colorectal cancer patients. Clin Gastroenterol Hepatol. 2004 Nov;2(11):1017-25. PMID: 15551255. EXC4

205. Chan JA, Meyerhardt JA, Niedzwiecki D, et al. Association of family history with cancer recurrence and survival among patients with stage III colon cancer. JAMA. 2008 Jun 4;299(21):2515-23. PMID: 18523220. EXC4

206. Chang EY, Dorsey PB, Frankhouse J, et al. Combination of microsatellite instability and lymphocytic infiltrate as a prognostic indicator in colon cancer. Arch Surg. 2009 Jun;144(6):511-5. PMID: 19528382. EXC4

207. Chang EY, Dorsey PB, Johnson N, et al. A prospective analysis of microsatellite instability as a molecular marker in colorectal cancer. Am J Surg. 2006 May;191(5):646-51. PMID: 16647353. EXC4

208. Chang JW, Liu HP, Hsieh MH, et al. Increased epidermal growth factor receptor (EGFR) gene copy number is strongly associated with EGFR mutations and adenocarcinoma in non-small cell lung cancers: a chromogenic in situ hybridization study of 182 patients. Lung Cancer. 2008 Sep;61(3):328-39. PMID: 18304690. EXC6

209. Chang KL, Lau SK. EGFR mutations in non-small cell lung carcinomas may predict response to gefitinib: extension of an emerging paradigm. Adv Anat Pathol. 2005 Mar;12(2):47-52. PMID: 15731572. EXC4

210. Chang MH, Ahn HK, Lee J, et al. Clinical impact of amphiregulin expression in patients with epidermal growth factor receptor (EGFR) wild-type nonsmall cell lung cancer treated with EGFR-tyrosine kinase inhibitors. Cancer. 2011;117(1):143-51. EXC4

211. Chang SC, Lin JK, Lin TC, et al. Loss of heterozygosity: an independent prognostic factor of colorectal cancer. World J Gastroenterol. 2005 Feb 14;11(6):778-84. PMID: 15682467. EXC4

212. Chang YS, Yeh KT, Hsu NC, et al. Detection of N-, H-, and KRAS codons 12, 13, and 61 mutations with universal RAS primer multiplex PCR and N-, H-, and KRAS-specific primer extension. Clin Biochem. 2010 Feb;43(3):296-301. PMID: 19879255. EXC6

213. Chapusot C, Martin L, Puig PL, et al. What is the best way to assess microsatellite instability status in colorectal cancer? Study on a population base of 462 colorectal cancers. Am J Surg Pathol. 2004 Dec;28(12):1553-9. PMID: 15577673. EXC4

214. Charara M, Edmonston TB, Burkholder S, et al. Microsatellite status and cell cycle associated markers in rectal cancer patients undergoing a combined regimen of 5-FU and CPT-11 chemotherapy and radiotherapy. Anticancer Res. 2004;24(5 B):3161-7. EXC6

215. Chaves P, Cruz C, Lage P, et al. Immunohistochemical detection of mismatch repair gene proteins as a useful tool for the identification of colorectal carcinoma with the mutator phenotype. J Pathol. 2000 Aug;191(4):355-60. PMID: 10918209. EXC4

216. Chen HJ, Mok TS, Chen ZH, et al. Clinicopathologic and molecular features of epidermal growth factor receptor T790M mutation and c-MET amplification in tyrosine kinase inhibitor-resistant Chinese non-small cell lung cancer. Pathol Oncol Res. 2009 Dec;15(4):651-8. PMID: 19381876. EXC6

217. Chen KY, Chen JH, Shih JY, et al. Octogenarians with advanced non-small cell lung cancer: treatment modalities, survival, and prognostic factors. J Thorac Oncol. 2010 Jan;5(1):82-9. PMID: 19884854. EXC4

218. Chen P, Wang L, Liu B, et al. EGFR-targeted therapies combined with chemotherapy for treating advanced non-small-cell lung cancer: a meta-analysis (Structured abstract). Eur J Clin Pharmacol. 2011(3):235-43. PMID: DARE-12011002949. EXC1

219. Chen TD, Chang IC, Liu HP, et al. Correlation of anaplastic lymphoma kinase overexpression and the EML4-ALK fusion gene in non-small cell lung cancer by immunohistochemical study. Chang Gung Med J. 2012 Jul-Aug;35(4):309-17. PMID: 22913857. EXC6

220. Chen WS, Chen JY, Liu JM, et al. Microsatellite instability in sporadic-colon-cancer patients with and without liver metastases. Int J Cancer. 1997 Aug 22;74(4):470-4. PMID: 9291442. EXC4

221. Chen X, Li W, Hu X, et al. Effect of gefitinib challenge to initial treatment with non-small cell lung cancer. Biomed Pharmacother. 2011 Dec;65(8):542-6. PMID: 21840160. EXC6

222. Chen YM, Tsai CM, Fan WC, et al. Phase II randomized trial of erlotinib or vinorelbine in chemonaive, advanced, non-small cell lung cancer patients aged 70 years or older. J Thorac Oncol. 2012 Feb;7(2):412-8. PMID: 22157367. EXC6

223. Cheng C, Wu YL, Gu LJ, et al. [Predicting efficacy of neoadjuvant cheomotherapy on resectable stage IIIA non-small cell lung cancer by multi-gene expressions]. Ai zheng = Aizheng = Chinese journal of cancer. 2005(7):846-9. PMID: CN-00569956. EXC2

224. Chiosea S, Shuai Y, Cieply K, et al. EGFR fluorescence in situ hybridization-positive lung adenocarcinoma: incidence of coexisting KRAS and BRAF mutations. Hum Pathol. 2010 Aug;41(8):1053-60. PMID: 20381121. EXC6

225. Chiosea SI, Sherer CK, Jelic T, et al. KRAS mutant allele-specific imbalance in lung adenocarcinoma. Mod Pathol. 2011 Dec;24(12):1571-7. PMID: 21743433. EXC5

226. Cho MC, Choi CM, Kim S, et al. Direct sequencing in cytological specimens as a useful strategy for detecting EGFR mutations in non-small cell lung cancer patients. Clin Chem Lab Med. 2012 Mar;50(3):565-72. PMID: 21899495. EXC4

227. Cho SH, Park LC, Ji JH, et al. Efficacy of EGFR tyrosine kinase inhibitors for non-adenocarcinoma NSCLC patients with EGFR mutation. Cancer Chemother Pharmacol. 2012;70(2):315-20. EXC3

228. Choi CM, Seo KW, Jang SJ, et al. Chromosomal instability is a risk factor for poor prognosis of adenocarcinoma of the lung: Fluorescence in situ hybridization analysis of paraffin-embedded tissue from Korean patients. Lung Cancer. 2009 Apr;64(1):66-70. PMID: 18814932. EXC4

229. Choi DR, Lee DH, Choi CM, et al. Erlotinib in first-line therapy for non-small cell lung cancer: a prospective phase II study. Anticancer Res. 2011 Oct;31(10):3457-62. PMID: 21965761. EXC6

230. Choi H, Kratz J, Pham P, et al. Development of a rapid and practical mutation screening assay for human lung adenocarcinoma. Int J Oncol. 2012 Jun;40(6):1900-6. PMID: 22407457. EXC4

231. Choi H, Paeng JC, Kim DW, et al. Metabolic and metastatic characteristics of ALK-rearranged lung adenocarcinoma on FDG PET/CT. Lung Cancer. 2013 Mar;79(3):242-7. PMID: 23261227. EXC6

232. Choi SW, Lee KJ, Bae YA, et al. Genetic classification of colorectal cancer based on chromosomal loss and microsatellite instability predicts survival. Clin Cancer Res. 2002 Jul;8(7):2311-22. PMID: 12114436. EXC4

233. Choi YJ, Cho BC, Jeong YH, et al. Correlation Between 18F-Fluorodeoxyglucose Uptake and Epidermal Growth Factor Receptor Mutations in Advanced Lung Cancer. Nuclear Med Molec Imaging. 2012;46(3):169-75. EXC3

234. Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med. 2010 Oct 28;363(18):1734-9. PMID: 20979473. EXC2

235. Cholongitas E, Kokolakis G, Ioannidou D. How important is the pus culture obtained from epithelial growth factor receptor (EGFR) inhibitors'associated rash? Int J Dermatol. 2008;47(11):1203-4. EXC8

236. Chou TY, Chiu CH, Li LH, et al. Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res. 2005 May 15;11(10):3750-7. PMID: 15897572. EXC6

237. Christensen M, Katballe N, Wikman F, et al. Antibody-based screening for hereditary nonpolyposis colorectal carcinoma compared with microsatellite analysis and sequencing. Cancer. 2002 Dec 1;95(11):2422-30. PMID: 12436451. EXC3

238. Chua W, Goldstein D, Lee CK, et al. Molecular markers of response and toxicity to FOLFOX chemotherapy in metastatic colorectal cancer. Br J Cancer. 2009 Sep 15;101(6):998-1004. PMID: 19672255. EXC3

239. Chuko J, Yeh MK, Chen BJ, et al. Efficacy of cetuximab on wild-type and mutant KRAS in colorectal cancer: systematic review and meta-analysis (Structured abstract). J Med Sciences. 2010(5):189-98. PMID: DARE-12010007964. EXC2

240. Chung CH, Seeley EH, Roder H, et al. Detection of tumor epidermal growth factor receptor pathway dependence by serum mass spectrometry in cancer patients. Cancer Epidemiology Biomarkers and Prevention. 2010;19(2):358-65. EXC4

241. Chung FT, Lee KY, Wang CW, et al. Tumor-associated macrophages correlate with response to epidermal growth factor receptor-tyrosine kinase inhibitors in advanced non-small cell lung cancer. Int J Cancer. 2012 Aug 1;131(3):E227-35. PMID: 22174092. EXC4

242. Chung JH, Choe G, Jheon S, et al. Epidermal growth factor receptor mutation and pathologic-radiologic correlation between multiple lung nodules with ground-glass opacity differentiates multicentric origin from intrapulmonary spread. J Thorac Oncol. 2009 Dec;4(12):1490-5. PMID: 19844187. EXC6

243. Chung KP, Wu SG, Wu JY, et al. Clinical outcomes in non-small cell lung cancers harboring different exon 19 deletions in EGFR. Clin Cancer Res. 2012;18(12):3470-7. EXC6

244. Chung KY, Kim NG, Li LS, et al. Clinicopathologic characteristics related to the high variability of coding mononucleotide repeat sequences in tumors with high-microsatellite instability. Oncol Rep. 2003 Mar-Apr;10(2):439-44. PMID: 12579286. EXC6

245. Cicek MS, Lindor NM, Gallinger S, et al. Quality assessment and correlation of microsatellite instability and immunohistochemical markers among population- and clinic-based colorectal tumors results from the Colon Cancer Family Registry. J Mol Diagn. 2011 May;13(3):271-81. PMID: 21497289. EXC4

246. Ciledag A, Kaya A, Yetkin O, et al. The prognostic value of serum epidermal growth factor receptor level in patients with non-small cell lung cancer. Tuberk Toraks. 2008;56(4):390-5. PMID: 19123074. EXC4

247. Clark GM, Zborowski DM, Santabarbara P, et al. Smoking history and epidermal growth factor receptor expression as predictors of survival benefit from erlotinib for patients with non-small-cell lung cancer in the National Cancer Institute of Canada Clinical Trials Group study BR.21. Clin Lung Cancer. 2006 May;7(6):389-94. PMID: 16800964. EXC5

248. Cobleigh MA, Tabesh B, Bitterman P, et al. Tumor gene expression and prognosis in breast cancer patients with 10 or more positive lymph nodes. Clin Cancer Res. 2005 Dec 15;11(24 Pt 1):8623-31. PMID: 16361546. EXC4

249. Cohn AL, Shumaker GC, Khandelwal P, et al. An open-label, single-arm, phase 2 trial of panitumumab plus FOLFIRI as second-line therapy in patients with metastatic colorectal cancer. Clin Colorectal Cancer. 2011 Sep;10(3):171-7. PMID: 21855038. EXC3

250. Cohn DE, Pavelka JC, Frankel WL, et al. Correlation between patient weight and defects in DNA mismatch repair: Is this the link between an increased risk of previous cancer in thinner women with endometrial cancer? Int J Gynecol Cancer. 2008;18(1):136-40. EXC5

251. Colebatch A, Hitchins M, Williams R, et al. The role of MYH and microsatellite instability in the development of sporadic colorectal cancer. Br J Cancer. 2006 Nov 6;95(9):1239-43. PMID: 17031395. EXC6

252. Colombino M, Cossu A, Manca A, et al. Prevalence and prognostic role of microsatellite instability in patients with rectal carcinoma. Ann Oncol. 2002 Sep;13(9):1447-53. PMID: 12196371. EXC7

253. Colucci G, Giuliani F, Garufi C, et al. Cetuximab plus FOLFOX-4 in untreated patients with advanced colorectal cancer: a Gruppo Oncologico dell'Italia Meridionale Multicenter phase II study. Oncology. 2010;79(5-6):415-22. PMID: 21474966. EXC3

254. Conde E, Angulo B, Tang M, et al. Molecular context of the EGFR mutations: evidence for the activation of mTOR/S6K signaling. Clin Cancer Res. 2006 Feb 1;12(3 Pt 1):710-7. PMID: 16467080. EXC6

255. Conklin CM, Craddock KJ, Have C, et al. Immunohistochemistry is a reliable screening tool for identification of ALK rearrangement in non-small-cell lung carcinoma and is antibody dependent. J Thorac Oncol. 2013 Jan;8(1):45-51. PMID: 23196275. EXC4

256. Coolbaugh-Murphy MI, Xu JP, Ramagli LS, et al. Microsatellite instability in the peripheral blood leukocytes of HNPCC patients. Hum Mutat. 2010 Mar;31(3):317-24. PMID: 20052760. EXC3

257. Cooper WA, Yu B, Yip PY, et al. EGFR mutant-specific immunohistochemistry has high specificity and sensitivity for detecting targeted activating EGFR mutations in lung adenocarcinoma. J Clin Pathol. 2013 Sep;66(9):744-8. PMID: 23757037. EXC4

258. Coppola D, Nebozhyn M, Khalil F, et al. Unique ectopic lymph node-like structures present in human primary colorectal carcinoma are identified by immune gene array profiling. Am J Pathol. 2011 Jul;179(1):37-45. PMID: 21703392. EXC4

259. Cornianu M, Tudose N. Immunohistochemical markers in the morphological diagnosis of lung carcinoma. Rom J Morphol Embryol. 1997 Jul-Dec;43(3-4):181-91. PMID: 9747120. EXC4

260. Cortas T, Eisenberg R, Fu P, et al. Activation state EGFR and STAT-3 as prognostic markers in resected non-small cell lung cancer. Lung Cancer. 2007 Mar;55(3):349-55. PMID: 17161498. EXC4

261. Cortot AB, Italiano A, Burel-Vandenbos F, et al. KRAS mutation status in primary nonsmall cell lung cancer and matched metastases. Cancer. 2010 Jun 1;116(11):2682-7. PMID: 20336783. EXC5

262. Cosler LE, Kuderer NM, Hornberger J, et al. 21-gene RT-PCR assay in lymph node negative (LN-), estrogen receptor positive (ER+) breast cancer: An economic analysis including prognostic and predictive information. J Clin Oncol. 2006 Jun 20;24(18):307s-s. PMID: ISI:000239009402097. EXC2

263. Cosler LE, Lyman GH. Economic analysis of gene expression profile data to guide adjuvant treatment in women with early-stage breast cancer. Cancer Invest. 2009 Dec;27(10):953-9. PMID: 19909009. EXC6

264. Cox G, Jones JL, Andi A, et al. A biological staging model for operable non-small cell lung cancer. Thorax. 2001 Jul;56(7):561-6. PMID: 11413356. EXC4

265. Cox G, Louise Jones J, Andi A, et al. Bcl-2 is an independent prognostic factor and adds to a biological model for predicting outcome in operable non-small cell lung cancer. Lung Cancer. 2001 Dec;34(3):417-26. PMID: 11714539. EXC4

266. CsToth I, Anthoine G, Berghmans T, et al. C-erbB-3 expression in non-small cell lung cancer (NSCLC) patients treated by Erlotinib. Anticancer Res. 2011 Jan;31(1):281-5. PMID: 21273611. EXC4

267. Cuadros-Celorrio M, Llanos-Mendez A, Villegas-Portero R. Mammaprint (Structured abstract). Health Technology Assessment Database. 2010(3)PMID: HTA-32011000518. EXC2

268. Cuffe S, Bourredjem A, Graziano S, et al. A pooled exploratory analysis of the effect of tumor size and KRAS mutations on survival benefit from adjuvant platinum-based chemotherapy in node-negative non-small cell lung cancer. J Thorac Oncol. 2012 Jun;7(6):963-72. PMID: 22588152. EXC6

269. Curtin K, Samowitz WS, Ulrich CM, et al. Nutrients in folate-mediated, one-carbon metabolism and the risk of rectal tumors in men and women. Nutr Cancer. 2011;63(3):357-66. PMID: 21462086. EXC6

270. Curtin K, Ulrich CM, Samowitz WS, et al. Thymidylate synthase polymorphisms and colon cancer: associations with tumor stage, tumor characteristics and survival. Int J Cancer. 2007 May 15;120(10):2226-32. PMID: 17290389. EXC6

271. Cushman-Vokoun AM, Crowley AM, Rapp SA, et al. Comparison study of the performance of the QIAGEN EGFR RGQ and EGFR pyro assays for mutation analysis in non-small cell lung cancer. Am J Clin Pathol. 2013 Jul;140(1):7-19. PMID: 23765529. EXC4

272. Cutsem E, Köhne CH, Láng I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011(15):2011-9. PMID: CN-00788862. EXC6

273. Dacic S, Flanagan M, Cieply K, et al. Significance of EGFR protein expression and gene amplification in non-small cell lung carcinoma. Am J Clin Pathol. 2006 Jun;125(6):860-5. PMID: 16690485. EXC4

274. Dacic S, Shuai Y, Yousem S, et al. Clinicopathological predictors of EGFR/KRAS mutational status in primary lung adenocarcinomas. Mod Pathol. 2010 Feb;23(2):159-68. PMID: 19855375. EXC6

275. Dahabreh IJ, Linardou H, Siannis F, et al. Somatic EGFR mutation and gene copy gain as predictive biomarkers for response to tyrosine kinase inhibitors in non-small cell lung cancer (Provisional abstract). Clin Cancer Res. 2010(1):291-303. PMID: DARE-12010001146. EXC1

276. Dahlin AM, Henriksson ML, Van Guelpen B, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. Mod Pathol. 2011;24(5):671-82. EXC4

277. Dahlin AM, Palmqvist R, Henriksson ML, et al. The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status. Clin Cancer Res. 2010 Mar 15;16(6):1845-55. PMID: 20197478. EXC6

278. Dahse R, Berndt A, Kosmehl H. PCR-based testing for therapy-related EGFR mutations in patients with non-small cell lung cancer. Anticancer Res. 2008 Jul-Aug;28(4B):2265-70. PMID: 18751405. EXC6

279. D'Angelo SP, Janjigian YY, Ahye N, et al. Distinct clinical course of EGFR-mutant resected lung cancers: results of testing of 1118 surgical specimens and effects of adjuvant gefitinib and erlotinib. J Thorac Oncol. 2012 Dec;7(12):1815-22. PMID: 23154553. EXC7

280. Daniele L, Cassoni P, Bacillo E, et al. Epidermal growth factor receptor gene in primary tumor and metastatic sites from non-small cell lung cancer. J Thorac Oncol. 2009 Jun;4(6):684-8. PMID: 19404216. EXC6

281. Daniely M, Rona R, Kaplan T, et al. Combined morphologic and fluorescence in situ hybridization analysis of voided urine samples for the detection and follow-up of bladder cancer in patients with benign urine cytology. Cancer. 2007;111(6):517-24. EXC5

282. Daniely M, Rona R, Kaplan T, et al. Combined analysis of morphology and fluorescence in situ hybridization significantly increases accuracy of bladder cancer detection in voided urine samples. Urology. 2005 Dec;66(6):1354-9. PMID: 16360483. EXC5

283. . A prospective clinical utility study of the impact of the 21-gene recurrence score assay (Oncotype DX) in estrogen receptor positive (ER+) node negative (pN0) breast cancer in academic Canadian centers. ASCO; 2012. Exc-2

284. Davies JM, Trembath D, Deal AM, et al. Phospho-ERK and AKT status, but not KRAS mutation status, are associated with outcomes in rectal cancer treated with chemoradiotherapy. Radiat Oncol. 2011;6:114. PMID: 21910869. EXC6

285. De Bruijn MT, Raats DAE, Tol J, et al. Combined KRAS and TP53 mutation status is not predictive in CAPOX-treated metastatic colorectal cancer. Anticancer Res. 2011;31(4):1379-85. EXC5

286. de Lima Lopes G, Chien R, Hornberger J. Cost-benefit of the 21-gene breast cancer recurrence score assay for patients in Singapore. Breast J. 2013 Mar-Apr;19(2):220-1. PMID: 23320386. EXC2

287. De Oliveira Duarte Achcar R, Nikiforova MN, Yousem SA. Micropapillary lung adenocarcinoma: EGFR, K-ras, and BRAF mutational profile. Am J Clin Pathol. 2009 May;131(5):694-700. PMID: 19369630. EXC6

288. De Pas T, Toffalorio F, Manzotti M, et al. Activity of epidermal growth factor receptor-tyrosine kinase inhibitors in patients with non-small cell lung cancer harboring rare epidermal growth factor receptor mutations. J Thorac Oncol. 2011 Nov;6(11):1895-901. PMID: 21841502. EXC4

289. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010 Aug;11(8):753-62. PMID: 20619739. EXC3

290. De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA. 2010 Oct 27;304(16):1812-20. PMID: 20978259. EXC3

291. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol. 2008 Mar;19(3):508-15. PMID: 17998284. EXC3

292. De Ruyck K, Sabbe N, Oberije C, et al. Development of a multicomponent prediction model for acute esophagitis in lung cancer patients receiving chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2011 Oct 1;81(2):537-44. PMID: 21605946. EXC4

293. de Vos tot Nederveen Cappel WH, Meulenbeld HJ, Kleibeuker JH, et al. Survival after adjuvant 5-FU treatment for stage III colon cancer in hereditary nonpolyposis colorectal cancer. Int J Cancer. 2004 Apr 10;109(3):468-71. PMID: 14961589. EXC4

294. Debniak T, Gorski B, Cybulski C, et al. Comparison of Alu-PCR, microsatellite instability, and immunohistochemical analyses in finding features characteristic for hereditary nonpolyposis colorectal cancer. J Cancer Res Clin Oncol. 2001;127(9):565-9. EXC3

295. Deeb G, Wang J, Ramnath N, et al. Altered E-cadherin and epidermal growth factor receptor expressions are associated with patient survival in lung cancer: a study utilizing high-density tissue microarray and immunohistochemistry. Mod Pathol. 2004 Apr;17(4):430-9. PMID: 14739904. EXC4

296. Denice Smith G, Sangle NA, Wilson A, et al. A retrospective review of UroVysion fish interpretations over 8.6 years: A major shift in the patient test population. Diagn Cytopathol. 2013 May;41(5):437-47. PMID: 2013255978 MEDLINE PMID 22865746 (http://www.ncbi.nlm.nih.gov/pubmed/22865746) FULL TEXT LINK http://dx.doi.org/10.1002/dc.22881. EXC6

297. Denzinger S, Stoehr R, Schwarz S, et al. Low level STK15 amplification in histologically benign urothelium of patients with bladder cancer adversely predicts patient outcome following cystectomy. Int J Oncol. 2007 Oct;31(4):793-802. PMID: 17786310. EXC6

298. Des Guetz G, Lecaille C, Mariani P, et al. Prognostic impact of microsatellite instability in colorectal cancer patients treated with adjuvant FOLFOX. Anticancer Res. 2010 Oct;30(10):4297-301. PMID: 21036755. EXC4

299. des Guetz G, Mariani P, Cucherousset J, et al. Microsatellite instability and sensitivitiy to FOLFOX treatment in metastatic colorectal cancer. Anticancer Res. 2007 Jul-Aug;27(4C):2715-9. PMID: 17695437. EXC3

300. Deschoolmeester V, Baay M, Van Marck E, et al. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC Immunol. 2010;11:19. PMID: 20385003. EXC4

301. Deschoolmeester V, Van Damme N, Baay M, et al. Microsatellite instability in sporadic colon carcinomas has no independent prognostic value in a Belgian study population. Eur J Cancer. 2008 Oct;44(15):2288-95. PMID: 18707864. EXC4

302. Dewdney A, Cunningham D, Tabernero J, et al. Multicenter randomized phase II clinical trial comparing neoadjuvant oxaliplatin, capecitabine, and preoperative radiotherapy with or without cetuximab followed by total mesorectal excision in patients with high-risk rectal cancer (EXPERT-C). J Clin Oncol. 2012 May 10;30(14):1620-7. PMID: 22473163. EXC5

303. Di Bartolomeo M, Dotti KF, Pietrantonio F, et al. Biological analysis of phase ii study evaluating the activity of cetuximab combined to oxaliplatin and fluoropirimidine (TEGAFOX-E) as first line treatment in metastatic colorectal cancer (mCRC) pts by the italian trials in medical oncology (I.T.M.O.) group. Eur J Cancer. 2011;47:S433. EXC6

304. Di Maio M, Leighl NB, Gallo C, et al. Quality of life analysis of TORCH, a randomized trial testing first-line erlotinib followed by second-line cisplatin/gemcitabine chemotherapy in advanced non-small-cell lung cancer. J Thorac Oncol. 2012 Dec;7(12):1830-44. PMID: 23154555. EXC3

305. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008 Dec 10;26(35):5705-12. PMID: 19001320. EXC3

306. Diaz-Rubio E, Gomez-Espana A, Massuti B, et al. Role of Kras Status in Patients with Metastatic Colorectal Cancer Receiving First-Line Chemotherapy plus Bevacizumab: A TTD Group Cooperative Study. PLoS One. 2012;7(10). EXC3

307. Dicuonzo G, Angeletti S, Garcia-Foncillas J, et al. Colorectal carcinomas and PTEN/MMAC1 gene mutations. Clin Cancer Res. 2001 Dec;7(12):4049-53. PMID: 11751500. EXC8

308. Dienstmann R, Serpico D, Rodon J, et al. Molecular profiling of patients with colorectal cancer and matched targeted therapy in phase I clinical trials. Molecul Cancer Therapeut. 2012;11(9):2062-71. EXC4

309. Diep CB, Thorstensen L, Meling GI, et al. Genetic tumor markers with prognostic impact in Dukes' stages B and C colorectal cancer patients. J Clin Oncol. 2003 Mar 1;21(5):820-9. PMID: 12610180. EXC4

310. Dietmaier W, Wallinger S, Bocker T, et al. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res. 1997 Nov 1;57(21):4749-56. PMID: 9354436. EXC4

311. Dimou A, Agarwal S, Anagnostou V, et al. Standardization of epidermal growth factor receptor (EGFR) measurement by quantitative immunofluorescence and impact on antibody-based mutation detection in non-small cell lung cancer. Am J Pathol. 2011 Aug;179(2):580-9. PMID: 21722621. EXC4

312. Dittadi R, Gion M, Pagan V, et al. Epidermal growth factor receptor in lung malignancies. Comparison between cancer and normal tissue. Br J Cancer. 1991 Oct;64(4):741-4. PMID: 1654986. EXC4

313. Dodurga Y, Avc ICB, S YI, et al. UroVysion fluorescence in situ hybridization (UroVysion FISH) assay for detection of bladder cancer in voided urine of Turkish patients: A preliminary study. Wspolczesna Onkologia. 2013 2013;17(2):156-60. PMID: 2013308443 FULL TEXT LINK http://dx.doi.org/10.5114/wo.2013.34619. EXC4

314. Doebele RC, Lu X, Sumey C, et al. Oncogene status predicts patterns of metastatic spread in treatment-naive nonsmall cell lung cancer. Cancer. 2012;118(18):4502-11. EXC3

315. Dogan S, Shen R, Ang DC, et al. Molecular epidemiology of EGFR and KRAS mutations in 3,026 lung adenocarcinomas: higher susceptibility of women to smoking-related KRAS-mutant cancers. Clin Cancer Res. 2012 Nov 15;18(22):6169-77. PMID: 23014527. EXC6

316. Doi T, Tahara M, Yoshino T, et al. Tumor KRAS status predicts responsiveness to panitumumab in Japanese patients with metastatic colorectal cancer. Jpn J Clin Oncol. 2011 Feb;41(2):210-6. PMID: 21169348. EXC3

317. Dolcetti R, Viel A, Doglioni C, et al. High prevalence of activated intraepithelial cytotoxic T lymphocytes and increased neoplastic cell apoptosis in colorectal carcinomas with microsatellite instability. Am J Pathol. 1999 Jun;154(6):1805-13. PMID: 10362805. EXC4

318. Dong HS, Dong KC, Kim YH, et al. Effectiveness of each bethesda marker in defining microsatellite instability when screening for Lynch syndrome. Hepatogastroenterology. 2009;56(91-92):672-6. EXC3

319. Dong J, Dai J, Shu Y, et al. Polymorphisms in EGFR and VEGF contribute to non-small-cell lung cancer survival in a Chinese population. Carcinogenesis. 2010;31(6):1080-6. EXC5

320. Dongiovanni D, Daniele L, Barone C, et al. Gefitinib (ZD1839): therapy in selected patients with non-small cell lung cancer (NSCLC)? Lung Cancer. 2008 Jul;61(1):73-81. PMID: 18243402. EXC6

321. Donovan MJ, Kotsianti A, Bayer-Zubek V, et al. A systems pathology model for predicting overall survival in patients with refractory, advanced non-small-cell lung cancer treated with gefitinib. Eur J Cancer. 2009 May;45(8):1518-26. PMID: 19272767. EXC6

322. Dorard C, De Thonel A, Collura A, et al. Expression of a mutant HSP110 sensitizes colorectal cancer cells to chemotherapy and improves disease prognosis. Nat Med. 2011;17(10):1283-9. EXC4

323. Dotan E, Meropol NJ, Zhu F, et al. Relationship of increased aurora kinase A gene copy number, prognosis and response to chemotherapy in patients with metastatic colorectal cancer. Br J Cancer. 2012;106(4):748-55. EXC4

324. Douillard JY, Shepherd FA, Hirsh V, et al. Molecular predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III INTEREST trial. J Clin Oncol. 2010(5):744-52. PMID: CN-00728915. EXC5

325. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol. 2010 Nov 1;28(31):4697-705. PMID: 20921465. EXC3

326. Dove-Edwin I, de Jong AE, Adams J, et al. Prospective Results of Surveillance Colonoscopy in Dominant Familial Colorectal Cancer With and Without Lynch Syndrome. Gastroenterology. 2006;130(7):1995-2000. EXC6

327. Dragani TA, Hirohashi S, Juji T, et al. Population-based mapping of pulmonary adenoma susceptibility 1 locus. Cancer Res. 2000 Sep 15;60(18):5017-20. PMID: 11016621. EXC4

328. Dragnev KH, Ma T, Cyrus J, et al. Bexarotene plus erlotinib suppress lung carcinogenesis independent of KRAS mutations in two clinical trials and transgenic models. Cancer Prev Res (Phila). 2011 Jun;4(6):818-28. PMID: 21636548. EXC4

329. Dragnev KH, Petty WJ, Shah S, et al. Bexarotene and erlotinib for aerodigestive tract cancer. J Clin Oncol. 2005 Dec 1;23(34):8757-64. PMID: 16314636. EXC6

330. Drukker CA, Bueno-de-Mesquita JM, Retel VP, et al. A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. Int J Cancer. 2013 Jan 31PMID: 23371464. EXC 6

331. Dudek AZ, Kmak KL, Koopmeiners J, et al. Skin rash and bronchoalveolar histology correlates with clinical benefit in patients treated with gefitinib as a therapy for previously treated advanced or metastatic non-small cell lung cancer. Lung Cancer. 2006 Jan;51(1):89-96. PMID: 16290256. EXC6

332. Dufort S, Richard MJ, Lantuejoul S, et al. Pyrosequencing, a method approved to detect the two major EGFR mutations for anti EGFR therapy in NSCLC. J Exp Clin Cancer Res. 2011;30:57. PMID: 21575212. EXC6

333. Dutu T, Michiels S, Fouret P, et al. Differential expression of biomarkers in lung adenocarcinoma: a comparative study between smokers and never-smokers. Ann Oncol. 2005 Dec;16(12):1906-14. PMID: 16219624. EXC4

334. Dziadziuszko R, Holm B, Skov BG, et al. Epidermal growth factor receptor gene copy number and protein level are not associated with outcome of non-small-cell lung cancer patients treated with chemotherapy. Ann Oncol. 2007 Mar;18(3):447-52. PMID: 17082511. EXC4

335. Dziadziuszko R, Witta SE, Cappuzzo F, et al. Epidermal growth factor receptor messenger RNA expression, gene dosage, and gefitinib sensitivity in non-small cell lung cancer. Clin Cancer Res. 2006 May 15;12(10):3078-84. PMID: 16707605. EXC6

336. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol. 2005 Sep 1;23(25):5900-9. PMID: 16043828. EXC6

337. Ebinger M, Sotlar K, Weber A, et al. Simplified detection of microsatellite instability in colorectal cancer without the need for corresponding germline DNA analysis. J Clin Pathol. 2006;59(10):1114-5. EXC4

338. Ecri. Microsatellite instability testing for hereditary nonpolyposis colorectal cancer (Structured abstract). Health Technology Assessment Database. 2002(3):28. PMID: HTA-32006000547. EXC3

339. Edmonston TB, Cuesta KH, Burkholder S, et al. Colorectal carcinomas with high microsatellite instability: defining a distinct immunologic and molecular entity with respect to prognostic markers. Hum Pathol. 2000 Dec;31(12):1506-14. PMID: 11150376. EXC8

340. Eichler AF, Kahle KT, Wang DL, et al. EGFR mutation status and survival after diagnosis of brain metastasis in nonsmall cell lung cancer. Neuro Oncol. 2010 Nov;12(11):1193-9. PMID: 20627894. EXC6

341. Ellison G, Donald E, McWalter G, et al. A comparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. J Exp Clin Cancer Res. 2010;29:132. PMID: 20925915. EXC3

342. El-Osta H, Falchook G, Tsimberidou A, et al. BRAF mutations in advanced cancers: Clinical characteristics and outcomes. PLoS One. 2011;6(10). EXC4

343. Elsaleh H, Cserni G, Iacopetta B. Extent of nodal involvement in Stage III colorectal carcinoma: relationship to clinicopathologic variables and genetic alterations. Dis Colon Rectum. 2002 Sep;45(9):1218-22. PMID: 12352240. EXC4

344. Elsaleh H, Iacopetta B. Microsatellite instability is a predictive marker for survival benefit from adjuvant chemotherapy in a population-based series of stage III colorectal carcinoma. Clin Colorectal Cancer. 2001 Aug;1(2):104-9. PMID: 12445368. EXC4

345. Elsaleh H, Powell B, McCaul K, et al. P53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. Clin Cancer Res. 2001 May;7(5):1343-9. PMID: 11350904. EXC4

346. Elsaleh H, Powell B, Soontrapornchai P, et al. p53 gene mutation, microsatellite instability and adjuvant chemotherapy: impact on survival of 388 patients with Dukes' C colon carcinoma. Oncology. 2000;58(1):52-9. PMID: 10644941. EXC4

347. Emterling A, Wallin A, Arbman G, et al. Clinicopathological significance of microsatellite instability and mutated RIZ in colorectal cancer. Ann Oncol. 2004 Feb;15(2):242-6. PMID: 14760116. EXC4

348. Endo K, Konishi A, Sasaki H, et al. Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay. Lung Cancer. 2005 Dec;50(3):375-84. PMID: 16199108. EXC6

349. Endo K, Sasaki H, Yano M, et al. Evaluation of the epidermal growth factor receptor gene mutation and copy number in non-small cell lung cancer with gefitinib therapy. Oncol Rep. 2006 Sep;16(3):533-41. PMID: 16865253. EXC6

350. Endoh H, Ishibashi Y, Yamaki E, et al. Immunohistochemical analysis of phosphorylated epidermal growth factor receptor might provide a surrogate marker of EGFR mutation. Lung Cancer. 2009 Feb;63(2):241-6. PMID: 18585821. EXC4

351. Endoh H, Yatabe Y, Kosaka T, et al. PTEN and PIK3CA expression is associated with prolonged survival after gefitinib treatment in EGFR-mutated lung cancer patients. J Thorac Oncol. 2006 Sep;1(7):629-34. PMID: 17409929. EXC6

352. Erb C, Fox K, Patel M. Evaluation of practice patterns in the treatment of node-negative, hormone-receptor positive breast cancer patients with the use of the Oncotype DX assay at the University of Pennsylvania. 30th Annual San Antonio Breast Cancer Symposium; 2007. Breast Cancer Res Treat; 106. p. A3082. EXC2

353. Erdamar S, Ucaryilmaz E, Demir G, et al. Importance of MutL homologue MLH1 and MutS homologue MSH2 expression in Turkish patients with sporadic colorectal cancer. World J Gastroenterol. 2007 Sep 7;13(33):4437-44. PMID: 17724798. EXC4

354. Esemuede I, Forslund A, Khan SA, et al. Improved testing for microsatellite instability in colorectal cancer using a simplified 3-marker assay. Ann Surg Oncol. 2010 Dec;17(12):3370-8. PMID: 20703819. EXC5

355. Espinel CF, Keating S, Hibshoosh H, et al. MammaPrint Feasibility in a Large Tertiary Urban Medical Center: An Initial Experience. Scientifica. 2012;2012:1-5. EXC6

356. Espinosa E, Sanchez-Navarro I, Gamez-Pozo A, et al. Comparison of prognostic gene profiles using qRT-PCR in paraffin samples: a retrospective study in patients with early breast cancer. PLoS One. 2009;4(6):e5911. PMID: 19547727. EXC5

357. Esserman LJ, Berry DA, Cheang MC, et al. Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). Breast Cancer Res Treat. 2012 Apr;132(3):1049-62. PMID: 22198468. EXC6

358. Esserman LJ, Berry DA, DeMichele A, et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL--CALGB 150007/150012, ACRIN 6657. J Clin Oncol. 2012 Sep 10;30(26):3242-9. PMID: 22649152. EXC4

359. Esserman LJ, Shieh Y, Rutgers EJT, et al. Impact of mammographic screening on the detection of good and poor prognosis breast cancers. Breast Cancer Res Treat. 2011;130(3):725-34. EXC5

360. Evertson S, Wallin A, Arbman G, et al. Microsatellite instability and MBD4 mutation in unselected colorectal cancer. Anticancer Res. 2003 Jul-Aug;23(4):3569-74. PMID: 12926109. EXC4

361. Fan X, Liu B, Xu H, et al. Immunostaining with EGFR mutation-specific antibodies: a reliable screening method for lung adenocarcinomas harboring EGFR mutation in biopsy and resection samples. Hum Pathol. 2013 Aug;44(8):1499-507. PMID: 23465272. EXC4

362. Farina G, Longo F, Martelli O, et al. Rationale for treatment and study design of tailor: a randomized phase III trial of second-line erlotinib versus docetaxel in the treatment of patients affected by advanced non-small-cell lung cancer with the absence of epidermal growth factor receptor mutations. Clin Lung Cancer. 2011(2):138-41. PMID: CN-00800187. EXC2

363. Farrington SM, Lin-Goerke J, Ling J, et al. Systematic analysis of hMSH2 and hMLH1 in young colon cancer patients and controls. Am J Hum Gene. 1998;63(3):749-59. EXC2

364. Farrington SM, McKinley AJ, Carothers AD, et al. Evidence for an age-related influence of microsatellite instability on colorectal cancer survival. Int J Cancer. 2002 Apr 20;98(6):844-50. PMID: 11948461. EXC4

365. Fassina A, Gazziero A, Zardo D, et al. Detection of EGFR and KRAS mutations on trans-thoracic needle aspiration of lung nodules by high resolution melting analysis. J Clin Pathol. 2009 Dec;62(12):1096-102. PMID: 19640859. EXC6

366. Feeley KM, Fullard JF, Heneghan MA, et al. Microsatellite instability in sporadic colorectal carcinoma is not an indicator of prognosis. J Pathol. 1999 May;188(1):14-7. PMID: 10398134. EXC4

367. Felip E, Rojo F, Reck M, et al. A phase II pharmacodynamic study of erlotinib in patients with advanced non-small cell lung cancer previously treated with platinum-based chemotherapy. Clin Cancer Res. 2008 Jun 15;14(12):3867-74. PMID: 18559607. EXC6

368. Feliu Batlle J, Cuadrado E, Castro J, et al. Irinotecan-cetuximab-bevacizumab as a salvage treatment in heavily pretreated metastatic colorectal cancer patients: A retrospective observational study. Chemotherapy. 2011;57(2):138-44. EXC8

369. Fernandez-Peralta AM, Nejda N, Oliart S, et al. Significance of mutations in TGFBR2 and BAX in neoplastic progression and patient outcome in sporadic colorectal tumors with high-frequency microsatellite instability. Cancer Genet Cytogenet. 2005;157(1):18-24. EXC4

370. Fernando Lopez-Rios F, Angulo B, Gomez B, et al. Comparison of molecular testing methods for the detection of EGFR mutations in formalin-fixed paraffin-embedded tissue (FFPET) specimens of non-small cell lung cancer (NSCLC). ESMO Lung; 2012 Geneva. EXC9

371. Fernebro E, Halvarsson B, Baldetorp B, et al. Predominance of CIN versus MSI in the development of rectal cancer at young age. BMC Cancer. 2002 Oct 14;2:25. PMID: 12379157. EXC6

372. Ferra S, Denley R, Herr H, et al. Reflex UroVysion testing in suspicious urine cytology cases. Cancer. 2009 Feb 25;117(1):7-14. PMID: 19347824. EXC3

373. Fidler MJ, Argiris A, Patel JD, et al. The potential predictive value of cyclooxygenase-2 expression and increased risk of gastrointestinal hemorrhage in advanced non-small cell lung cancer patients treated with erlotinib and celecoxib. Clin Cancer Res. 2008 Apr 1;14(7):2088-94. PMID: 18381949. EXC6

374. Fidler MJ, Morrison LE, Basu S, et al. PTEN and PIK3CA gene copy numbers and poor outcomes in non-small cell lung cancer patients with gefitinib therapy. Br J Cancer. 2011 Dec 6;105(12):1920-6. PMID: 22095222. EXC3

375. Finberg KE, Sequist LV, Joshi VA, et al. Mucinous differentiation correlates with absence of EGFR mutation and presence of KRAS mutation in lung adenocarcinomas with bronchioloalveolar features. J Mol Diagn. 2007 Jul;9(3):320-6. PMID: 17591931. EXC6

376. Firestein R, Shima K, Nosho K, et al. CDK8 expression in 470 colorectal cancers in relation to beta-catenin activation, other molecular alterations and patient survival. Int J Cancer. 2010 Jun 15;126(12):2863-73. PMID: 19790197. EXC6

377. Fisher B, Dignam J, Wolmark N, et al. Tamoxifen and chemotherapy for lymph node-negative, estrogen receptor-positive breast cancer. J Natl Cancer Inst. 1997 Nov 19;89(22):1673-82. PMID: 9390536. EXC6

378. . Evaluation of Oncotype DX testing and subsequent patterns of care in patients (pts) with early-stage breast cancer (ESBC). ASCO; 2012. EXC6

379. Flaig TW, Wilson S, van Bokhoven A, et al. Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma. Urology. 2011 Oct;78(4):863-7. PMID: 21813167. EXC5

380. Flanagan MB, Dabbs DJ, Brufsky AM, et al. Histopathologic variables predict Oncotype DX recurrence score. Mod Pathol. 2008 Oct;21(10):1255-61. PMID: 18360352. EXC6

381. Fong KM, Zimmerman PV, Smith PJ. KRAS codon 12 mutations in Australian non-small cell lung cancer. Aust N Z J Med. 1998 Apr;28(2):184-9. PMID: 9612526. EXC6

382. Fontanini G, De Laurentiis M, Vignati S, et al. Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I-IIIA non-small-cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. Clin Cancer Res. 1998 Jan;4(1):241-9. PMID: 9516978. EXC4

383. Fornaro L, Baldi GG, Masi G, et al. Cetuximab plus irinotecan after irinotecan failure in elderly metastatic colorectal cancer patients: clinical outcome according to KRAS and BRAF mutational status. Crit Rev Oncol Hematol. 2011 Jun;78(3):243-51. PMID: 20619672. EXC3

384. Forster S, Sattler HP, Hack M, et al. Microsatellite instability in sporadic carcinomas of the proximal colon: association with diploid DNA content, negative protein expression of p53, and distinct histomorphologic features. Surgery. 1998 Jan;123(1):13-8. PMID: 9457218. EXC4

385. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer. 2007 Oct 22;97(8):1139-45. PMID: 17940504. EXC6

386. Freidlin B, McShane LM, Korn EL. Randomized clinical trials with biomarkers: design issues. J Natl Cancer Inst. 2010 Feb 3;102(3):152-60. PMID: 20075367. EXC7

387. French AJ, Sargent DJ, Burgart LJ, et al. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. Clin Cancer Res. 2008 Jun 1;14(11):3408-15. PMID: 18519771. EXC7

388. Frigerio S, Padberg BC, Strebel RT, et al. Improved detection of bladder carcinoma cells in voided urine by standardized microsatellite analysis. Int J Cancer. 2007 Jul 15;121(2):329-38. PMID: 17373664. EXC6

389. Fu XL, Zhu XZ, Shi DR, et al. Study of prognostic predictors for non-small cell lung cancer. Lung Cancer. 1999 Feb;23(2):143-52. PMID: 10217618. EXC4

390. Fujimoto K, Shitaune M, Shigeta M, et al. Combinatorial detection of urinary tract cancer in voided urine by cytology, nmp22, and urovysion fish test. Acta Cytol. 2013 May;57 SUPPL. 1:149. EXC6

391. Fujino S, Enokibori T, Katsura A, et al. Relationship between epidermal growth factor receptor expression and various prognostic factors in human non-small cell lung cancer. Japan J Lung Cancer. 1994;34(1):89-94. EXC2

392. Fujino S, Enokibori T, Tezuka N, et al. A comparison of epidermal growth factor receptor levels and other prognostic parameters in non-small cell lung cancer. Eur J Cancer. 1996 Nov;32A(12):2070-4. PMID: 9014747. EXC4

393. Fujita Y, Suda K, Kimura H, et al. Highly sensitive detection of EGFR T790M mutation using colony hybridization predicts favorable prognosis of patients with lung cancer harboring activating EGFR mutation. J Thorac Oncol. 2012 Nov;7(11):1640-4. PMID: 22899358. EXC6

394. Fujiwara Y, Kiura K, Toyooka S, et al. Elevated serum level of sialylated glycoprotein KL-6 predicts a poor prognosis in patients with non-small cell lung cancer treated with gefitinib. Lung Cancer. 2008 Jan;59(1):81-7. PMID: 17765355. EXC6

395. Fukui T, Ohe Y, Tsuta K, et al. Prospective study of the accuracy of EGFR mutational analysis by high-resolution melting analysis in small samples obtained from patients with non-small cell lung cancer. Clin Cancer Res. 2008 Aug 1;14(15):4751-7. PMID: 18676744. EXC4

396. Fukui T, Tsuta K, Furuta K, et al. Epidermal growth factor receptor mutation status and clinicopathological features of combined small cell carcinoma with adenocarcinoma of the lung. Cancer Sci. 2007 Nov;98(11):1714-9. PMID: 17784875. EXC6

397. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol. 2011 Jul 20;29(21):2866-74. PMID: 21670455. EXC5

398. Furlan D, Carnevali IW, Bernasconi B, et al. Hierarchical clustering analysis of pathologic and molecular data identifies prognostically and biologically distinct groups of colorectal carcinomas. Mod Pathol. 2011 Jan;24(1):126-37. PMID: 20852594. EXC6

399. Furlan D, Tibiletti MG, Taborelli M, et al. The value of microsatellite instability in the detection of HNPCC families and of sporadic colorectal cancers with special biological features: an investigation on a series of 100 consecutive cases. Ann Oncol. 1998 Aug;9(8):901-6. PMID: 9789614. EXC6

400. Gadgeel SM, Ruckdeschel JC, Heath EI, et al. Phase II study of gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), and celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, in patients with platinum refractory non-small cell lung cancer (NSCLC). J Thorac Oncol. 2007 Apr;2(4):299-305. PMID: 17409801. EXC6

401. Gaedcke J, Grade M, Jung K, et al. KRAS and BRAF mutations in patients with rectal cancer treated with preoperative chemoradiotherapy. Radiother Oncol. 2010 Jan;94(1):76-81. PMID: 19913317. EXC7

402. Gafa R, Maestri I, Matteuzzi M, et al. Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. Cancer. 2000 Nov 15;89(10):2025-37. PMID: 11066042. EXC4

403. Galvan AB, Salido M, Espinet B, et al. A multicolor fluorescence in situ hybridization assay: A monitoring tool in the surveillance of patients with a history of non-muscle-invasive urothelial cell carcinoma: A prospective study. Cancer Cytopathol. 2011 Dec 25;119(6):395-403. PMID: 21717592. EXC6

404. Gandara DR, Grimminger P, Mack PC, et al. Association of epidermal growth factor receptor activating mutations with low ERCC1 gene expression in non-small cell lung cancer. J Thorac Oncol. 2010 Dec;5(12):1933-8. PMID: 20975603. EXC6

405. Gao B, Sun Y, Zhang J, et al. Spectrum of LKB1, EGFR, and KRAS mutations in Chinese lung adenocarcinomas. J Thorac Oncol. 2010;5(8):1130-5. EXC6

406. Gao J, Wang TT, Yu JW, et al. Wild-Type KRAS and BRAF could predict response to cetuximab in Chinese colorectal cancer patients. Chin J Cancer Res. 2011;23(4):271-5. EXC7

407. Gao JF, Arbman G, Wadhra TI, et al. Relationships of tumor inflammatory infiltration and necrosis with microsatellite instability in colorectal cancers. World J Gastroenterol. 2005 Apr 14;11(14):2179-83. PMID: 15810089. EXC4

408. Garcia-Aguilar J, Chen Z, Smith DD, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. Ann Surg. 2011 Sep;254(3):486-92; discussion 92-3. PMID: 21865946. EXC6

409. Garcia-Albeniz X, Pericay C, Alonso-Espinaco V, et al. Serum matrilysin correlates with poor survival independently of KRAS and BRAF status in refractory advanced colorectal cancer patients treated with irinotecan plus cetuximab. Tumour Biol. 2011 Apr;32(2):417-24. PMID: 21104178. EXC3

410. . Can Oncotype DX recurrence score (RS) be used in luminal A and luminal B breast cancer patients (pts) to predict the likely benefit of chemotherapy? A retrospective study in the Spanish population. ASCO; 2012. EXC6

411. Garcia-Solano J, Conesa-Zamora P, Carbonell P, et al. Microsatellite pathologic score does not efficiently identify high microsatellite instability in colorectal serrated adenocarcinoma. Hum Pathol. 2013 May;44(5):759-65. PMID: 23089493. EXC6

412. Garm Spindler KL, Pallisgaard N, Rasmussen AA, et al. The importance of KRAS mutations and EGF61A>G polymorphism to the effect of cetuximab and irinotecan in metastatic colorectal cancer. Ann Oncol. 2009 May;20(5):879-84. PMID: 19179548. EXC3

413. Garrido-Laguna I, Hong DS, Janku F, et al. KRASness and PIK3CAness in patients with advanced colorectal cancer: outcome after treatment with early-phase trials with targeted pathway inhibitors. PLoS One. 2012;7(5):e38033. PMID: 22675430. EXC3

414. Gasinska A, Kolodziejski L, Niemiec J, et al. Clinical significance of biological differences between cavitated and solid form of squamous cell lung cancer. Lung Cancer. 2005 Aug;49(2):171-9. PMID: 16022910. EXC4

415. Gausachs M, Mur P, Corral J, et al. MLH1 promoter hypermethylation in the analytical algorithm of Lynch syndrome: a cost-effectiveness study. Eur J Hum Genet. 2012 Jul;20(7):762-8. PMID: 22274583. EXC3

416. Gautschi O, Huegli B, Ziegler A, et al. Origin and prognostic value of circulating KRAS mutations in lung cancer patients. Cancer Lett. 2007 Sep 8;254(2):265-73. PMID: 17449174. EXC4

417. Gayed BA, Seideman C, Lotan Y. Cost-effectiveness of fluorescence in situ hybridization in patients with atypical cytology for the detection of urothelial carcinoma. J Urol. 2013 Oct;190(4):1181-6. PMID: 23583531. EXC7

418. Geffen DB, Amir N, Sion-Vardy N, et al. Stage I breast cancer in a regional oncology practice in Israel 2002-2006: clinicopathologic features, risk estimation and planned therapy of 328 consecutive patients. Breast. 2009 Oct;18(5):316-21. PMID: 19819143. EXC7

419. Genomic Health. Scientific Publications and Presentations. OncotypeDX Breast Cancer Assay. Uncover the Unexpected; 2012. EXC2

420. Georgieva M, Krasteva M, Angelova E, et al. Analysis of the K-ras/B-raf/Erk signal cascade, p53 and CMAP as markers for tumor progression in colorectal cancer patients. Oncol Rep. 2008 Jul;20(1):3-11. PMID: 18575712. EXC8

421. Gerson Cwilich R, Alban de la Torre LF, Villalobos Prieto A, et al. [Clinicopathological features, prognosis and influence in the adjuvant treatment of the risk recurrence groups determined by the 21 gene expression profile, Oncotype Dx(R), in early breast cancer]. Gac Med Mex. 2012 Mar-Apr;148(2):117-24. PMID: 22622310. EXC2

422. Gervaz P, Cerottini JP, Bouzourene H, et al. Comparison of microsatellite instability and chromosomal instability in predicting survival of patients with T3N0 colorectal cancer. Surgery. 2002 Feb;131(2):190-7. PMID: 11854698. EXC7

423. Gevensleben H, Gohring UJ, Buttner R, et al. Comparison of MammaPrint and TargetPrint results with clinical parameters in German patients with early stage breast cancer. Int J Mol Med. 2010 Dec;26(6):837-43. PMID: 21042777. EXC5

424. Giaccone G. The role of EGFR-TK inhibition in non-small cell lung cancer. Onkologie. 2005 Dec;28(12):619-20. PMID: 16370043. EXC3

425. Gianni L, Zambetti M, Clark K, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. J Clin Oncol. 2005 Oct 10;23(29):7265-77. PMID: 16145055. EXC4

426. Giatromanolaki A, Koukourakis MI, O'Byrne K, et al. Non-small cell lung cancer: c-erbB-2 overexpression correlates with low angiogenesis and poor prognosis. Anticancer Res. 1996 Nov-Dec;16(6B):3819-25. PMID: 9042264. EXC6

427. Gille JJP, Hogervorst FBL, Pals G, et al. Genomic deletions of MSH2 and MLH1 in colorectal cancer families detected by a novel mutation detection approach. Br J Cancer. 2002;87(8):892-7. EXC3

428. Gillern SM, Chua TC, Stojadinovic A, et al. KRAS status in patients with colorectal cancer peritoneal carcinomatosis and its impact on outcome. Am J Clin Oncol. 2010 Oct;33(5):456-60. PMID: 19952717. EXC3

429. Giovannetti E, Zucali PA, Peters GJ, et al. Association of polymorphisms in AKT1 and EGFR with clinical outcome and toxicity in non-small cell lung cancer patients treated with gefitinib. Mol Cancer Ther. 2010 Mar;9(3):581-93. PMID: 20159991. EXC6

430. Girard N, Deshpande C, Azzoli CG, et al. Use of epidermal growth factor receptor/Kirsten rat sarcoma 2 viral oncogene homolog mutation testing to define clonal relationships among multiple lung adenocarcinomas: comparison with clinical guidelines. Chest. 2010 Jan;137(1):46-52. PMID: 19376842. EXC2

431. Girard N, Jacoulet P, Gainet M, et al. Third-line chemotherapy in advanced non-small cell lung cancer: identifying the candidates for routine practice. J Thorac Oncol. 2009 Dec;4(12):1544-9. PMID: 19884862. EXC6

432. Girard N, Sima CS, Jackman DM, et al. Nomogram to predict the presence of EGFR activating mutation in lung adenocarcinoma. Eur Respir J. 2012 Feb;39(2):366-72. PMID: 21778168. EXC2

433. Glas AM, Floore A, Delahaye LJ, et al. Converting a breast cancer microarray signature into a high-throughput diagnostic test. BMC Genomics. 2006;7:278. PMID: 17074082. EXC7

434. Gluck S, de Snoo F, Peeters J, et al. Molecular subtyping of early-stage breast cancer identifies a group of patients who do not benefit from neoadjuvant chemotherapy. Breast Cancer Res Treat. 2013 Jun;139(3):759-67. PMID: 23756626. EXC6

435. . Prospective comparison of recurrence score and independent central pathology assessment of prognostic tools in early breast cancer (BC): Focus on HER2, ER, PR, Ki-67 results from the phase III WSG-Plan B trial. ASCO; 2012. EXC6

436. . Prospective comparison of Recurrence Score and different definitions of luminal subtypes by central pathology assessment of single markers in early breast cancer: results from the phase III WSG-planB. SABCS; 2012. EXC6

437. Gnanasampanthan G, Elsaleh H, McCaul K, et al. Ki-ras mutation type and the survival benefit from adjuvant chemotherapy in Dukes' C colorectal cancer. J Pathol. 2001;195(5):543-8. EXC6

438. Goel A, Nagasaka T, Hamelin R, et al. An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. PLoS One. 2010;5(2):e9393. PMID: 20195377. EXC5

439. Gofrit ON, Zorn KC, Silvestre J, et al. The predictive value of multi-targeted fluorescent in-situ hybridization in patients with history of bladder cancer. Urol Oncol. 2008 May-Jun;26(3):246-9. PMID: 18452813. EXC6

440. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013 Sep;24(9):2206-23. PMID: 23917950. EXC7

441. Goncalves A, Esteyries S, Taylor-Smedra B, et al. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. BMC Cancer. 2008;8:169. PMID: 18544172. EXC3

442. Gormally E, Vineis P, Matullo G, et al. TP53 and KRAS2 mutations in plasma DNA of healthy subjects and subsequent cancer occurrence: a prospective study. Cancer Res. 2006 Jul 1;66(13):6871-6. PMID: 16818665. EXC4

443. Goss G, Ferry D, Wierzbicki R, et al. Randomized phase II study of gefitinib compared with placebo in chemotherapy-naive patients with advanced non-small-cell lung cancer and poor performance status. J Clin Oncol. 2009 May 1;27(13):2253-60. PMID: 19289623. EXC6

444. Goto K, Ichinose Y, Ohe Y, et al. Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. J Thorac Oncol. 2012 Jan;7(1):115-21. PMID: 21900837. EXC4

445. Gow CH, Chang YL, Hsu YC, et al. Comparison of epidermal growth factor receptor mutations between primary and corresponding metastatic tumors in tyrosine kinase inhibitor-naive non-small-cell lung cancer. Ann Oncol. 2009 Apr;20(4):696-702. PMID: 19088172. EXC5

446. Gow CH, Chien CR, Chang YL, et al. Radiotherapy in lung adenocarcinoma with brain metastases: effects of activating epidermal growth factor receptor mutations on clinical response. Clin Cancer Res. 2008 Jan 1;14(1):162-8. PMID: 18172267. EXC6

447. Gray RG, Quirke P, Handley K, et al. Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. J Clin Oncol. 2011 Dec 10;29(35):4611-9. PMID: 22067390. EXC6

448. Graziano F, Canestrari E, Loupakis F, et al. Genetic modulation of the Let-7 microRNA binding to KRAS 3'-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. Pharmacogenomics J. 2010 Oct;10(5):458-64. PMID: 20177422. EXC3

449. Greenson JK, Huang SC, Herron C, et al. Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg Pathol. 2009 Jan;33(1):126-33. PMID: 18830122. EXC6

450. Gregorc V, Ceresoli GL, Floriani I, et al. Effects of gefitinib on serum epidermal growth factor receptor and HER2 in patients with advanced non-small cell lung cancer. Clin Cancer Res. 2004 Sep 15;10(18 Pt 1):6006-12. PMID: 15447984. EXC4

451. Grob TJ, Hoenig T, Clauditz TS, et al. Frequent intratumoral heterogeneity of EGFR gene copy gain in non-small cell lung cancer. Lung Cancer. 2013 Mar;79(3):221-7. PMID: 23238037. EXC6

452. Grommes C, Oxnard GR, Kris MG, et al. "Pulsatile" high-dose weekly erlotinib for CNS metastases from EGFR mutant non-small cell lung cancer. Neuro Oncol. 2011 Dec;13(12):1364-9. PMID: 21865399. EXC3

453. Grossi F, Spizzo R, Bordo D, et al. Prognostic stratification of stage IIIA pN2 non-small cell lung cancer by hierarchical clustering analysis of tissue microarray immunostaining data: an Alpe Adria Thoracic Oncology Multidisciplinary Group study (ATOM 014). J Thorac Oncol. 2010 Sep;5(9):1354-60. PMID: 20631638. EXC4

454. Guastadisegni C, Colafranceschi M, Ottini L, et al. Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data (Provisional abstract). Eur J Cancer. 2010(15):2788-98. PMID: DARE-12010007142. EXC3

455. Guo GF, Jiang WQ, Zhang B, et al. Autophagy-related proteins Beclin-1 and LC3 predict cetuximab efficacy in advanced colorectal cancer. World J Gastroenterol. 2011 Nov 21;17(43):4779-86. PMID: 22147978. EXC3

456. Guo H, Wan Y, Tian G, et al. EGFR mutations predict a favorable outcome for malignant pleural effusion of lung adenocarcinoma with Tarceva therapy. Oncol Rep. 2012 Mar;27(3):880-90. PMID: 22134479. EXC3

457. Gupta AK, Soto DE, Feldman MD, et al. Signaling pathways in NSCLC as a predictor of outcome and response to therapy. Lung. 2004;182(3):151-62. PMID: 15526754. EXC4

458. Gupta S, Ashfaq R, Kapur P, et al. Microsatellite instability among individuals of Hispanic origin with colorectal cancer. Cancer. 2010 Nov 1;116(21):4965-72. PMID: 20665498. EXC5

459. Gustafsson B, Angelini S, Sander B, et al. Mutations in the BRAF and N-ras genes in childhood acute lymphoblastic leukaemia [11]. Leukemia. 2005;19(2):310-2. EXC3

460. Gustafsson SB, Palmqvist R, Henriksson ML, et al. High tumour cannabinoid CB1 receptor immunoreactivity negatively impacts disease-specific survival in stage II microsatellite stable colorectal cancer. PLoS One. 2011;6(8):e23003. PMID: 21901119. EXC4

461. Gwin K, Pinto M, Tavassoli FA. Complementary value of the Ki-67 proliferation index to the oncotype DX recurrence score. Int J Surg Pathol. 2009 Aug;17(4):303-10. PMID: 19578051. EXC7

462. Ha D, Choi H, Almeida FA, et al. Histologic and molecular characterization of lung cancer with tissue obtained by electromagnetic navigation bronchoscopy. J Bronchology Interv Pulmonol. 2013 Jan;20(1):10-5. PMID: 23328135. EXC6

463. Habel L, Quesenberry C, Jacobs M, et al. Gene expression and breast cancer mortality in Northern California Kaiser Permanente patients: a large population-based case control study. Proc Am Soc Clin Oncol; 2005. 24. p. 603a. EXC2

464. Haddad R, Ogilvie RT, Croitoru M, et al. Microsatellite instability as a prognostic factor in resected colorectal cancer liver metastases. Ann Surg Oncol. 2004 Nov;11(11):977-82. PMID: 15525826. EXC5

465. Hadziavdic V, Pavlovic-Calic N, Eminovic I. Molecular analysis: microsatellity instability and loss of heterozygosity of tumor suppressor gene in hereditary non-polyposis colorectal cancers (HNPCC). Bosn J Basic Med Sci. 2009 Feb;9(1):10-8. PMID: 19284389. EXC6

466. Haibe-Kains B, Desmedt C, Piette F, et al. Comparison of prognostic gene expression signatures for breast cancer. BMC Genomics. 2008;9:394. PMID: 18717985. EXC5

467. Hall G, Clarkson A, Shi A, et al. Immunohistochemistry for PMS2 and MSH6 alone can replace a four antibody panel for mismatch repair deficiency screening in colorectal adenocarcinoma. Pathology (Phila). 2010;42(5):409-13. PMID: 20632815. EXC6

468. Hall MJ, Manne SL, Winkel G, et al. Effects of a decision support intervention on decisional conflict associated with microsatellite instability testing. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011(2):249-54. PMID: CN-00780300. EXC4

469. Hall PS, McCabe C, Stein RC, et al. Economic evaluation of genomic test-directed chemotherapy for early-stage lymph node-positive breast cancer. J Natl Cancer Inst. 2012 Jan 4;104(1):56-66. PMID: 22138097. EXC7

470. Halling KC, French AJ, McDonnell SK, et al. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. J Natl Cancer Inst. 1999 Aug 4;91(15):1295-303. PMID: 10433618. EXC4

471. Halling KC, King W, Sokolova IA, et al. A comparison of BTA stat, hemoglobin dipstick, telomerase and Vysis UroVysion assays for the detection of urothelial carcinoma in urine. J Urol. 2002 May;167(5):2001-6. PMID: 11956427. EXC6

472. Halling KC, King W, Sokolova IA, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol. 2000 Nov;164(5):1768-75. PMID: 11025767. EXC6

473. Halling KC, Rickman OB, Kipp BR, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of lung cancer in bronchoscopic specimens. Chest. 2006 Sep;130(3):694-701. PMID: 16963665. EXC4

474. Hallqvist A, Wagenius G, Rylander H, et al. Concurrent cetuximab and radiotherapy after docetaxel-cisplatin induction chemotherapy in stage III NSCLC: satellite--a phase II study from the Swedish Lung Cancer Study Group. Lung Cancer. 2011 Feb;71(2):166-72. PMID: 20541833. EXC4

475. Halvarsson B, Anderson H, Domanska K, et al. Clinicopathologic factors identify sporadic mismatch repair-defective colon cancers. Am J Clin Pathol. 2008 Feb;129(2):238-44. PMID: 18208804. EXC6

476. Halvarsson B, Lindblom A, Rambech E, et al. Microsatellite instability analysis and/or immunostaining for the diagnosis of hereditary nonpolyposis colorectal cancer? Virchows Arch. 2004 Feb;444(2):135-41. PMID: 14652751. EXC5

477. Hameed F, Goldberg PA, Hall P, et al. Immunohistochemistry detects mismatch repair gene defects in colorectal cancer. Colorectal Dis. 2006 Jun;8(5):411-7. PMID: 16684085. EXC6

478. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res. 2006 Aug 1;66(15):7810-7. PMID: 16885385. EXC5

479. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol. 2008 Dec 10;26(35):5783-8. PMID: 18809606. EXC4

480. Han HS, Lim SN, An JY, et al. Detection of EGFR mutation status in lung adenocarcinoma specimens with different proportions of tumor cells using two methods of differential sensitivity. J Thorac Oncol. 2012 Feb;7(2):355-64. PMID: 22157369. EXC6

481. Han SW, Hwang PG, Chung DH, et al. Epidermal growth factor receptor (EGFR) downstream molecules as response predictive markers for gefitinib (Iressa, ZD1839) in chemotherapy-resistant non-small cell lung cancer. Int J Cancer. 2005 Jan 1;113(1):109-15. PMID: 15386420. EXC6

482. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol. 2005 Apr 10;23(11):2493-501. PMID: 15710947. EXC6

483. Han SW, Kim TY, Jeon YK, et al. Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. Clin Cancer Res. 2006 Apr 15;12(8):2538-44. PMID: 16638863. EXC6

484. Han SW, Kim TY, Lee KH, et al. Clinical predictors versus epidermal growth factor receptor mutation in gefitinib-treated non-small-cell lung cancer patients. Lung Cancer. 2006 Nov;54(2):201-7. PMID: 16956694. EXC6

485. Han SW, Lee HJ, Bae JM, et al. Methylation and microsatellite status and recurrence following adjuvant FOLFOX in colorectal cancer. Int J Cancer. 2013 May 1;132(9):2209-16. PMID: 23034738. EXC3

486. Han Y, Xu JM, Duan HQ, et al. Epidermal growth factor receptor mutations, HER2/3 protein expressions and clinical outcome in the chinese patients with advanced nonsmall cell lung cancer treated with gefitinib. Chinese Journal of Cancer. 2010;29(1):64-70. EXC3

487. Haneda H, Sasaki H, Lindeman N, et al. A correlation between EGFR gene mutation status and bronchioloalveolar carcinoma features in Japanese patients with adenocarcinoma. Jpn J Clin Oncol. 2006 Feb;36(2):69-75. PMID: 16449241. EXC6

488. Haneda H, Sasaki H, Shimizu S, et al. Epidermal growth factor receptor gene mutation defines distinct subsets among small adenocarcinomas of the lung. Lung Cancer. 2006 Apr;52(1):47-52. PMID: 16503085. EXC6

489. Hanna N, Lilenbaum R, Ansari R, et al. Phase II trial of cetuximab in patients with previously treated non-small-cell lung cancer. J Clin Oncol. 2006 Nov 20;24(33):5253-8. PMID: 17114658. EXC6

490. Hannouf MB, Xie B, Brackstone M, et al. Cost-effectiveness of a 21-gene recurrence score assay versus Canadian clinical practice in women with early-stage estrogen- or progesterone-receptor-positive, axillary lymph-node negative breast cancer. BMC Cancer. 2012;12:447. PMID: 23031196. EXC7

491. Harle A, Busser B, Rouyer M, et al. Comparison of COBAS 4800 KRAS, TaqMan PCR and high resolution melting PCR assays for the detection of KRAS somatic mutations in formalin-fixed paraffin embedded colorectal carcinomas. Virchows Arch. 2013 Mar;462(3):329-35. PMID: 23400679. EXC3

492. Harrell FE, Lee KL, Mark DB. Tutorial in Biostatistics. Multivariable prognostic models: Issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15:361-87. EXC2

493. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. 2007 Nov 20;25(33):5287-312. PMID: 17954709. EXC7

494. Hartmann S, Gerber B, Elling D, et al. The 70-gene signature as prognostic factor for elderly women with hormone receptor-positive, HER2-negative breast cancer. Breast Care. 2012;7(1):19-24. EXC5

495. Hassett MJ, O'Malley AJ, Pakes JR, et al. Frequency and cost of chemotherapy-related serious adverse effects in a population sample of women with breast cancer. J Natl Cancer Inst. 2006 Aug 16;98(16):1108-17. PMID: 16912263. EXC6

496. Hassett MJ, Silver SM, Hughes ME, et al. Adoption of gene expression profile testing and association with use of chemotherapy among women with breast cancer. J Clin Oncol. 2012 Jun 20;30(18):2218-26. PMID: 22585699. Exc-6

497. Hata A, Yoshioka H, Fujita S, et al. Complex mutations in the epidermal growth factor receptor gene in non-small cell lung cancer. J Thorac Oncol. 2010 Oct;5(10):1524-8. PMID: 20808254. EXC6

498. Hatch SB, Lightfoot HM, Jr., Garwacki CP, et al. Microsatellite instability testing in colorectal carcinoma: choice of markers affects sensitivity of detection of mismatch repair-deficient tumors. Clin Cancer Res. 2005 Mar 15;11(6):2180-7. PMID: 15788665. EXC5

499. Hawkins NJ, Tomlinson I, Meagher A, et al. Microsatellite-stable diploid carcinoma: a biologically distinct and aggressive subset of sporadic colorectal cancer. Br J Cancer. 2001 Jan;84(2):232-6. PMID: 11161382. EXC7

500. Hawkins NJ, Ward RL. Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. J Natl Cancer Inst. 2001 Sep 5;93(17):1307-13. PMID: 11535705. EXC6

501. Hayashi H, Okamoto I, Kimura H, et al. Clinical outcomes of thoracic radiotherapy for locally advanced NSCLC with EGFR mutations or EML4-ALK rearrangement. Anticancer Res. 2012 Oct;32(10):4533-7. PMID: 23060582. EXC3

502. Hayes, Inc. Ancillary UroVysion fluorescence in situ hybridization (FISH) testing for bladder cancer screening and detection (Structured abstract). Health Technology Assessment Database. 2008(3)PMID: HTA-32009100606. EXC2

503. Hayes, Inc. KRAS sequence variant analysis for non-small cell lung cancer (NSCLC) (Structured abstract). Health Technology Assessment Database. 2008(3)PMID: HTA-32010000083. EXC2

504. Hayes, Inc. Oncotype DX for prognosis of breast cancer recurrence (Structured abstract). Health Technology Assessment Database. 2010(3)PMID: HTA-32010001399. EXC2

505. Hayes, Inc. MammaPrint for prognosis of breast cancer recurrence (Structured abstract). Health Technology Assessment Database. 2011(3)PMID: HTA-32011001341. EXC2

506. Hayes, Inc. VeriStrat® Test to predict response to Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors (TKIs) in Non-Small Cell Lung Cancer (NSCLC) (Structured abstract). Health Technology Assessment Database. 2012(3)PMID: HTA-32012000128. EXC2

507. Hayes, Inc. Epidermal Growth Factor Receptor (EGFR) sequence variant analysis for predicting response to Non-Small Cell Lung Cancer (NSCLC) drug therapy (Structured abstract). Health Technology Assessment Database. 2012(3)PMID: HTA-32012000085. EXC2

508. Hayes, Inc. Ki-67 (MKI67) proliferation marker testing in ductal carcinoma in situ (DCIS) and breast cancer (Structured abstract). Health Technology Assessment Database. 2012(4)PMID: HTA-32013000310. EXC9

509. Hayes, Inc. Anaplastic lymphoma kinase (ALK) gene rearrangement testing in non-small cell lung cancer (NSCLC) (Structured abstract). Health Technology Assessment Database. 2012(4)PMID: HTA-32013000111. EXC9

510. Hayes, Inc. KRAS sequence variant analysis for predicting response to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in the treatment of non-small cell lung cancer (NSCLC) (Structured abstract). Health Technology Assessment Database. 2012(4)PMID: HTA-32012000582. EXC9

511. Hayes I. KRAS sequence variant analysis for predicting response to colorectal cancer drug therapy (Structured abstract). Health Technology Assessment Database. 2008(3)PMID: HTA-32010000084. EXC2

512. Hayes I. BRAF p.Val600Glu (V600E) testing for assessment of treatment options in metastatic colorectal cancer (Structured abstract). Health Technology Assessment Database. 2010(3)PMID: HTA-32010001003. EXC2

513. Hayes I. Neuroblastoma RAS Viral Oncogene (NRAS) testing to predict treatment response in colorectal cancer (Structured abstract). Health Technology Assessment Database. 2012(3)PMID: HTA-32012000124. EXC2

514. He C, Liu M, Zhou C, et al. Detection of epidermal growth factor receptor mutations in plasma by mutant-enriched PCR assay for prediction of the response to gefitinib in patients with non-small-cell lung cancer. Int J Cancer. 2009 Nov 15;125(10):2393-9. PMID: 19530244. EXC4

515. He M, Capelletti M, Nafa K, et al. EGFR exon 19 insertions: a new family of sensitizing EGFR mutations in lung adenocarcinoma. Clin Cancer Res. 2012 Mar 15;18(6):1790-7. PMID: 22190593. EXC6

516. He Y, Van't Veer LJ, Mikolajewska-Hanclich I, et al. PIK3CA mutations predict local recurrences in rectal cancer patients. Clin Cancer Res. 2009 Nov 15;15(22):6956-62. PMID: 19903786. EXC6

517. Hecht JR, Mitchell E, Chidiac T, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol. 2009 Feb 10;27(5):672-80. PMID: 19114685. EXC3

518. Hecht JR, Mitchell E, Neubauer MA, et al. Lack of correlation between epidermal growth factor receptor status and response to Panitumumab monotherapy in metastatic colorectal cancer. Clin Cancer Res. 2010 Apr 1;16(7):2205-13. PMID: 20332321. EXC5

519. Hellebrekers DM, Lentjes MH, van den Bosch SM, et al. GATA4 and GATA5 are potential tumor suppressors and biomarkers in colorectal cancer. Clin Cancer Res. 2009 Jun 15;15(12):3990-7. PMID: 19509152. EXC5

520. Hemminki A, Mecklin JP, Jarvinen H, et al. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. Gastroenterology. 2000;119(4):921-8. EXC8

521. Hendriks Y, Franken P, Dierssen JW, et al. Conventional and tissue microarray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors. Am J Pathol. 2003;162(2):469-77. EXC3

522. Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology. 2004 Jul;127(1):17-25. PMID: 15236168. EXC6

523. Heon S, Yeap BY, Britt GJ, et al. Development of central nervous system metastases in patients with advanced non-small cell lung cancer and somatic EGFR mutations treated with gefitinib or erlotinib. Clin Cancer Res. 2010 Dec 1;16(23):5873-82. PMID: 21030498. EXC6

524. Heon S, Yeap BY, Lindeman NI, et al. The impact of initial gefitinib or erlotinib versus chemotherapy on central nervous system progression in advanced non-small cell lung cancer with EGFR mutations. Clin Cancer Res. 2012;18(16):4406-14. EXC5

525. Herbst RS, Kelly K, Chansky K, et al. Phase II selection design trial of concurrent chemotherapy and cetuximab versus chemotherapy followed by cetuximab in advanced-stage non-small-cell lung cancer: Southwest Oncology Group study S0342. J Clin Oncol. 2010 Nov 1;28(31):4747-54. PMID: 20921467. EXC6

526. Herbst RS, Maddox AM, Rothenberg ML, et al. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. J Clin Oncol. 2002 Sep 15;20(18):3815-25. PMID: 12228201. EXC4

527. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol. 2005 Sep 1;23(25):5892-9. PMID: 16043829. EXC6

528. Hijiya N, Miyawaki M, Kawahara K, et al. Phosphorylation status of epidermal growth factor receptor is closely associated with responsiveness to gefitinib in pulmonary adenocarcinoma. Hum Pathol. 2008 Mar;39(3):316-23. PMID: 18261621. EXC6

529. Hirsch FR, Dziadziuszko R, Thatcher N, et al. Epidermal growth factor receptor immunohistochemistry: comparison of antibodies and cutoff points to predict benefit from gefitinib in a phase 3 placebo-controlled study in advanced nonsmall-cell lung cancer. Cancer. 2008(5):1114-21. PMID: CN-00629599. EXC4

530. Hirsch FR, Herbst RS, Olsen C, et al. Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. J Clin Oncol. 2008 Jul 10;26(20):3351-7. PMID: 18612151. EXC4

531. Hirsch FR, Varella-Garcia M, Bunn PA, Jr., et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol. 2006 Nov 1;24(31):5034-42. PMID: 17075123. EXC6

532. Hirsch FR, Varella-Garcia M, Dziadziuszko R, et al. Fluorescence in situ hybridization subgroup analysis of TRIBUTE, a phase III trial of erlotinib plus carboplatin and paclitaxel in non-small cell lung cancer. Clin Cancer Res. 2008 Oct 1;14(19):6317-23. PMID: 18829515. EXC4

533. Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol. 2005 Oct 1;23(28):6838-45. PMID: 15998906. EXC4

534. Ho JW, Yuen ST, Chung LP, et al. Distinct clinical features associated with microsatellite instability in colorectal cancers of young patients. Int J Cancer. 2000 Jul 20;89(4):356-60. PMID: 10956410. EXC6

535. Hochstein N, Honsel D, Kappmeier C, et al. Pyrosequencing and its applications. QIAGEN; Hilden, Germany. EXC2

536. Hoedema R, Monroe T, Bos C, et al. testing for hereditary nonpolyposis colorectal cancer. Am Surg. 2003 May;69(5):387-91; discussion 91-2. PMID: 12769209. EXC6

537. Holdhoff M, Schmidt K, Diehl F, et al. Detection of tumor DNA at the margins of colorectal cancer liver metastasis. Clin Cancer Res. 2011 Jun 1;17(11):3551-7. PMID: 21531819. EXC6

538. Holdhoff M, Schmidt K, Donehower R, et al. Analysis of circulating tumor DNA to confirm somatic KRAS mutations. J Natl Cancer Inst. 2009 Sep 16;101(18):1284-5. PMID: 19641175. EXC2

539. Hollebecque A, Levy A, Broutin S, et al. First case report of intrathecal panitumumab for treatment of meningeal carcinomatousis in an EGFR mutant lung adenocarcinoma patient. Lung Cancer. 2013 Apr;80(1):113-4. PMID: 23352031. EXC7

540. . Cost-effectiveness evaluation of the Oncotype DX® breast cancer assay in clinical practice in the UK. San Antonio Breast Cancer Symposium; 2011 December; San Antonio, TX. EXC6

541. Hong T, Zhang R, Cai D, et al. Second-line epidermal growth factor receptor inhibitors followed by third-line pemetrexed or the reverse sequence: a retrospective analysis of 83 Chinese patients with advanced lung adenocarcinoma. J Cancer Res Clin Oncol. 2012 Feb;138(2):285-91. PMID: 22116317. EXC4

542. Hoogerbrugge N, Willems R, Van Krieken HJ, et al. Very low incidence of microsatellite instability in rectal cancers from families at risk for HNPCC. Clin Genet. 2003 Jan;63(1):64-70. PMID: 12519374. EXC6

543. Ho-Pun-Cheung A, Assenat E, Bascoul-Mollevi C, et al. EGFR and HER3 mRNA expression levels predict distant metastases in locally advanced rectal cancer. Int J Cancer. 2011 Jun 15;128(12):2938-46. PMID: 20824716. EXC4

544. Hoque MO, Brait M, Rosenbaum E, et al. Genetic and epigenetic analysis of erbB signaling pathway genes in lung cancer. J Thorac Oncol. 2010 Dec;5(12):1887-93. PMID: 21102258. EXC6

545. Horiike A, Kimura H, Nishio K, et al. Detection of epidermal growth factor receptor mutation in transbronchial needle aspirates of non-small cell lung cancer. Chest. 2007 Jun;131(6):1628-34. PMID: 17565015. EXC5

546. Hornberger J, Chien R, Krebs K, et al. US insurance program's experience with a multigene assay for early-stage breast cancer (Structured abstract). Journal of Oncology Practice. 2011(3 Supplement):e38s-e45s. PMID: NHSEED-22011001691. EXC5

547. Hornberger J, Chien R, Krebs K, et al. US insurance program's experience with a multigene assay for early-stage breast cancer. Am J Manag Care. 2011 May;17(5 Spec No):e194-202. PMID: 21711071. EXC6

548. Hornberger J, Lyman GH, Chien R. Economic implications of 21-gene recurrence score assay: US multicenter experience. J Clin Oncol. 2010 Aug 1;28(22):e382; author reply e3. PMID: 20498396. EXC2

549. Horndler C, Gallego R, Garcia-Albeniz X, et al. Co-expression of matrix metalloproteinase-7 (MMP-7) and phosphorylated insulin growth factor receptor I (pIGF-1R) correlates with poor prognosis in patients with wild-type KRAS treated with cetuximab or panitumumab: a GEMCAD study. Cancer Biol Ther. 2011 Jan 15;11(2):177-83. PMID: 21099348. EXC4

550. Horstmann M, Patschan O, Hennenlotter J, et al. Combinations of urine-based tumour markers in bladder cancer surveillance. Scand J Urol Nephrol. 2009;43(6):461-6. PMID: 19903092. EXC6

551. Hoshi K, Takakura H, Mitani Y, et al. Rapid detection of epidermal growth factor receptor mutations in lung cancer by the SMart-Amplification Process. Clin Cancer Res. 2007 Sep 1;13(17):4974-83. PMID: 17785547. EXC4

552. Hosokawa S, Toyooka S, Fujiwara Y, et al. Comprehensive analysis of EGFR signaling pathways in Japanese patients with non-small cell lung cancer. Lung Cancer. 2009 Oct;66(1):107-13. PMID: 19185949. EXC6

553. Hotta K, Kiura K, Toyooka S, et al. Clinical significance of epidermal growth factor receptor gene mutations on treatment outcome after first-line cytotoxic chemotherapy in Japanese patients with non-small cell lung cancer. J Thorac Oncol. 2007 Jul;2(7):632-7. PMID: 17607119. EXC6

554. Hou MM, Huang SF, Kuo HP, et al. Erlotinib treatment in patients with advanced lung adenocarcinoma with CISH-positive and CISH-negative EGFR gene alterations. Anticancer Res. 2012 Mar;32(3):1107-12. PMID: 22399641. EXC4

555. Houskova L, Zemanova Z, Babjuk M, et al. Molecular cytogenetic characterization and diagnostics of bladder cancer. Neoplasma. 2007;54(6):511-6. PMID: 17949235. EXC6

556. Howlader N, Noone AM, Krapcho M, et al., eds. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). Bethesda, MD: National Cancer Institute; 2012. EXC2

557. Hsieh RK, Lim KH, Kuo HT, et al. Female sex and bronchioloalveolar pathologic subtype predict EGFR mutations in non-small cell lung cancer. Chest. 2005 Jul;128(1):317-21. PMID: 16002952. EXC6

558. Hu C, Liu X, Chen Y, et al. Direct serum and tissue assay for EGFR mutation in non-small cell lung cancer by high-resolution melting analysis. Oncol Rep. 2012;28(5):1815-21. EXC4

559. Hu P, Lee CW, Xu JP, et al. Microsatellite instability in saliva from patients with hereditary non-polyposis colon cancer and siblings carrying germline mismatch repair gene mutations. Ann Clin Lab Sci. 2011 Fall;41(4):321-30. PMID: 22166501. EXC6

560. Huang CT, Yen RF, Cheng MF, et al. Correlation of F-18 fluorodeoxyglucose-positron emission tomography maximal standardized uptake value and EGFR mutations in advanced lung adenocarcinoma. Med Oncol. 2010 Mar;27(1):9-15. PMID: 19130320. EXC6

561. Huang J, Morehouse C, Streicher K, et al. Altered expression of Insulin receptor isoforms in breast cancer. PLoS One. 2011;6(10). EXC4

562. Huang SF, Liu HP, Li LH, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res. 2004 Dec 15;10(24):8195-203. PMID: 15623594. EXC6

563. Huang Z, Wang Z, Bai H, et al. The detection of EGFR mutation status in plasma is reproducible and can dynamically predict the efficacy of EGFR-TKI. Thoracic Cancer. 2012 November;3(4):334-40. PMID: 2012655463 FULL TEXT LINK http://dx.doi.org/10.1111/j.1759-7714.2012.00133.x. EXC6

564. Hubner RA, Lubbe S, Chandler I, et al. MTHFR C677T has differential influence on risk of MSI and MSS colorectal cancer. Hum Mol Genet. 2007;16(9):1072-7. EXC4

565. Huh JW, Park YS, Lee JH, et al. CD133 mRNA expression and microsatellite instability in colorectal carcinoma. J Surg Oncol. 2010 Dec 1;102(7):765-70. PMID: 20872808. EXC4

566. Hu-Lieskovan S, Vallbohmer D, Zhang W, et al. EGF61 polymorphism predicts complete pathologic response to cetuximab-based chemoradiation independent of KRAS status in locally advanced rectal cancer patients. Clin Cancer Res. 2011 Aug 1;17(15):5161-9. PMID: 21673069. EXC4

567. Hunt JD, Strimas A, Martin JE, et al. Differences in KRAS mutation spectrum in lung cancer cases between African Americans and Caucasians after occupational or environmental exposure to known carcinogens. Cancer Epidemiol Biomarkers Prev. 2002 Nov;11(11):1405-12. PMID: 12433719. EXC6

568. Hutchins G, Southward K, Handley K, et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol. 2011(10):1261-70. PMID: CN-00786147. EXC6

569. Huysentruyt CJ, Baldewijns MM, Ruland AM, et al. Modified UroVysion scoring criteria increase the urothelial carcinoma detection rate in cases of equivocal urinary cytology. Histopathology. 2011 Jun;58(7):1048-53. PMID: 21707706. EXC6

570. Hyde A, Fontaine D, Stuckless S, et al. A histology-based model for predicting microsatellite instability in colorectal cancers. Am J Surg Pathol. 2010 Dec;34(12):1820-9. PMID: 21107088. EXC6

571. Ichihara S, Toyooka S, Fujiwara Y, et al. The impact of epidermal growth factor receptor gene status on gefitinib-treated Japanese patients with non-small-cell lung cancer. Int J Cancer. 2007 Mar 15;120(6):1239-47. PMID: 17192902. EXC6

572. Ide T, Kitajima Y, Ohtaka K, et al. Expression of the hMLH1 gene is a possible predictor for the clinical response to 5-fluorouracil after a surgical resection in colorectal cancer. Oncol Rep. 2008 Jun;19(6):1571-6. PMID: 18497967. EXC4

573. Iglesias D, Fernandez-Peralta AM, Nejda N, et al. RIS1, a gene with trinucleotide repeats, is a target in the mutator pathway of colorectal carcinogenesis. Cancer Genet Cytogenet. 2006 Jun;167(2):138-44. PMID: 16737913. EXC4

574. Iida S, Kato S, Ishiguro M, et al. PIK3CA mutation and methylation influences the outcome of colorectal cancer. Oncology Letters. 2012;3(3):565-70. EXC4

575. Ikeda K, Nomori H, Ohba Y, et al. Epidermal growth factor receptor mutations in multicentric lung adenocarcinomas and atypical adenomatous hyperplasias. J Thorac Oncol. 2008 May;3(5):467-71. PMID: 18448997. EXC6

576. Ikeda S, Takabe K, Inagaki M, et al. Detection of gene point mutation in paraffin sections using in situ loop-mediated isothermal amplification. Pathol Int. 2007 Sep;57(9):594-9. PMID: 17685931. EXC4

577. Ilie M, Long E, Hofman V, et al. Diagnostic value of immunohistochemistry for the detection of the BRAFV600E mutation in primary lung adenocarcinoma Caucasian patients. Ann Oncol. 2013 Mar;24(3):742-8. PMID: 23131393. EXC4

578. Ilie MI, Hofman V, Bonnetaud C, et al. Usefulness of tissue microarrays for assessment of protein expression, gene copy number and mutational status of EGFR in lung adenocarcinoma. Virchows Arch. 2010 Oct;457(4):483-95. PMID: 20803030. EXC4

579. Imielinski M, Berger AH, Hammerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell. 2012 Sep 14;150(6):1107-20. PMID: 22980975. EXC6

580. Inamura K, Togashi Y, Nomura K, et al. Up-regulation of PTEN at the transcriptional level is an adverse prognostic factor in female lung adenocarcinomas. Lung Cancer. 2007;57(2):201-6. EXC6

581. Iniesta P, de Juan C, Caldes T, et al. Genetic abnormalities and microsatellite instability in colorectal cancer. Cancer Detect Prev. 1998;22(5):383-95. PMID: 9727619. EXC5

582. Inno A, Di Salvatore M, Cenci T, et al. Is there a role for IGF1R and c-MET pathways in resistance to cetuximab in metastatic colorectal cancer? Clinical Colorectal Cancer. 2011;10(4):325-32. EXC3

583. Inoue A, Suzuki T, Fukuhara T, et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. J Clin Oncol. 2006 Jul 20;24(21):3340-6. PMID: 16785471. EXC6

584. Inoue Y, Saigusa S, Iwata T, et al. The prognostic value of KRAS mutations in patients with colorectal cancer. Oncol Rep. 2012;28(5):1579-84. EXC5

585. Iranzo V, Sirera R, Carrato A, et al. Phase II clinical trial with gemcitabine and paclitaxel sequential monotherapy as first-line treatment for advanced non-small-cell lung cancer (SLCG 01-04). Clin Transl Oncol. 2011 Jun;13(6):411-8. PMID: 21680302. EXC4

586. Irisa K, Masago K, Togashi Y, et al. Significance of pretreatment comorbidities in elderly patients with advanced non-small-cell lung cancer treated with chemotherapy or epidermal growth factor receptor-tyrosine kinase inhibitor. Med Oncol. 2012 Mar;29(1):185-92. PMID: 21136210. EXC4

587. Ishii T, Notohara K, Umapathy A, et al. Tubular adenomas with minor villous changes show molecular features characteristic of tubulovillous adenomas. Am J Surg Pathol. 2011 Feb;35(2):212-20. PMID: 21263241. EXC4

588. Ishitobi M, Goranova TE, Komoike Y, et al. Clinical utility of the 70-gene MammaPrint profile in a Japanese population. Jpn J Clin Oncol. 2010 Jun;40(6):508-12. PMID: 20110242. EXC7

589. Italiano A, Cortot AB, Ilie M, et al. EGFR and KRAS status of primary sarcomatoid carcinomas of the lung: Implications for anti-EGFR treatment of a rare lung malignancy. Int J Cancer. 2009;125(10):2479-82. EXC6

590. Italiano A, Vandenbos FB, Otto J, et al. Comparison of the epidermal growth factor receptor gene and protein in primary non-small-cell-lung cancer and metastatic sites: implications for treatment with EGFR-inhibitors. Ann Oncol. 2006 Jun;17(6):981-5. PMID: 16524970. EXC4

591. Iwata T, Sugio K, Uramoto H, et al. Detection of EGFR and K-ras mutations for diagnosis of multiple lung adenocarcinomas. Front Biosci. 2011;16:2961-9. PMID: 21622214. EXC5

592. Jackman DM, Miller VA, Cioffredi LA, et al. Impact of epidermal growth factor receptor and KRAS mutations on clinical outcomes in previously untreated non-small cell lung cancer patients: results of an online tumor registry of clinical trials. Clin Cancer Res. 2009 Aug 15;15(16):5267-73. PMID: 19671843. EXC6

593. Jackman DM, Yeap BY, Sequist LV, et al. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. Clin Cancer Res. 2006 Jul 1;12(13):3908-14. PMID: 16818686. EXC6

594. Jacot W, Pujol JL, Boher JM, et al. Serum EGF-receptor and HER-2 extracellular domains and prognosis of non-small-cell lung cancer. Br J Cancer. 2004 Aug 2;91(3):430-3. PMID: 15226769. EXC4

595. Janjigian YY, Park BJ, Zakowski MF, et al. Impact on disease-free survival of adjuvant erlotinib or gefitinib in patients with resected lung adenocarcinomas that harbor EGFR mutations. J Thorac Oncol. 2011 Mar;6(3):569-75. PMID: 21150674. EXC6

596. Janku F, Wheler JJ, Naing A, et al. PIK3CA mutations in advanced cancers: Characteristics and outcomes. Oncotarget. 2012 December;3(12):1566-75. PMID: 2013150050 MEDLINE PMID 23248156 (http://www.ncbi.nlm.nih.gov/pubmed/23248156). EXC3

597. Janne PA, Gurubhagavatula S, Yeap BY, et al. Outcomes of patients with advanced non-small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study. Lung Cancer. 2004 May;44(2):221-30. PMID: 15084387. EXC6

598. Janne PA, Wang X, Socinski MA, et al. Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. J Clin Oncol. 2012 Jun 10;30(17):2063-9. PMID: 22547605. EXC6

599. Jantus-Lewintre E, Sirera R, Cabrera A, et al. Analysis of the prognostic value of soluble epidermal growth factor receptor plasma concentration in advanced non-small-cell lung cancer patients. Clin Lung Cancer. 2011 Sep;12(5):320-7. PMID: 21729651. EXC4

600. Jass JR, Pokos V, Arnold JL, et al. Colorectal neoplasms detected colonoscopically in at-risk members of colorectal cancer families stratified by the demonstration of DNA microsatellite instability. J Mol Med (Berl). 1996 Sep;74(9):547-51. PMID: 8892060. EXC4

601. Jemal A, Center MM, DeSantis C, et al. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev. 2010 Aug;19(8):1893-907. PMID: 20647400. EXC4

602. Jenkins MA, Hayashi S, O'Shea AM, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology. 2007 Jul;133(1):48-56. PMID: 17631130. EXC4

603. Jensen AD, Munter MW, Bischoff H, et al. Treatment of non-small cell lung cancer with intensity-modulated radiation therapy in combination with cetuximab: the NEAR protocol (NCT00115518). BMC Cancer. 2006;6:122. PMID: 16681848. EXC6

604. Jensen LH, Dysager L, Lindebjerg J, et al. Molecular biology from bench-to-bedside - which colorectal cancer patients should be referred for genetic counselling and risk assessment. Eur J Cancer. 2010 Jul;46(10):1823-8. PMID: 20417091. EXC6

605. Jensen LH, Kuramochi H, Cruger DG, et al. Gene expression of the mismatch repair gene MSH2 in primary colorectal cancer. Tumour Biol. 2011 Oct;32(5):977-83. PMID: 21732224. EXC6

606. Jensen LH, Lindebjerg J, Byriel L, et al. Strategy in clinical practice for classification of unselected colorectal tumours based on mismatch repair deficiency. Colorectal Dis. 2008 Jun;10(5):490-7. PMID: 17868408. EXC6

607. Jeon CH, Lee HI, Shin IH, et al. Genetic alterations of APC, K-ras, p53, MSI, and MAGE in Korean colorectal cancer patients. Int J Colorectal Dis. 2008 Jan;23(1):29-35. PMID: 17704924. EXC5

608. Jeong SY, Shin KH, Shin JH, et al. Microsatellite instability and mutations in DNA mismatch repair genes in sporadic colorectal cancers. Dis Colon Rectum. 2003 Aug;46(8):1069-77. PMID: 12907901. EXC4

609. Jia XL, Chen G. EGFR and KRAS mutations in Chinese patients with adenosquamous carcinoma of the lung. Lung Cancer. 2011;74(3):396-400. EXC6

610. Jian G, Songwen Z, Ling Z, et al. Prediction of epidermal growth factor receptor mutations in the plasma/pleural effusion to efficacy of gefitinib treatment in advanced non-small cell lung cancer. J Cancer Res Clin Oncol. 2010 Sep;136(9):1341-7. PMID: 20155428. EXC4

611. Jiang X, Liu Y, Chen C, et al. The value of biomarkers in patients with sarcomatoid carcinoma of the lung: molecular analysis of 33 cases. Clin Lung Cancer. 2012 Jul;13(4):288-96. PMID: 22169481. EXC6

612. Jin P, Meng XM, Sheng JQ, et al. Clinicopathological features of non-familial colorectal cancer with high-frequency microsatellite instability. Chin Med Sci J. 2010;25(4):228-32. EXC6

613. Jin Y, Li JP, Tang LY, et al. Protein expression and significance of VEGF, EGFR and MMP-9 in non-small cell lung carcinomas. Asian Pac J Cancer Prev. 2011;12(6):1473-6. PMID: 22126484. EXC4

614. Joerger M, deJong D, Burylo A, et al. Tubulin, BRCA1, ERCC1, Abraxas, RAP80 mRNA expression, p53/p21 immunohistochemistry and clinical outcome in patients with advanced non small-cell lung cancer receiving first-line platinum-gemcitabine chemotherapy. Lung Cancer. 2011 Nov;74(2):310-7. PMID: 21529986. EXC4

615. John T, Kohler D, Pintilie M, et al. The ability to form primary tumor xenografts is predictive of increased risk of disease recurrence in early-stage non-small cell lung cancer. Clin Cancer Res. 2011 Jan 1;17(1):134-41. PMID: 21081655. EXC6

616. Johnson FM, Bekele BN, Feng L, et al. Phase II study of dasatinib in patients with advanced non-small-cell lung cancer. J Clin Oncol. 2010 Oct 20;28(30):4609-15. PMID: 20855820. EXC6

617. Johnson ML, Riely GJ, Rizvi NA, et al. Phase II trial of dasatinib for patients with acquired resistance to treatment with the epidermal growth factor receptor tyrosine kinase inhibitors erlotinib or gefitinib. J Thorac Oncol. 2011 Jun;6(6):1128-31. PMID: 21623279. EXC6

618. Johnson ML, Sima CS, Chaft J, et al. Association of KRAS and EGFR mutations with survival in patients with advanced lung adenocarcinomas. Cancer. 2013 Jan 15;119(2):356-62. PMID: 22810899. EXC3

619. Johnson V, Lipton LR, Cummings C, et al. Analysis of somatic molecular changes, clinicopathological features, family history, and germline mutations in colorectal cancer families: evidence for efficient diagnosis of HNPCC and for the existence of distinct groups of non-HNPCC families. J Med Genet. 2005 Oct;42(10):756-62. PMID: 15788729. EXC6

620. Jones JS. DNA-based molecular cytology for bladder cancer surveillance. Urology. 2006 Mar;67(3 Suppl 1):35-45; discussion -7. PMID: 16530074. EXC6

621. Jones TD, Zhang S, Lopez-Beltran A, et al. Urothelial carcinoma with an inverted growth pattern can be distinguished from inverted papilloma by fluorescence in situ hybridization, immunohistochemistry, and morphologic analysis. Am J Surg Pathol. 2007 Dec;31(12):1861-7. PMID: 18043040. EXC5

622. Jonsson M, Ekstrand A, Edekling T, et al. Experiences from treatment-predictive KRAS testing; High mutation frequency in rectal cancers from females and concurrent mutations in the same tumor. BMC Clinical Pathology. 2009;9(1). EXC6

623. Jou YS, Lo YL, Hsiao CF, et al. Association of an EGFR intron 1 SNP with never-smoking female lung adenocarcinoma patients. Lung Cancer. 2009 Jun;64(3):251-6. PMID: 19026460. EXC6

624. Jover R, Paya A, Alenda C, et al. Defective mismatch-repair colorectal cancer: clinicopathologic characteristics and usefulness of immunohistochemical analysis for diagnosis. Am J Clin Pathol. 2004 Sep;122(3):389-94. PMID: 15362369. EXC4

625. Jover R, Zapater P, Castells A, et al. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. Eur J Cancer. 2009 Feb;45(3):365-73. PMID: 18722765. EXC4

626. Jover R, Zapater P, Castells A, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut. 2006;55(6):848-55. EXC6

627. Jung M, Kim SH, Hong S, et al. Prognostic and predictive value of carcinoembryonic antigen and cytokeratin-19 fragments levels in advanced non-small cell lung cancer patients treated with gefitinib or erlotinib. Yonsei Med J. 2012;53(5):931-9. EXC2

628. Jung M, Kim SH, Lee YJ, et al. Prognostic and predictive value of CEA and CYFRA 21-1 levels in advanced non-small cell lung cancer patients treated with geftinib or erlotinib. Experimental and Therapeutic Medicine. 2011;2(4):685-93. EXC4

629. Jung SB, Lee HI, Oh HK, et al. Clinico-pathologic parameters for prediction of microsatellite instability in colorectal cancer. Cancer Research and Treatment. 2012 2012;44(3):179-86. PMID: 2013121114 FULL TEXT LINK http://dx.doi.org/10.4143/crt.2012.44.3.179. EXC6

630. Junker K, Fritsch T, Hartmann A, et al. Multicolor fluorescence in situ hybridization (M-FISH) on cells from urine for the detection of bladder cancer. Cytogenet Genome Res. 2006;114(3-4):279-83. PMID: 16954667. EXC6

631. Just PA, Cazes A, Audebourg A, et al. Histologic subtypes, immunohistochemistry, FISH or molecular screening for the accurate diagnosis of ALK-rearrangement in lung cancer: a comprehensive study of Caucasian non-smokers. Lung Cancer. 2012 Jun;76(3):309-15. PMID: 22153831. EXC6

632. Kadiyska TK, Konstantinova DV, Atanasov VR, et al. Frequency and application of the hot spot BRAF gene mutation (p.V600E) in the diagnostic strategy for Hereditary Nonpolyposis Colorectal Cancer. Cancer Detect Prev. 2007;31(3):254-6. PMID: 17566669. EXC5

633. Kaira K, Horie Y, Ayabe E, et al. Pulmonary pleomorphic carcinoma: a clinicopathological study including EGFR mutation analysis. J Thorac Oncol. 2010 Apr;5(4):460-5. PMID: 20107421. EXC6

634. Kaira K, Nakagawa K, Ohde Y, et al. Depolarized MUC1 expression is closely associated with hypoxic markers and poor outcome in resected non-small cell lung cancer. Int J Surg Pathol. 2012 Jun;20(3):223-32. PMID: 22108499. EXC4

635. Kaira K, Oriuchi N, Takahashi T, et al. L-type amino acid transporter 1 (LAT1) expression in malignant pleural mesothelioma. Anticancer Res. 2011 Dec;31(12):4075-82. PMID: 22199264. EXC6

636. Kaji E, Kato J, Suzuki H, et al. Analysis of K-ras, BRAF, and PIK3CA mutations in laterally-spreading tumors of the colorectum. J Gastroenterol Hepatol. 2011 Mar;26(3):599-607. PMID: 21332555. EXC6

637. Kakar S, Aksoy S, Burgart LJ, et al. Mucinous carcinoma of the colon: correlation of loss of mismatch repair enzymes with clinicopathologic features and survival. Mod Pathol. 2004 Jun;17(6):696-700. PMID: 15017435. EXC4

638. Kakar S, Deng G, Smyrk TC, et al. Loss of heterozygosity, aberrant methylation, BRAF mutation and KRAS mutation in colorectal signet ring cell carcinoma. Mod Pathol. 2012 Jul;25(7):1040-7. PMID: 22522845. EXC6

639. Kakar S, Smyrk TC. Signet ring cell carcinoma of the colorectum: correlations between microsatellite instability, clinicopathologic features and survival. Mod Pathol. 2005 Feb;18(2):244-9. PMID: 15492759. EXC6

640. Kakegawa S, Shimizu K, Sugano M, et al. Clinicopathological features of lung adenocarcinoma with KRAS mutations. Cancer. 2011;117(18):4257-66. EXC6

641. Kalady MF, Sanchez JA, Manilich E, et al. Divergent oncogenic changes influence survival differences between colon and rectal adenocarcinomas. Dis Colon Rectum. 2009 Jun;52(6):1039-45. PMID: 19581844. EXC4

642. Kalikaki A, Koutsopoulos A, Hatzidaki D, et al. Clinical outcome of patients with non-small cell lung cancer receiving front-line chemotherapy according to EGFR and K-RAS mutation status. Lung Cancer. 2010 Jul;69(1):110-5. PMID: 19854533. EXC6

643. Kamat AM, Dickstein RJ, Messetti F, et al. Use of fluorescence in situ hybridization to predict response to bacillus Calmette-Guerin therapy for bladder cancer: results of a prospective trial. J Urol. 2012 Mar;187(3):862-7. PMID: 22245325. Inc

644. Kamat AM, Hegarty PK, Gee JR, et al. ICUD-EAU International Consultation on Bladder Cancer 2012: Screening, diagnosis, and molecular markers. Eur Urol. 2013 Jan;63(1):4-15. PMID: 23083902. EXC6

645. Kamat AM, Karam JA, Grossman HB, et al. Prospective trial to identify optimal bladder cancer surveillance protocol: reducing costs while maximizing sensitivity. BJU Int. 2011 Oct;108(7):1119-23. PMID: 21426474. EXC5

646. Kanaji N, Bandoh S, Ishii T, et al. Detection of EML4-ALK fusion genes in a few cancer cells from transbronchial cytological specimens utilizing immediate cytology during bronchoscopy. Lung Cancer. 2012;77(2):293-8. EXC4

647. Kanaji N, Bandoh S, Nagamura N, et al. Significance of an epidermal growth factor receptor mutation in cerebrospinal fluid for carcinomatous meningitis. Intern Med. 2007;46(19):1651-5. PMID: 17917328. EXC2

648. Kaneda H, Okamoto I, Sakai K, et al. Marked response to both S-1 and pemetrexed in a patient with echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase-positive lung adenocarcinoma. Acta Oncol. 2012;51(7):942-4. EXC2

649. Kaneda H, Uemura Y, Nakano T, et al. Lesions in patients with multifocal adenocarcinoma are more frequently in the right upper lobes. Interact Cardiovasc Thorac Surg. 2012 Oct;15(4):627-32. PMID: 22733594. EXC6

650. Kanematsu T, Yano S, Uehara H, et al. Phosphorylation, but not overexpression, of epidermal growth factor receptor is associated with poor prognosis of non-small cell lung cancer patients. Oncol Res. 2003;13(5):289-98. PMID: 12688680. EXC4

651. Kang BW, Kim JG, Lee SJ, et al. Clinical significance of microsatellite instability for stage II or III colorectal cancer following adjuvant therapy with doxifluridine. Med Oncol. 2011 Dec;28 Suppl 1:S214-8. PMID: 20953739. EXC4

652. Kang JU, Koo SH, Kwon KC, et al. Gain of the EGFR gene located on 7p12 is a frequent and early event in squamous cell carcinoma of the lung. Cancer Genet Cytogenet. 2008 Jul;184(1):31-7. PMID: 18558286. EXC6

653. Kappers I, Vollebergh MA, Van Tinteren H, et al. Soluble epidermal growth factor receptor (sEGFR) and carcinoembryonic antigen (CEA) concentration in patients with nonsmall cell lung cancer: Correlation with survival after erlotinib and gefitinib treatment. ecancermedicalscience. 2010;4(1). EXC4

654. Karim B, Florence C, Kamel R, et al. KRAS mutation detection in Tunisian sporadic coloractal cancer patients with direct sequencing, high resolution melting and denaturating high performance liquid chromatography. Cancer Biomark. 2010;8(6):331-40. PMID: 22072121. EXC5

655. Karnwal A, Venegas R, Shuch B, et al. The role of fluorescence in situ hybridization assay for surveillance of non-muscle invasive bladder cancer. Can J Urol. 2010 Apr;17(2):5077-81. PMID: 20398445. EXC5

656. Karpinski P, Szmida E, Misiak B, et al. Assessment of three epigenotypes in colorectal cancer by combined bisulfite restriction analysis. Mol Carcinog. 2012 Dec;51(12):1003-8. PMID: 22006538. EXC6

657. Kasahara K, Arao T, Sakai K, et al. Impact of serum hepatocyte growth factor on treatment response to epidermal growth factor receptor tyrosine kinase inhibitors in patients with non-small cell lung adenocarcinoma. Clin Cancer Res. 2010 Sep 15;16(18):4616-24. PMID: 20679350. EXC6

658. Kashihara M, Azuma K, Kawahara A, et al. Nuclear Y-box binding protein-1, a predictive marker of prognosis, is correlated with expression of HER2/ErbB2 and HER3/ErbB3 in non-small cell lung cancer. J Thorac Oncol. 2009 Sep;4(9):1066-74. PMID: 19648825. EXC4

659. Katballe N, Christensen M, Wikman FP, et al. Frequency of hereditary non-polyposis colorectal cancer in Danish colorectal cancer patients. Gut. 2002 Jan;50(1):43-51. PMID: 11772966. EXC3

660. Katkoori VR, Shanmugam C, Jia X, et al. Prognostic significance and gene expression profiles of p53 mutations in microsatellite-stable stage III colorectal adenocarcinomas. PLoS One. 2012;7(1):e30020. PMID: 22276141. EXC4

661. Kawada K, Nakamoto Y, Kawada M, et al. Relationship between 18F-fluorodeoxyglucose accumulation and KRAS/BRAF mutations in colorectal cancer. Clin Cancer Res. 2012 Mar 15;18(6):1696-703. PMID: 22282467. EXC6

662. Kawahara A, Taira T, Azuma K, et al. A diagnostic algorithm using EGFR mutation-specific antibodies for rapid response EGFR-TKI treatment in patients with non-small cell lung cancer. Lung Cancer. 2012;78(1):39-44. EXC4

663. Kawai H, Ishii A, Washiya K, et al. Combined overexpression of EGFR and estrogen receptor alpha correlates with a poor outcome in lung cancer. Anticancer Res. 2005 Nov-Dec;25(6C):4693-8. PMID: 16334162. EXC4

664. Kawano Y, Ohyanagi F, Yanagitani N, et al. Pemetrexed and cisplatin for advanced non-squamous non-small cell lung cancer in Japanese patients: phase II study. Anticancer Res. 2013 Aug;33(8):3327-33. PMID: 23898099. EXC3

665. Kayton ML, He M, Zakowski MF, et al. Primary lung adenocarcinomas in children and adolescents treated for pediatric malignancies. J Thorac Oncol. 2010;5(11):1764-71. EXC3

666. Kazama Y, Watanabe T, Kanazawa T, et al. Mucinous colorectal cancers with chromosomal instability: A biologically distinct and aggressive subtype. Diagn Mol Pathol. 2006;15(1):30-4. EXC5

667. Kazama Y, Watanabe T, Kanazawa T, et al. Microsatellite instability in poorly differentiated adenocarcinomas of the colon and rectum: relationship to clinicopathological features. J Clin Pathol. 2007 Jun;60(6):701-4. PMID: 17557871. EXC6

668. Ke HG, Zhou XY, Shen Y, et al. The relationship between EGFR gene mutation status and ERCC1 in lung adenocarcinoma of Chinese patients receiving platinum-based neoadjuvant chemotherapy. Oncol Res. 2012;20(5-6):221-9. PMID: 23581229. EXC7

669. Kehinde EO, Al-Mulla F, Kapila K, et al. Comparison of the sensitivity and specificity of urine cytology, urinary nuclear matrix protein-22 and multitarget fluorescence in situ hybridization assay in the detection of bladder cancer. Scand J Urol Nephrol. 2011 Mar;45(2):113-21. PMID: 21091091. EXC6

670. . Impact of the recurrence score (RS) result and mismatch repair status (MMR) on agreement between oncologists (MDs) for stage II colon cancer (CC) recurrence risk (RR) assessment: A novel clinical utility endpoint for prognostic markers. ASCO; 2013. EXC6

671. Kelly CM, Krishnamurthy S, Bianchini G, et al. Utility of oncotype DX risk estimates in clinically intermediate risk hormone receptor-positive, HER2-normal, grade II, lymph node-negative breast cancers. Cancer. 2010 Nov 15;116(22):5161-7. PMID: 20665886. EXC6

672. Kelly RJ, Rajan A, Force J, et al. Evaluation of KRAS mutations, angiogenic biomarkers, and DCE-MRI in patients with advanced non-small-cell lung cancer receiving sorafenib. Clin Cancer Res. 2011 Mar 1;17(5):1190-9. PMID: 21224376. EXC6

673. Kennecke HF, Speers CH, Ennis CA, et al. Impact of routine pathology review on treatment for node-negative breast cancer. J Clin Oncol. 2012 Jun 20;30(18):2227-31. PMID: 22564990. EXC4

674. Kets CM, Hoogerbrugge N, Bodmer D, et al. Unfavorable pathological characteristics in familial colorectal cancer with low-level microsatellite instability. Mod Pathol. 2006 Dec;19(12):1624-30. PMID: 16980941. EXC3

675. Kets CM, Van Krieken JHJM, Van Erp PEJ, et al. Is early-onset microsatellite and chromosomally stable colorectal cancer a hallmark of a genetic susceptibility syndrome? Int J Cancer. 2008;122(4):796-801. EXC6

676. Khambata-Ford S, Harbison CT, Hart LL, et al. Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small-cell lung cancer. J Clin Oncol. 2010 Feb 20;28(6):918-27. PMID: 20100958. EXC6

677. Khan SA, Morris M, Idrees K, et al. The biology of early-onset colorectal cancer: An examination of tumor markers, pathology, and survival in a large cohort of patients. J Clin Oncol. 2011;29(15). EXC6

678. Khode R, Larsen DA, Culbreath BC, et al. Comparative study of epidermal growth factor receptor mutation analysis on cytology smears and surgical pathology specimens from primary and metastatic lung carcinomas. Cancer Cytopathol. 2013 Jul;121(7):361-9. PMID: 23364874. EXC4

679. Kidwell KM, Yothers G, Ganz PA, et al. Long-term neurotoxicity effects of oxaliplatin added to fluorouracil and leucovorin as adjuvant therapy for colon cancer: results from National Surgical Adjuvant Breast and Bowel Project trials C-07 and LTS-01. Cancer. 2012 Nov 15;118(22):5614-22. PMID: 22569841. EXC6

680. Kim DW, Lee SH, Lee JS, et al. A multicenter phase II study to evaluate the efficacy and safety of gefitinib as first-line treatment for Korean patients with advanced pulmonary adenocarcinoma harboring EGFR mutations. Lung Cancer. 2011 Jan;71(1):65-9. PMID: 20430469. EXC6

681. Kim DW, Min HS, Lee KH, et al. High tumour islet macrophage infiltration correlates with improved patient survival but not with EGFR mutations, gene copy number or protein expression in resected non-small cell lung cancer. Br J Cancer. 2008 Mar 25;98(6):1118-24. PMID: 18283317. EXC6

682. Kim H, Piao Z, Kim JW, et al. Expression of hMSH2 and hMLH1 in colorectal carcinomas with microsatellite instability. Pathol Res Pract. 1998;194(1):3-9. PMID: 9542742. EXC4

683. Kim H, Xu X, Yoo SB, et al. Discordance between anaplastic lymphoma kinase status in primary non-small-cell lung cancers and their corresponding metastases. Histopathology. 2013 Jan;62(2):305-14. PMID: 23020707. EXC6

684. Kim H, Yoo SB, Choe JY, et al. Detection of ALK gene rearrangement in non-small cell lung cancer: a comparison of fluorescence in situ hybridization and chromogenic in situ hybridization with correlation of ALK protein expression. J Thorac Oncol. 2011 Aug;6(8):1359-66. PMID: 21587085. EXC5

685. Kim HJ, Lee KY, Kim YC, et al. Detection and comparison of peptide nucleic acid-mediated real-time polymerase chain reaction clamping and direct gene sequencing for epidermal growth factor receptor mutations in patients with non-small cell lung cancer. Lung Cancer. 2012 Mar;75(3):321-5. PMID: 21930325. EXC6

686. Kim HJ, Oh SY, Kim WS, et al. Clinical investigation of EGFR mutation detection by pyrosequencing in lung cancer patients. Oncology Letters. 2012 2012;5(1):271-6. PMID: 2012665743 FULL TEXT LINK http://dx.doi.org/10.3892/ol.2012.950. EXC6

687. Kim HR, Shim HS, Chung JH, et al. Distinct clinical features and outcomes in never-smokers with nonsmall cell lung cancer who harbor EGFR or KRAS mutations or ALK rearrangement. Cancer. 2012 Feb 1;118(3):729-39. PMID: 21720997. EXC6

688. Kim HS, Park YH, Lee J, et al. Clinical impact of phosphorylated signal transducer and activator of transcription 3, epidermal growth factor receptor, p53, and vascular endothelial growth factor receptor 1 expression in resected adenocarcinoma of lung by using tissue microarray. Cancer. 2010 Feb 1;116(3):676-85. PMID: 20052735. EXC4

689. Kim JC, Roh SA, Cho DH, et al. Chemoresponsiveness associated with canonical molecular changes in colorectal adenocarcinomas. Anticancer Res. 2009 Aug;29(8):3115-23. PMID: 19661324. EXC5

690. Kim JE, Hong YS, Ryu MH, et al. Association between deficient mismatch repair system and efficacy to irinotecan-containing chemotherapy in metastatic colon cancer. Cancer Sci. 2011 Sep;102(9):1706-11. PMID: 21679278. EXC5

691. Kim JH, Shin SH, Kwon HJ, et al. Prognostic implications of CpG island hypermethylator phenotype in colorectal cancers. Virchows Arch. 2009 Dec;455(6):485-94. PMID: 19911194. EXC5

692. Kim KS, Jeong JY, Kim YC, et al. Predictors of the response to gefitinib in refractory non-small cell lung cancer. Clin Cancer Res. 2005 Mar 15;11(6):2244-51. PMID: 15788673. EXC6

693. Kim SJ, Rabbani ZN, Dong F, et al. Phosphorylated epidermal growth factor receptor and cyclooxygenase-2 expression in localized non-small cell lung cancer. Med Oncol_. 2010;27(1):91-7. EXC5

694. Kim ST, Choi YJ, Park KH, et al. Epidermal growth factor receptor mutations as a prognostic factor in korean patients with advanced lung adenocarcinoma who had not been treated with received epidermal growth factor receptor tyrosine kinase inhibitors. Chemotherapy. 2011;57(2):108-14. EXC3

695. Kim ST, Jung HY, Sung JS, et al. Can serum be used for analyzing the EGFR mutation status in patients with advanced non-small cell lung cancer? Am J Clin Oncol. 2013 Feb;36(1):57-63. PMID: 22237146. EXC3

696. Kim ST, Lee J, Kim JH, et al. Comparison of gefitinib versus erlotinib in patients with nonsmall cell lung cancer who failed previous chemotherapy. Cancer. 2010 Jun 15;116(12):3025-33. PMID: 20564408. EXC6

697. Kim ST, Sung JS, Jo UH, et al. Can mutations of EGFR and KRAS in serum be predictive and prognostic markers in patients with advanced non-small cell lung cancer (NSCLC)? Med Oncol. 2013 Mar;30(1):328. PMID: 23307237. EXC7

698. Kim SY, Shim EK, Yeo HY, et al. KRAS mutation status and clinical outcome of preoperative chemoradiation with cetuximab in locally advanced rectal cancer: a pooled analysis of 2 phase II trials. Int J Radiat Oncol Biol Phys. 2013 Jan 1;85(1):201-7. PMID: 22672749. EXC4

699. Kim YH, Min BH, Kim SJ, et al. Difference between proximal and distal microsatellite-unstable sporadic colorectal cancers: analysis of clinicopathological and molecular features and prognoses. Ann Surg Oncol. 2010 May;17(5):1435-41. PMID: 20049642. EXC4

700. Kim YH, Song SY, Kwon YD, et al. Microsatellite instable double primary cancers of the colorectum and stomach exhibit less favorable outcome. World J Gastroenterol. 2005 Jul 14;11(26):3998-4002. PMID: 15996022. EXC3

701. Kimura H, Fujiwara Y, Sone T, et al. High sensitivity detection of epidermal growth factor receptor mutations in the pleural effusion of non-small cell lung cancer patients. Cancer Sci. 2006 Jul;97(7):642-8. PMID: 16827805. EXC6

702. Kimura H, Kasahara K, Kawaishi M, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. Clin Cancer Res. 2006 Jul 1;12(13):3915-21. PMID: 16818687. EXC6

703. Kimura H, Nakajima T, Takeuchi K, et al. ALK fusion gene positive lung cancer and 3 cases treated with an inhibitor for ALK kinase activity. Lung Cancer. 2012 Jan;75(1):66-72. PMID: 21757253. EXC6

704. Kimura H, Suminoe M, Kasahara K, et al. Evaluation of epidermal growth factor receptor mutation status in serum DNA as a predictor of response to gefitinib (IRESSA). Br J Cancer. 2007 Sep 17;97(6):778-84. PMID: 17848912. EXC6

705. Kimura T, Okamoto K, Miyamoto H, et al. Clinical benefit of high-sensitivity KRAS mutation testing in metastatic colorectal cancer treated with anti-EGFR antibody therapy. Oncology. 2012;82(5):298-304. PMID: 22555244. EXC3

706. Kipp BR, Karnes RJ, Brankley SM, et al. Monitoring intravesical therapy for superficial bladder cancer using fluorescence in situ hybridization. J Urol. 2005 Feb;173(2):401-4. PMID: 15643180. EXC7

707. Kirk R. Risk factors. Oncotype DX assay predicts local recurrence in breast cancer. Nat Rev Clin Oncol. 2010 Jun;7(6):300. PMID: 20527688. EXC2

708. Kitano S, Nakayama M, Yamane A, et al. Detection of DNA mutations by fluorescence resonance energy transfer-based preferential homoduplex formation assay. Anal Biochem. 2011 Jan 15;408(2):197-205. PMID: 20850410. EXC4

709. Klarskov L, Holck S, Bernstein I, et al. Challenges in the identification of MSH6-associated colorectal cancer: rectal location, less typical histology, and a subset with retained mismatch repair function. Am J Surg Pathol. 2011 Sep;35(9):1391-9. PMID: 21836479. EXC5

710. Klump B, Nehls O, Okech T, et al. Molecular lesions in colorectal cancer: Impact on prognosis? Original data and review of the literature. Int J Colorectal Dis. 2004;19(1):23-42. EXC1

711. Knauer M, Mook S, Rutgers EJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. Breast Cancer Res Treat. 2010 Apr;120(3):655-61. PMID: 20204499. EXC6

712. Knijn N, Mekenkamp LJ, Klomp M, et al. KRAS mutation analysis: a comparison between primary tumours and matched liver metastases in 305 colorectal cancer patients. Br J Cancer. 2011 Mar 15;104(6):1020-6. PMID: 21364579. EXC6

713. Kobayashi M, Sonobe M, Takahashi T, et al. Detection of ALK fusion in lung cancer using fluorescence in situ hybridization. Asian Cardiovasc Thorac Ann. 2012 Aug;20(4):426-31. PMID: 22879549. EXC6

714. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005 Feb 24;352(8):786-92. PMID: 15728811. EXC2

715. Koelzer VH, Karamitopoulou E, Dawson H, et al. Geographic analysis of RKIP expression and its clinical relevance in colorectal cancer. Br J Cancer. 2013 May 28;108(10):2088-96. PMID: 23632477. EXC4

716. Koga T, Takeshita M, Ijichi K, et al. CHFR aberrant methylation involves a subset of human lung adenocarcinoma associated with poor clinical outcomes. Hum Pathol. 2013 Jul;44(7):1382-90. PMID: 23415374. EXC7

717. Koh Y, Kim DW, Kim TM, et al. Clinicopathologic characteristics and outcomes of patients with anaplastic lymphoma kinase-positive advanced pulmonary adenocarcinoma: suggestion for an effective screening strategy for these tumors. J Thorac Oncol. 2011 May;6(5):905-12. PMID: 21358343. EXC6

718. Kohonen-Corish MR, Daniel JJ, Chan C, et al. Low microsatellite instability is associated with poor prognosis in stage C colon cancer. J Clin Oncol. 2005 Apr 1;23(10):2318-24. PMID: 15800322. EXC3

719. Koinuma K, Kaneda R, Toyota M, et al. Screening for genomic fragments that are methylated specifically in colorectal carcinoma with a methylated MLH1 promoter. Carcinogenesis. 2005 Dec;26(12):2078-85. PMID: 16033773. EXC3

720. Kok M, Koornstra RH, Mook S, et al. Additional value of the 70-gene signature and levels of ER and PR for the prediction of outcome in tamoxifen-treated ER-positive breast cancer. Breast. 2012 Dec;21(6):769-78. PMID: 22738860. EXC3

721. Kondo M, Hoshi SL, Ishiguro H, et al. Economic evaluation of the 70-gene prognosis-signature (MammaPrint(registered trademark)) in hormone receptor-positive, lymph node-negative, human epidermal growth factor receptor type 2-negative early stage breast cancer in Japan. Breast Cancer Res Treat. 2012;133(2):759-68. EXC2

722. Kondo M, Hoshi SL, Ishiguro H, et al. Economic evaluation of 21-gene reverse transcriptase-polymerase chain reaction assay in lymph-node-negative, estrogen-receptor-positive, early-stage breast cancer in Japan (Structured abstract). Breast Cancer Res Treat. 2008(1):175-87. PMID: NHSEED-22008102336. EXC2

723. Kondo M, Hoshi SL, Yamanaka T, et al. Economic evaluation of the 21-gene signature (Oncotype DX) in lymph node-negative/positive, hormone receptor-positive early-stage breast cancer based on Japanese validation study (JBCRG-TR03) (Structured abstract). Breast Cancer Res Treat. 2011(3):739-49. PMID: NHSEED-22011001103. EXC2

724. Konigsberg R, Hulla W, Klimpfinger M, et al. Clinical and economic aspects of KRAS mutational status as predictor for epidermal growth factor receptor inhibitor therapy in metastatic colorectal cancer patients. Oncology. 2011;81(5-6):359-64. PMID: 22248908. EXC4

725. Konishi J, Yamazaki K, Kinoshita I, et al. Analysis of the response and toxicity to gefitinib of non-small cell lung cancer. Anticancer Res. 2005 Jan-Feb;25(1B):435-41. PMID: 15816608. EXC4

726. Koopman M, Kortman GA, Mekenkamp L, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer. 2009 Jan 27;100(2):266-73. PMID: 19165197. EXC4

727. . Genomic Classifiers (ColoPrint/MSI-Print) to Predict Outcome and Chemotherapy Benefit in Stage II and III Colon Cancer Patients. ASCO Gastrointestinal Symposium; 2013; San Francisco, CA. EXC4

728. Kordiak J, Szemraj J, Hamara K, et al. Complete surgical resection of lung tumor decreases exhalation of mutated KRAS oncogene. Respir Med. 2012;106(9):1293-300. EXC4

729. Korpanty G, Mulligan N, Carney DN. Complete radiological response of metastatic anaplastic lymphoma kinase-positive signet ring lung adenocarcinoma to systemic chemotherapy. J Thorac Oncol. 2011 May;6(5):963-5. PMID: 21623269. EXC2

730. Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res. 2004 Dec 15;64(24):8919-23. PMID: 15604253. EXC4

731. Kosaka T, Yatabe Y, Onozato R, et al. Prognostic implication of EGFR, KRAS, and TP53 gene mutations in a large cohort of Japanese patients with surgically treated lung adenocarcinoma. J Thorac Oncol. 2009 Jan;4(1):22-9. PMID: 19096302. EXC4

732. Koukourakis MI, Giatromanolaki A, O'Byrne KJ, et al. Potential role of bcl-2 as a suppressor of tumour angiogenesis in non-small-cell lung cancer. Int J Cancer. 1997 Dec 19;74(6):565-70. PMID: 9421349. EXC4

733. Koyama N, Jinn Y, Takabe K, et al. The characterization of gefitinib sensitivity and adverse events in patients with non-small cell lung cancer. Anticancer Res. 2006 Nov-Dec;26(6B):4519-25. PMID: 17201173. EXC6

734. Kozu Y, Tsuta K, Kohno T, et al. The usefulness of mutation-specific antibodies in detecting epidermal growth factor receptor mutations and in predicting response to tyrosine kinase inhibitor therapy in lung adenocarcinoma. Lung Cancer. 2011 Jul;73(1):45-50. PMID: 21129809. EXC6

735. Krajewska M, Kim H, Kim C, et al. Analysis of apoptosis protein expression in early-stage colorectal cancer suggests opportunities for new prognostic biomarkers. Clin Cancer Res. 2005;11(15):5451-61. EXC4

736. Krawczyk P, Kowalski DM, Wojas-Krawczyk K, et al. The qualification of docetaxel or erlotinib for second-line therapy should be based on clinical and molecular predictive factors. Chemotherapy. 2012;58(1):60-9. PMID: 22338650. EXC6

737. Krawczyk P, Wojas-Krawczyk K, Mlak R, et al. Predictive value of ERCC1 single-nucleotide polymorphism in patients receiving platinum-based chemotherapy for locally-advanced and advanced non-small cell lung cancer - a pilot study. Folia Histochem Cytobiol. 2012;50(1):80-6. EXC4

738. Kriegl L, Jung A, Horst D, et al. Microsatellite instability, KRAS mutations and cellular distribution of TRAIL-receptors in early stage colorectal cancer. PLoS One. 2012;7(12):e51654. PMID: 23284732. EXC4

739. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA. 2003 Oct 22;290(16):2149-58. PMID: 14570950. EXC6

740. Krishnaswamy S, Kanteti R, Duke-Cohan JS, et al. Ethnic differences and functional analysis of MET mutations in lung cancer. Clin Cancer Res. 2009 Sep 15;15(18):5714-23. PMID: 19723643. EXC6

741. Krivokapic Z, Markovic S, Antic J, et al. Clinical and pathological tools for identifying microsatellite instability in colorectal cancer. Croat Med J. 2012 Aug;53(4):328-35. PMID: 22911525. EXC4

742. Krol LC, t Hart NA, Methorst N, et al. Concordance in KRAS and BRAF mutations in endoscopic biopsy samples and resection specimens of colorectal adenocarcinoma. Eur J Cancer. 2012 May;48(7):1108-15. PMID: 22446020. EXC6

743. Kruger S, Mess F, Bohle A, et al. Numerical aberrations of chromosome 17 and the 9p21 locus are independent predictors of tumor recurrence in non-invasive transitional cell carcinoma of the urinary bladder. Int J Oncol. 2003 Jul;23(1):41-8. PMID: 12792774. EXC6

744. Kruhoffer M, Jensen JL, Laiho P, et al. Gene expression signatures for colorectal cancer microsatellite status and HNPCC. Br J Cancer. 2005 Jun 20;92(12):2240-8. PMID: 15956967. EXC5

745. Kruschewski M, Noske A, Haier J, et al. Is reduced expression of mismatch repair genes MLH1 and MSH2 in patients with sporadic colorectal cancer related to their prognosis? Clin Exp Metastasis. 2002;19(1):71-7. PMID: 11918085. EXC5

746. Kuebler JP, Colangelo L, O'Connell MJ, et al. Severe enteropathy among patients with stage II/III colon cancer treated on a randomized trial of bolus 5-fluorouracil/leucovorin plus or minus oxaliplatin: a prospective analysis. Cancer. 2007 Nov 1;110(9):1945-50. PMID: 17853393. EXC6

747. Kumar K, Brim H, Giardiello F, et al. Distinct BRAF (V600E) and KRAS mutations in high microsatellite instability sporadic colorectal cancer in African Americans. Clin Cancer Res. 2009 Feb 15;15(4):1155-61. PMID: 19190129. EXC6

748. Kumar S, Chang EY, Frankhouse J, et al. Combination of microsatellite instability and lymphocytic infiltrate as a prognostic indicator for adjuvant therapy in colon cancer. Arch Surg. 2009 Sep;144(9):835-40. PMID: 19797108. EXC5

749. Kunz G. Use of a genomic test (MammaPrint) in daily clinical practice to assist in risk stratification of young breast cancer patients. Arch Gynecol Obstet. 2011 Mar;283(3):597-602. PMID: 20383789. EXC5

750. Kuo CH, Lin SM, Lee KY, et al. Subsequent chemotherapy improves survival outcome in advanced non-small-cell lung cancer with acquired tyrosine kinase inhibitor resistance. Clin Lung Cancer. 2010 Jan;11(1):51-6. PMID: 20085868. EXC6

751. Kuo YW, Wu SG, Ho CC, et al. Good response to gefitinib in lung adenocarcinoma harboring coexisting EML4-ALK fusion gene and EGFR mutation. J Thorac Oncol. 2010;5(12):2039-40. EXC2

752. Kuramochi H, Nakajima G, Kaneko Y, et al. Amphiregulin and Epiregulin mRNA expression in primary colorectal cancer and corresponding liver metastases. BMC Cancer. 2012;12:88. PMID: 22409860. EXC6

753. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med. 2010 Oct 28;363(18):1693-703. PMID: 20979469. EXC6

754. Kwon MJ, Lee SE, Kang SY, et al. Frequency of KRAS, BRAF, and PIK3CA mutations in advanced colorectal cancers: Comparison of peptide nucleic acid-mediated PCR clamping and direct sequencing in formalin-fixed, paraffin-embedded tissue. Pathol Res Pract. 2011 Dec 15;207(12):762-8. PMID: 22070922. EXC3

755. . Cost-utility of the 21-gene breast cancer assay (Oncotype DX®) in the Irish healthcare setting. San Antonio Breast Cancer Symposium; 2011 December; San Antonio, TX. EXC6

756. Lacouture ME, Basti S, Patel J, et al. The SERIES clinic: An interdisciplinary approach to the management of toxicities of EGFR inhibitors. Journal of Supportive Oncology. 2006;4(5):236-8. EXC2

757. Laghi L, Bianchi P, Delconte G, et al. MSH3 protein expression and nodal status in MLH1-deficient colorectal cancers. Clin Cancer Res. 2012 Jun 1;18(11):3142-53. PMID: 22496206. EXC4

758. Laghi L, Bianchi P, Miranda E, et al. CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study. Lancet Oncol. 2009 Sep;10(9):877-84. PMID: 19656725. EXC5

759. Laiho P, Launonen V, Lahermo P, et al. Low-level microsatellite instability in most colorectal carcinomas. Cancer Res. 2002 Feb 15;62(4):1166-70. PMID: 11861399. EXC4

760. Lamberti C, Kruse R, Ruelfs C, et al. Microsatellite instability-a useful diagnostic tool to select patients at high risk for hereditary non-polyposis colorectal cancer: a study in different groups of patients with colorectal cancer. Gut. 1999 Jun;44(6):839-43. PMID: 10323887. EXC4

761. Lamberti C, Lundin S, Bogdanow M, et al. Microsatellite instability did not predict individual survival of unselected patients with colorectal cancer. Int J Colorectal Dis. 2007 Feb;22(2):145-52. PMID: 16724208. EXC4

762. Lancashire LJ, Powe DG, Reis-Filho JS, et al. A validated gene expression profile for detecting clinical outcome in breast cancer using artificial neural networks. Breast Cancer Res Treat. 2010 Feb;120(1):83-93. PMID: 19347577. EXC4

763. Lanza G, Gafa R, Maestri I, et al. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol. 2002 Jul;15(7):741-9. PMID: 12118112. EXC6

764. Lanza G, Gafa R, Santini A, et al. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. J Clin Oncol. 2006 May 20;24(15):2359-67. PMID: 16710035. EXC4

765. Larsen FO, Pfeiffer P, Nielsen D, et al. Bevacizumab in combination with cetuximab and irinotecan after failure of cetuximab and irinotecan in patients with metastatic colorectal cancer. Acta Oncol. 2011 May;50(4):574-7. PMID: 21529301. EXC5

766. Lastella P, Patruno M, Forte G, et al. Identification and surveillance of 19 Lynch syndrome families in southern Italy: report of six novel germline mutations and a common founder mutation. Fam Cancer. 2011 Jun;10(2):285-95. PMID: 21286823. EXC3

767. Laszlo L. Predictive and prognostic factors in the complex treatment of patients with colorectal cancer. Magy Onkol. 2010 Dec;54(4):383-94. PMID: 21163770. EXC5

768. Laudadio J, Keane TE, Reeves HM, et al. Fluorescence in situ hybridization for detecting transitional cell carcinoma: implications for clinical practice. BJU Int. 2005 Dec;96(9):1280-5. PMID: 16287445. EXC5

769. Lawes DA, Pearson T, Sengupta S, et al. Is MSI-H of value in predicting the development of metachronous colorectal cancer? Eur J Cancer. 2006 Mar;42(4):473-6. PMID: 16427780. EXC4

770. LeCaer H, Greillier L, Corre R, et al. A multicenter phase II randomized trial of gemcitabine followed by erlotinib at progression, versus the reverse sequence, in vulnerable elderly patients with advanced non small-cell lung cancer selected with a comprehensive geriatric assessment (the GFPC 0505 study). Lung Cancer. 2012 Jul;77(1):97-103. PMID: 22405570. EXC4

771. Lecomte T, Berger A, Zinzindohoue F, et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. Int J Cancer. 2002 Aug 10;100(5):542-8. PMID: 12124803. EXC5

772. Lee DH, Lee GK, Kong SY, et al. Epidermal growth factor receptor status in anaplastic thyroid carcinoma. J Clin Pathol. 2007;60(8):881-4. EXC3

773. Lee HL, Chung TS, Ting LL, et al. EGFR mutations are associated with favorable intracranial response and progression-free survival following brain irradiation in non-small cell lung cancer patients with brain metastases. Radiation Oncology. 2012;7(1)PMID: 2013053048 FULL TEXT LINK http://dx.doi.org/10.1186/1748-717X-7-181. EXC3

774. Lee HY, Ahn HK, Jeong JY, et al. Favorable clinical outcomes of pemetrexed treatment in anaplastic lymphoma kinase positive non-small-cell lung cancer. Lung Cancer. 2013 Jan;79(1):40-5. PMID: 23103072. EXC6

775. Lee JJ, Shen J. Is the Oncotype DX assay necessary in strongly estrogen receptor-positive breast cancers? Am Surg. 2011 Oct;77(10):1364-7. PMID: 22127090. EXC5

776. Lee JK, Park HS, Kim DW, et al. Comparative analyses of overall survival in patients with anaplastic lymphoma kinase-positive and matched wild-type advanced nonsmall cell lung cancer. Cancer. 2012 Jul 15;118(14):3579-86. PMID: 22086654. EXC3

777. Lee JS, Hirsh V, Park K, et al. Vandetanib Versus placebo in patients with advanced non-small-cell lung cancer after prior therapy with an epidermal growth factor receptor tyrosine kinase inhibitor: a randomized, double-blind phase III trial (ZEPHYR). J Clin Oncol. 2012 Apr 1;30(10):1114-21. PMID: 22370318. EXC5

778. Lee JW, Soung YH, Kim SY, et al. ERBB2 kinase domain mutation in the lung squamous cell carcinoma. Cancer Lett. 2006 Jun 8;237(1):89-94. PMID: 16029927. EXC4

779. Lee KH, Lee JS, Nam JH, et al. Promoter methylation status of hMLH1, hMSH2, and MGMT genes in colorectal cancer associated with adenoma-carcinoma sequence. Langenbecks Arch Surg. 2011 Oct;396(7):1017-26. PMID: 21706233. EXC4

780. Lee S, Cho NY, Choi M, et al. Clinicopathological features of CpG island methylator phenotype-positive colorectal cancer and its adverse prognosis in relation to KRAS/BRAF mutation. Pathol Int. 2008 Feb;58(2):104-13. PMID: 18199160. EXC5

781. Lee S, Kim Y, Sun JM, et al. Molecular profiles of EGFR, K-ras, c-met, and FGFR in pulmonary pleomorphic carcinoma, a rare lung malignancy. J Cancer Res Clin Oncol. 2011 Aug;137(8):1203-11. PMID: 21626008. EXC6

782. Lee Y, Lee HJ, Kim YT, et al. Imaging characteristics of stage I non-small cell lung cancer on CT and FDG-PET: relationship with epidermal growth factor receptor protein expression status and survival. Korean J Radiol. 2013 Mar-Apr;14(2):375-83. PMID: 23483676. EXC4

783. Lee Y, Shim HS, Park MS, et al. High EGFR gene copy number and skin rash as predictive markers for EGFR tyrosine kinase inhibitors in patients with advanced squamous cell lung carcinoma. Clin Cancer Res. 2012 Mar 15;18(6):1760-8. PMID: 22271877. EXC4

784. Lee YJ, Park IK, Park MS, et al. Activating mutations within the EGFR kinase domain: a molecular predictor of disease-free survival in resected pulmonary adenocarcinoma. J Cancer Res Clin Oncol. 2009 Dec;135(12):1647-54. PMID: 19517135. EXC6

785. Lee YJ, Shim HS, Kang YA, et al. Dose effect of cigarette smoking on frequency and spectrum of epidermal growth factor receptor gene mutations in Korean patients with non-small cell lung cancer. J Cancer Res Clin Oncol. 2010 Dec;136(12):1937-44. PMID: 20237940. EXC4

786. Leidner RS, Fu P, Clifford B, et al. Genetic abnormalities of the EGFR pathway in African American Patients with non-small-cell lung cancer. J Clin Oncol. 2009 Nov 20;27(33):5620-6. PMID: 19786660. EXC6

787. Leite SM, Gomes KB, Pardini VC, et al. Assessment of microsatellite instability in colorectal cancer patients from Brazil. Mol Biol Rep. 2010 Jan;37(1):375-80. PMID: 19784864. EXC4

788. Leung WK, To KF, Man EP, et al. Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal cancer. Am J Gastroenterol. 2005 Oct;100(10):2274-9. PMID: 16181380. EXC6

789. Levallet G, Bergot E, Antoine M, et al. High TUBB3 expression, an independent prognostic marker in patients with early non-small cell lung cancer treated by preoperative chemotherapy, is regulated by K-ras signaling pathway. Molecul Cancer Therapeut. 2012;11(5):1203-13. EXC4

790. Levine AJ, Win AK, Buchanan DD, et al. Cancer risks for the relatives of colorectal cancer cases with a methylated MLH1 promoter region: data from the Colorectal Cancer Family Registry. Cancer Prev Res (Phila). 2012 Feb;5(2):328-35. PMID: 22144422. EXC6

791. Levy M, Benesova L, Lipska L, et al. Utility of cell-free tumour DNA for post-surgical follow-up of colorectal cancer patients. Anticancer Res. 2012 May;32(5):1621-6. PMID: 22593440. EXC5

792. Li AR, Chitale D, Riely GJ, et al. Clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. J Molec Diagnost. 2008;10(3):242-8. EXC6

793. Li BS, Wang XY, Xu AG, et al. High-resolution melting assay (HRMA) is a simple and sensitive stool-based DNA Test for the detection of mutations in colorectal neoplasms. Clin Colorectal Cancer. 2012 Dec;11(4):280-90. PMID: 22609129. EXC4

794. Li C, Fang R, Sun Y, et al. Spectrum of oncogenic driver mutations in lung adenocarcinomas from East Asian never smokers. PLoS One. 2011;6(11):e28204. PMID: 22140546. EXC6

795. Li C, Sun Y, Fang R, et al. Lung adenocarcinomas with HER2-activating mutations are associated with distinct clinical features and HER2/EGFR copy number gains. J Thorac Oncol. 2012 Jan;7(1):85-9. PMID: 22071781. EXC6

796. Li C, Sun Y, Fang Z, et al. Comprehensive analysis of epidermal growth factor receptor gene status in lung adenocarcinoma. J Thorac Oncol. 2011 Jun;6(6):1016-21. PMID: 21532505. EXC6

797. Li F, Liu Y, Chen H, et al. EGFR and COX-2 protein expression in non-small cell lung cancer and the correlation with clinical features. J Exp Clin Cancer Res. 2011;30(1). EXC4

798. Li FH, Shen L, Li ZH, et al. Impact of KRAS mutation and PTEN expression on cetuximab-treated colorectal cancer. World J Gastroenterol. 2010 Dec 14;16(46):5881-8. PMID: 21155011. EXC3

799. Li H, Pan Y, Li Y, et al. Frequency of well-identified oncogenic driver mutations in lung adenocarcinoma of smokers varies with histological subtypes and graduated smoking dose. Lung Cancer. 2013 Jan;79(1):8-13. PMID: 23098378. EXC6

800. Li M, Liu L, Liu Z, et al. The status of KRAS mutations in patients with non-small cell lung cancers from mainland China. Oncol Rep. 2009 Nov;22(5):1013-20. PMID: 19787214. EXC4

801. Li M, Zhang Q, Liu L, et al. The different clinical significance of EGFR mutations in exon 19 and 21 in non-small cell lung cancer patients of China. Neoplasma. 2011;58(1):74-81. PMID: 21067269. EXC6

802. Li P, Fang YJ, Li F, et al. ERCC1, defective mismatch repair status as predictive biomarkers of survival for stage III colon cancer patients receiving oxaliplatin-based adjuvant chemotherapy. Br J Cancer. 2013 Apr 2;108(6):1238-44. PMID: 23481186. EXC3

803. Li YH, Wang F, Shen L, et al. EGFR fluorescence in situ hybridization pattern of chromosome 7 disomy predicts resistance to cetuximab in KRAS wild-type metastatic colorectal cancer patients. Clin Cancer Res. 2011 Jan 15;17(2):382-90. PMID: 20884623. EXC4

804. Liang H, Brufsky A, Lembersky B, et al. A retrospective analysis of the impact of oncotype DX low recurrence score results on treatment decisions in a single academic breast cancer center. 30th Annual San Antonio Breast Cancer Symposium; 2007 December 13-16, 2007; San Antonio, TX. Breast Cancer Research and Treatment; 106. pp. 13-6. EXC2

805. Liang JT, Huang KC, Lai HS, et al. High-frequency microsatellite instability predicts better chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV sporadic colorectal cancer after palliative bowel resection. Int J Cancer. 2002 Oct 20;101(6):519-25. PMID: 12237891. EXC5

806. Liang Z, Zhang J, Zeng X, et al. Relationship between EGFR expression, copy number and mutation in lung adenocarcinomas. BMC Cancer. 2010;10:376. PMID: 20637128. EXC6

807. Liao W, Liao Y, Zhou JX, et al. Gene mutations in epidermal growth factor receptor signaling network and their association with survival in Chinese patients with metastatic colorectal cancers. Anat Rec (Hoboken). 2010 Sep;293(9):1506-11. PMID: 20652941. EXC5

808. Licar A, Cerkovnik P, Ocvirk J, et al. KRAS mutations in Slovene patients with colorectal cancer: frequency, distribution and correlation with the response to treatment. Int J Oncol. 2010 May;36(5):1137-44. PMID: 20372787. EXC3

809. Lievre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol. 2008 Jan 20;26(3):374-9. PMID: 18202412. EXC5

810. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006 Apr 15;66(8):3992-5. PMID: 16618717. EXC3

811. Lim EH, Zhang SL, Li JL, et al. Using whole genome amplification (WGA) of low-volume biopsies to assess the prognostic role of EGFR, KRAS, p53, and CMET mutations in advanced-stage non-small cell lung cancer (NSCLC). J Thorac Oncol. 2009 Jan;4(1):12-21. PMID: 19096301. EXC3

812. Lim KH, Huang MJ, Liu HC, et al. Lack of prognostic value of EGFR mutations in primary resected non-small cell lung cancer. Med Oncol. 2007;24(4):388-93. PMID: 17917087. EXC6

813. Lim SB, Jeong SY, Lee MR, et al. Prognostic significance of microsatellite instability in sporadic colorectal cancer. Int J Colorectal Dis. 2004 Nov;19(6):533-7. PMID: 15175889. EXC4

814. Lin AY, Buckley NS, Lu AT, et al. Effect of KRAS mutational status in advanced colorectal cancer on the outcomes of anti-epidermal growth factor receptor monoclonal antibody therapy: a systematic review and meta-analysis (Provisional abstract). Clinical Colorectal Cancer. 2011(1):63-9. PMID: DARE-12011004577. EXC2

815. Lin CC, Hsu HH, Sun CT, et al. Chemotherapy response in East Asian non-small cell lung cancer patients harboring wild-type or activating mutation of epidermal growth factor receptors. J Thorac Oncol. 2010 Sep;5(9):1424-9. PMID: 20631634. EXC6

816. Lin CH, Lin JK, Chang SC, et al. Molecular profile and copy number analysis of sporadic colorectal cancer in Taiwan. J Biomed Sci. 2011;18:36. PMID: 21645411. EXC6

817. Lin G, Zheng XW, Li C, et al. KRAS mutation and NF-(kappa)B activation indicates tolerance of chemotherapy and poor prognosis in colorectal cancer. Dig Dis Sci. 2012;57(9):2325-33. EXC4

818. Lin JK, Lin AJ, Lin CC, et al. The status of EGFR-associated genes could predict the outcome and tumor response of chemo-refractory metastatic colorectal patients using cetuximab and chemotherapy. J Surg Oncol. 2011 Nov 1;104(6):661-6. PMID: 21671463. EXC3

819. Lind JS, Dingemans AM, Groen HJ, et al. A multicenter phase II study of erlotinib and sorafenib in chemotherapy-naive patients with advanced non-small cell lung cancer. Clin Cancer Res. 2010 Jun 1;16(11):3078-87. PMID: 20395213. EXC6

820. Lipton LR, Johnson V, Cummings C, et al. Refining the Amsterdam Criteria and Bethesda Guidelines: testing algorithms for the prediction of mismatch repair mutation status in the familial cancer clinic. J Clin Oncol. 2004 Dec 15;22(24):4934-43. PMID: 15611508. EXC3

821. Liu G, Cheng D, Ding K, et al. Pharmacogenetic analysis of BR.21, a placebo-controlled randomized phase III clinical trial of erlotinib in advanced non-small cell lung cancer. J Thorac Oncol. 2012 Feb;7(2):316-22. PMID: 22237259. EXC4

822. Liu G, Gurubhagavatula S, Zhou W, et al. Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib. Pharmacogenomics J. 2008 Apr;8(2):129-38. PMID: 17375033. EXC4

823. Liu T, Wahlberg S, Burek E, et al. Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer. Genes Chromosomes Cancer. 2000 Jan;27(1):17-25. PMID: 10564582. EXC4

824. Liu X, Goldblum JR, Zhao Z, et al. Distinct clinicohistologic features of inflammatory bowel disease-associated colorectal adenocarcinoma: in comparison with sporadic microsatellite-stable and Lynch syndrome-related colorectal adenocarcinoma. Am J Surg Pathol. 2012 Aug;36(8):1228-33. PMID: 22790862. EXC4

825. Liu Y, Liu B, Li XY, et al. A comparison of ARMS and direct sequencing for EGFR mutation analysis and Tyrosine Kinase Inhibitors treatment prediction in body fluid samples of Non-Small-Cell Lung Cancer patients. J Exp Clin Cancer Res. 2011;30(1). EXC6

826. Liu Y, Xia Q, Jia Y, et al. Significance of differential expression of thymidylate synthase in normal and primary tumor tissues from patients with colorectal cancer. J Hematol Oncol. 2011;4:33. PMID: 21824439. EXC5

827. Liu Y, Xu ML, Zhong HH, et al. EGFR mutations are more frequent in well-differentiated than in poor-differentiated lung adenocarcinomas. Pathology and Oncology Research. 2008;14(4):373-9. EXC7

828. London S. Novel drug active against ALK gene rearrangements. Oncology Report. 2010(MARCH-APRIL):37-8. EXC3

829. Lopez-Crapez E, Mineur L, Emptas H, et al. KRAS status analysis and anti-EGFR therapies: is comprehensiveness a biologist's fancy or a clinical necessity? Br J Cancer. 2010 Mar 16;102(6):1074-5; author reply 6-7. PMID: 20160721. EXC2

830. Lotan Y, Bensalah K, Ruddell T, et al. Prospective evaluation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. J Urol. 2008 Jun;179(6):2164-9. PMID: 18423745. EXC5

831. Loughrey MB, Waring PM, Tan A, et al. Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer. Fam Cancer. 2007;6(3):301-10. PMID: 17453358. EXC3

832. Loukola A, de la Chapelle A, Aaltonen LA. Strategies for screening for hereditary non-polyposis colorectal cancer. J Med Genet. 1999 Nov;36(11):819-22. PMID: 10544224. EXC4

833. Loukola A, Eklin K, Laiho P, et al. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). Cancer Res. 2001 Jun 1;61(11):4545-9. PMID: 11389088. EXC5

834. Loukola A, Salovaara R, Kristo P, et al. Microsatellite instability in adenomas as a marker for hereditary nonpolyposis colorectal cancer. Am J Pathol. 1999 Dec;155(6):1849-53. PMID: 10595914. EXC4

835. Loupakis F, Pollina L, Stasi I, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol. 2009 Jun 1;27(16):2622-9. PMID: 19398573. EXC3

836. Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer. 2009 Aug 18;101(4):715-21. PMID: 19603018. EXC5

837. Lovig T, Thorstensen L, Hofstad B, et al. Genetic and protein markers related to in situ growth and multiplicity in small sporadic colorectal adenomas. Scand J Gastroenterol. 2003 Mar;38(3):298-306. PMID: 12737446. EXC6

838. Lowery MA, Gallagher DJ, Capanu M, et al. Clinical outcomes in patients age 40 or younger at diagnosis of synchronous metastatic colorectal cancer: A 20-year experience at Memorial Sloan-Kettering Cancer Center. J Clin Oncol. 2011;29(15). EXC6

839. Lozano MD, Zulueta JJ, Echeveste JI, et al. Assessment of epidermal growth factor receptor and K-ras mutation status in cytological stained smears of non-small cell lung cancer patients: correlation with clinical outcomes. Oncologist. 2011;16(6):877-85. PMID: 21572125. EXC5

840. Lu HY, Mao WM, Cheng QY, et al. Mutation status of epidermal growth factor receptor and clinical features of patients with combined small cell lung cancer who received surgical treatment. Oncology Letters. 2012;3(6):1288-92. EXC7

841. Lucci-Cordisco E, Rovella V, Carrara S, et al. Mutations of the 'minor' mismatch repair gene MSH6 in typical and atypical hereditary nonpolyposis colorectal cancer. Fam Cancer. 2001;1(2):93-9. PMID: 14574004. EXC4

842. Ludovini V, Bellezza G, Pistola L, et al. High coexpression of both insulin-like growth factor receptor-1 (IGFR-1) and epidermal growth factor receptor (EGFR) is associated with shorter disease-free survival in resected non-small-cell lung cancer patients. Ann Oncol. 2009 May;20(5):842-9. PMID: 19153117. EXC4

843. Lund MJ, Mosunjac M, Davis KM, et al. 21-Gene recurrence scores: racial differences in testing, scores, treatment, and outcome. Cancer. 2012 Feb 1;118(3):788-96. PMID: 21720988. EXC6

844. Luo D, Huang M, Zhang X, et al. Salvage treatment with erlotinib after gefitinib failure in advanced non-small-cell lung cancer patients with poor performance status: A matched-pair case-control study. Thoracic Cancer. 2012;3(1):27-33. EXC6

845. Luo YH, Wu CH, Wu WS, et al. Association between tumor epidermal growth factor receptor mutation and pulmonary tuberculosis in patients with adenocarcinoma of the lungs. J Thorac Oncol. 2012 Feb;7(2):299-305. PMID: 22173705. EXC6

846. Lyda MH, Noffsinger A, Belli J, et al. Microsatellite instability and K-ras mutations in patients with ulcerative colitis. Hum Pathol. 2000 Jun;31(6):665-71. PMID: 10872658. EXC4

847. Ma ESK, Ng WK, Wong CLP. EGFR gene mutation study in cytology specimens. Acta Cytol. 2012 November-December;56(6):661-8. PMID: 2012718080 MEDLINE PMID 23207445 (http://www.ncbi.nlm.nih.gov/pubmed/23207445) FULL TEXT LINK http://dx.doi.org/10.1159/000343606. EXC6

848. Ma F, Sun T, Shi Y, et al. Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with Gefitinib. Lung Cancer. 2009 Oct;66(1):114-9. PMID: 19201048. EXC3

849. Macedo MP, Andrade Lde B, Coudry R, et al. Multiple mutations in the Kras gene in colorectal cancer: review of the literature with two case reports. Int J Colorectal Dis. 2011 Oct;26(10):1241-8. PMID: 21603900. EXC7

850. Mack PC, Holland WS, Burich RA, et al. EGFR mutations detected in plasma are associated with patient outcomes in erlotinib plus docetaxel-treated non-small cell lung cancer. J Thorac Oncol. 2009 Dec;4(12):1466-72. PMID: 19884861. EXC4

851. . The impact of chemotherapeutic regiments on the cost-utility analysis of Onctype DX® assay. The European Breast Cancer Conference; 2012 March; Vienna, Austria. EXC5

852. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 2010 Jun 24;362(25):2380-8. PMID: 20573926. EXC4

853. Maffezzini M, Campodonico F, Capponi G, et al. Prognostic significance of fluorescent in situ hybridisation in the follow-up of non-muscle-invasive bladder cancer. Anticancer Res. 2010 Nov;30(11):4761-5. PMID: 21115937. EXC7

854. Maffezzini M, Capponi G, Casazza S, et al. The UroVysion F.I.S.H. test compared to standard cytology for surveillance of non-muscle invasive bladder cancer. Arch Ital Urol Androl. 2008 Dec;80(4):127-31. PMID: 19235427. EXC7

855. Magnin S, Viel E, Baraquin A, et al. A multiplex SNaPshot assay as a rapid method for detecting KRAS and BRAF mutations in advanced colorectal cancers. J Molec Diagnost. 2011;13(5):485-92. EXC4

856. Mak RH, Digumarthy SR, Muzikansky A, et al. Role of 18F-fluorodeoxyglucose positron emission tomography in predicting epidermal growth factor receptor mutations in non-small cell lung cancer. Oncologist. 2011;16(3):319-26. PMID: 21339258. EXC8

857. Malapelle U, Bellevicine C, Salatiello M, et al. Sanger sequencing in routine KRAS testing: A review of 1720 cases from a pathologist's perspective. J Clin Pathol. 2012;65(10):940-4. EXC6

858. Malapelle U, Carlomagno C, Salatiello M, et al. KRAS mutation detection by high-resolution melting analysis significantly predicts clinical benefit of cetuximab in metastatic colorectal cancer. Br J Cancer. 2012 Aug 7;107(4):626-31. PMID: 22805329. EXC6

859. Malesci A, Laghi L, Bianchi P, et al. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. Clin Cancer Res. 2007 Jul 1;13(13):3831-9. PMID: 17606714. EXC4

860. Malo TL, Lipkus I, Wilson T, et al. Treatment Choices Based on OncotypeDx in the Breast Oncology Care Setting. J Cancer Epidemiol. 2012;2012:941495. PMID: 22927848. Exc-6

861. Mamounas E, Budd GT, Miller K. Incorporating the Oncotype DX breast cancer assay into community practice: An expert Q & A and case study sampling. Clin Adv Hematol Oncol. 2008 Feb;6(2):s1-s8. PMID: 18516819. EXC2

862. Manders P, Spruijt L, Kets CM, et al. Young age and a positive family history of colorectal cancer are complementary selection criteria for the identification of Lynch syndrome. Eur J Cancer. 2011 Jun;47(9):1407-13. PMID: 21273057. EXC3

863. Manne SL, Chung DC, Weinberg DS, et al. Knowledge and attitudes about microsatellite instability testing among high-risk individuals diagnosed with colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2007 Oct;16(10):2110-7. PMID: 17932359. EXC6

864. Mao C, Zhou J, Yang Z, et al. KRAS, BRAF and PIK3CA mutations and the loss of PTEN expression in Chinese patients with colorectal cancer. PLoS One. 2012;7(5):e36653. PMID: 22586484. EXC6

865. Marchetti A, Felicioni L, Malatesta S, et al. Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. J Clin Oncol. 2011;29(26):3574-9. EXC4

866. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol. 2005 Feb 1;23(4):857-65. PMID: 15681531. EXC6

867. Marchetti A, Milella M, Felicioni L, et al. Clinical implications of KRAS mutations in lung cancer patients treated with tyrosine kinase inhibitors: an important role for mutations in minor clones. Neoplasia. 2009 Oct;11(10):1084-92. PMID: 19794967. EXC3

868. Marchionni L, Wilson RF, Marinopoulos SS, et al. Impact of gene expression profiling tests on breast cancer outcomes (Structured abstract). Health Technology Assessment Database. 2008(3):230. PMID: HTA-32008000072. EXC1

869. Marcus VA, Madlensky L, Gryfe R, et al. Immunohistochemistry for hMLH1 and hMSH2: a practical test for DNA mismatch repair-deficient tumors. Am J Surg Pathol. 1999 Oct;23(10):1248-55. PMID: 10524526. EXC4

870. Mariani P, Lae M, Degeorges A, et al. Concordant analysis of KRAS status in primary colon carcinoma and matched metastasis. Anticancer Res. 2010 Oct;30(10):4229-35. PMID: 21036746. EXC6

871. Marisa L, de Reynies A, Duval A, et al. Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. PLoS Med. 2013;10(5):e1001453. PMID: 23700391. EXC4

872. Markopoulos C, Xepapadakis G, Venizelos V, et al. Clinical experience of using Oncotype DX as an additional treatment decision tool in early breast cancer - a retrospective analysis from 5 Greek institutions. Eur J Surg Oncol. 2012 May;38(5):413-9. PMID: 22425282. EXC6

873. Markovic S, Antic J, Dragicevic N, et al. High-frequency microsatellite instability and BRAF mutation (V600E) in unselected Serbian patients with colorectal cancer. J Mol Histol. 2012 Apr;43(2):137-43. PMID: 22210186. EXC7

874. Marks JL, Broderick S, Zhou Q, et al. Prognostic and therapeutic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. J Thorac Oncol. 2008 Feb;3(2):111-6. PMID: 18303429. EXC6

875. Martin V, Landi L, Molinari F, et al. HER2 gene copy number status may influence clinical efficacy to anti-EGFR monoclonal antibodies in metastatic colorectal cancer patients. Br J Cancer. 2013 Feb 19;108(3):668-75. PMID: 23348520. EXC3

876. Martinez-Lopez E, Abad A, Font A, et al. Allelic loss on chromosome 18q as a prognostic marker in stage II colorectal cancer. Gastroenterology. 1998 Jun;114(6):1180-7. PMID: 9609754. EXC4

877. Martinez-Navarro EM, Rebollo J, Gonzalez-Manzano R, et al. Epidermal growth factor receptor (EGFR) mutations in a series of non-small-cell lung cancer (NSCLC) patients and response rate to EGFR-specific tyrosine kinase inhibitors (TKIs). Clin Transl Oncol. 2011 Nov;13(11):812-8. PMID: 22082647. EXC3

878. Masago K, Fujita S, Mio T, et al. Accuracy of epidermal growth factor receptor gene mutation analysis by direct sequencing method based on small biopsy specimens from patients with non-small cell lung cancer: analysis of results in 19 patients. Int J Clin Oncol. 2008 Oct;13(5):442-6. PMID: 18946755. EXC5

879. Masago K, Fujita S, Togashi Y, et al. Clinical significance of epidermal growth factor receptor mutations and insulin-like growth factor 1 and its binding protein 3 in advanced non-squamous non-small cell lung cancer. Oncol Rep. 2011 Oct;26(4):795-803. PMID: 21805046. EXC6

880. Masago K, Fujita S, Togashi Y, et al. Clinicopathologic factors affecting the progression-free survival of patients with advanced non-small-cell lung cancer after gefitinib therapy. Clin Lung Cancer. 2011 Jan;12(1):56-61. PMID: 21273181. EXC6

881. Mascaux C, Wynes MW, Kato Y, et al. EGFR protein expression in non-small cell lung cancer predicts response to an EGFR tyrosine kinase inhibitor--a novel antibody for immunohistochemistry or AQUA technology. Clin Cancer Res. 2011 Dec 15;17(24):7796-807. PMID: 21994417. EXC4

882. Massa MJ, Iniesta P, Gonzalez-Quevedo R, et al. Differential prognosis of replication error phenotype and loss of heterozygosity in sporadic colorectal cancer. Eur J Cancer. 1999 Nov;35(12):1676-82. PMID: 10674012. EXC4

883. Masubuchi S, Konishi F, Togashi K, et al. The significance of microsatellite instability in predicting the development of metachronous multiple colorectal carcinomas in patients with nonfamilial colorectal carcinoma. Cancer. 1999 May 1;85(9):1917-24. PMID: 10223230. EXC4

884. Masuda H, Abe Y, Takayama T. Microsatellite instability in poorly differentiated colorectal adenocarcinoma, particularly in relation to two subtypes. Hepatogastroenterology. 2005 Jan-Feb;52(61):82-5. PMID: 15783000. EXC4

885. Matsumoto S, Iwakawa R, Kohno T, et al. Frequent EGFR mutations in noninvasive bronchioloalveolar carcinoma. Int J Cancer. 2006 May 15;118(10):2498-504. PMID: 16353158. Inc

886. Matsuo K, Hiraki A, Ito H, et al. Soy consumption reduces the risk of non-small-cell lung cancers with epidermal growth factor receptor mutations among Japanese. Cancer Sci. 2008 Jun;99(6):1202-8. PMID: 18429954. EXC8

887. Matsuo K, Ito H, Yatabe Y, et al. Risk factors differ for non-small-cell lung cancers with and without EGFR mutation: assessment of smoking and sex by a case-control study in Japanese. Cancer Sci. 2007 Jan;98(1):96-101. PMID: 17054433. EXC8

888. Matsuoka H, Kurata T, Okamoto I, et al. Clinical response to crizotinib retreatment after acquisition of drug resistance. J Clin Oncol. 2013 Jul 1;31(19):e322-3. PMID: 23715571. EXC7

889. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet. 2011 Jun 18;377(9783):2103-14. PMID: 21641636. EXC6

890. Maus MKH, Stephens C, Zeger G, et al. Identification of novel variant of EML4-ALK fusion gene in NSCLC: Potential benefits of the RT-PCR method. International Journal of Biomedical Science. 2012;8(1):1-6. EXC6

891. Mazieres J, Peters S, Lepage B, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol. 2013 Jun 1;31(16):1997-2003. PMID: 23610105. EXC4

892. Mazzoni F, Rotella V, Pratesi N, et al. From clinical trials to clinical practice: predictors of response to erlotinib in advanced non-small cell lung cancer patients pretreated with chemotherapy. Tumori. 2011 Mar-Apr;97(2):160-5. PMID: 21617709. EXC3

893. McGregor DK, Wu TT, Rashid A, et al. Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. Am J Surg Pathol. 2004 Jun;28(6):712-8. PMID: 15166663. EXC6

894. McLeer-Florin A, Moro-Sibilot D, Melis A, et al. Dual IHC and FISH testing for ALK gene rearrangement in lung adenocarcinomas in a routine practice: a French study. J Thorac Oncol. 2012 Feb;7(2):348-54. PMID: 22071784. EXC6

895. McMillen E, Ye F, Li G, et al. Epidermal growth factor receptor (EGFR) mutation and p-EGFR expression in resected non-small cell lung cancer. Exp Lung Res. 2010 Nov;36(9):531-7. PMID: 20939760. EXC6

896. McPhillips M, Meldrum CJ, Creegan R, et al. Deletion mutations in an Australian series of HNPCC patients. Hereditary Cancer in Clinical Practice. 2005;3(1):43-7. EXC4

897. McShane LM, Cavenagh MM, Lively TG, et al. Criteria for the use of omics-based predictors in clinical trials. Nature. 2013 Oct 17;502(7471):317-20. PMID: 24132288. EXC7

898. Mead LJ, Jenkins MA, Young J, et al. Microsatellite instability markers for identifying early-onset colorectal cancers caused by germ-line mutations in DNA mismatch repair genes. Clin Cancer Res. 2007 May 15;13(10):2865-9. PMID: 17504984. EXC4

899. Mekenkamp LJ, Heesterbeek KJ, Koopman M, et al. Mucinous adenocarcinomas: poor prognosis in metastatic colorectal cancer. Eur J Cancer. 2012 Mar;48(4):501-9. PMID: 22226571. EXC4

900. Melcher R, Hartmann E, Zopf W, et al. LOH and copy neutral LOH (cnLOH) act as alternative mechanism in sporadic colorectal cancers with chromosomal and microsatellite instability. Carcinogenesis. 2011 Apr;32(4):636-42. PMID: 21297112. EXC4

901. Melloni G, Doglioni C, Bandiera A, et al. Prognostic factors and analysis of microsatellite instability in resected pulmonary metastases from colorectal carcinoma. Ann Thorac Surg. 2006 Jun;81(6):2008-13. PMID: 16731121. EXC5

902. Mencoboni M, Bergaglio M, Serra M, et al. Maintenance therapy with gefitinib after first-line chemotherapy in patients affected by advanced non-small cell lung cancer. Anticancer Res. 2007 Nov-Dec;27(6C):4425-9. PMID: 18214055. EXC4

903. Meng WJ, Sun XF, Tian C, et al. Microsatellite instability did not predict individual survival in sporadic stage II and III rectal cancer patients. Oncology. 2007;72(1-2):82-8. PMID: 18004081. EXC5

904. Meng WJ, Wang L, Tian C, et al. Novel mutations and sequence variants in exons 3-9 of human T cell factor-4 gene in sporadic rectal cancer patients stratified by microsatellite instability. World J Gastroenterol. 2007 Jul 21;13(27):3747-51. PMID: 17659738. EXC6

905. Meng X, Loo BW, Jr., Ma L, et al. Molecular imaging with 11C-PD153035 PET/CT predicts survival in non-small cell lung cancer treated with EGFR-TKI: a pilot study. J Nucl Med. 2011 Oct;52(10):1573-9. PMID: 21903741. EXC4

906. Mengual L, Marin-Aguilera M, Ribal MJ, et al. Clinical utility of fluorescent in situ hybridization for the surveillance of bladder cancer patients treated with bacillus Calmette-Guerin therapy. Eur Urol. 2007 Sep;52(3):752-9. PMID: 17379395. EXC3

907. Menon AG, Morreau H, Tollenaar RA, et al. Down-regulation of HLA-A expression correlates with a better prognosis in colorectal cancer patients. Lab Invest. 2002 Dec;82(12):1725-33. PMID: 12480922. EXC5

908. Merrick DT, Kittelson J, Winterhalder R, et al. Analysis of c-ErbB1/epidermal growth factor receptor and c-ErbB2/HER-2 expression in bronchial dysplasia: evaluation of potential targets for chemoprevention of lung cancer. Clin Cancer Res. 2006 Apr 1;12(7 Pt 1):2281-8. PMID: 16609045. EXC3

909. Messerini L, Ciantelli M, Baglioni S, et al. Prognostic significance of microsatellite instability in sporadic mucinous colorectal cancers. Hum Pathol. 1999 Jun;30(6):629-34. PMID: 10374769. EXC4

910. Messerini L, Vitelli F, De Vitis LR, et al. Microsatellite instability in sporadic mucinous colorectal carcinomas: relationship to clinico-pathological variables. J Pathol. 1997 Aug;182(4):380-4. PMID: 9306957. EXC6

911. Messick CA, Sanchez J, Dejulius KL, et al. Genetic and molecular diversity of colon cancer hepatic metastases. Surgery. 2009 Aug;146(2):227-31. PMID: 19628078. EXC3

912. Metro G, Chiari R, Duranti S, et al. Impact of specific mutant KRAS on clinical outcome of EGFR-TKI-treated advanced non-small cell lung cancer patients with an EGFR wild type genotype. Lung Cancer. 2012;78(1):81-6. EXC3

913. Metro G, Crino L. The LUX-Lung clinical trial program of afatinib for non-small-cell lung cancer. Expert Rev Anticancer Ther. 2011 May;11(5):673-82. PMID: 21554040. EXC6

914. Michael-Robinson JM, Biemer-Huttmann A, Purdie DM, et al. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. Gut. 2001 Mar;48(3):360-6. PMID: 11171826. EXC4

915. Michael-Robinson JM, Pandeya N, Walsh MD, et al. Characterization of tumour-infiltrating lymphocytes and apoptosis in colitis-associated neoplasia: comparison with sporadic colorectal cancer. J Pathol. 2006 Feb;208(3):381-7. PMID: 16315333. EXC4

916. Michael-Robinson JM, Reid LE, Purdie DM, et al. Proliferation, apoptosis, and survival in high-level microsatellite instability sporadic colorectal cancer. Clin Cancer Res. 2001 Aug;7(8):2347-56. PMID: 11489812. EXC4

917. Michalopoulos NV, Saetta AA, Lazaris AC, et al. Microsatellite instability in sporadic and inherited colon adenocarcinomas from Greek patients: correlation with several clinicopathological characteristics. Acta Gastroenterol Belg. 2005 Jul-Sep;68(3):294-301. PMID: 16268414. EXC4

918. Midgley RS, Church D, Kerr DJ. The emergence of 'omics for the management of colorectal cancer. Curr Opin Oncol. 2011 Jul;23(4):410-4. PMID: 21577111. EXC2

919. Miglio U, Mezzapelle R, Paganotti A, et al. Mutation analysis of KRAS in primary colorectal cancer and matched metastases by means of highly sensitivity molecular assay. Pathol Res Pract. 2013 Apr;209(4):233-6. PMID: 23538047. EXC3

920. Mihaylova Z, Ludovini V, Gregorg V, et al. Serum level changes of matrix metalloproteinases 2 and 9, vascular endothelial growth factor and epidermal growth factor receptor during platinum-based chemotherapy in advanced non-small cell lung cancer patients. J BUON. 2007 Jan-Mar;12(1):105-11. PMID: 17436410. EXC6

921. Miladi-Abdennadher I, Abdelmaksoud-Damak R, Ayadi L, et al. Expression of p16INK4a, alone or combined with p53, is predictive of better prognosis in colorectal adenocarcinoma in Tunisian patients. Appl Immunohistochem Mol Morphol. 2011 Dec;19(6):562-8. PMID: 22095233. EXC4

922. Miller VA, Hirsh V, Cadranel J, et al. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol. 2012 May;13(5):528-38. PMID: 22452896. EXC3

923. Miller VA, Riely GJ, Zakowski MF, et al. Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. J Clin Oncol. 2008 Mar 20;26(9):1472-8. PMID: 18349398. EXC3

924. Milne RL, Antoniou AC. Genetic modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. Ann Oncol. 2011 Jan;22 Suppl 1:i11-7. PMID: 21285145. EXC7

925. Milton DT, Azzoli CG, Heelan RT, et al. A phase I/II study of weekly high-dose erlotinib in previously treated patients with nonsmall cell lung cancer. Cancer. 2006 Sep 1;107(5):1034-41. PMID: 16878326. EXC3

926. Mimae T, Tsuta K, Maeshima AM, et al. Cathepsin D as a potential prognostic marker for lung adenocarcinoma. Pathology Research and Practice. 2012;208(9):534-40. EXC4

927. Mina L, Soule SE, Badve S, et al. Predicting response to primary chemotherapy: gene expression profiling of paraffin-embedded core biopsy tissue. Breast Cancer Res Treat. 2007 Jun;103(2):197-208. PMID: 17039265. EXC6

928. Mino-Kenudson M, Mark EJ. Reflex testing for epidermal growth factor receptor mutation and anaplastic lymphoma kinase fluorescence in situ hybridization in non-small cell lung cancer. Arch Pathol Lab Med. 2011 May;135(5):655-64. PMID: 21526964. EXC1

929. Miquel C, Jacob S, Grandjouan S, et al. Frequent alteration of DNA damage signalling and repair pathways in human colorectal cancers with microsatellite instability. Oncogene. 2007 Aug 30;26(40):5919-26. PMID: 17384679. EXC5

930. Mirchev MB, Kahl P, Friedrichs N, et al. DNA methylation in patients with colorectal cancer--correlation with some clinical and morphological features and with local tumour invasion. Folia Med (Plovdiv). 2010 Apr-Jun;52(2):22-30. PMID: 20836393. EXC6

931. Mitchell EP, Piperdi B, Lacouture ME, et al. The efficacy and safety of panitumumab administered concomitantly with FOLFIRI or Irinotecan in second-line therapy for metastatic colorectal cancer: the secondary analysis from STEPP (Skin Toxicity Evaluation Protocol With Panitumumab) by KRAS status. Clin Colorectal Cancer. 2011 Dec;10(4):333-9. PMID: 22000810. EXC4

932. Mitiushkina NV, Iyevleva AG, Poltoratskiy AN, et al. Detection of EGFR mutations and EML4-ALK rearrangements in lung adenocarcinomas using archived cytological slides. Cancer Cytopathol. 2013 Jul;121(7):370-6. PMID: 23408463. EXC4

933. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol. 2010 Feb;11(2):121-8. PMID: 20022809. EXC6

934. Mittmann N, Au HJ, Tu D, et al. Prospective cost-effectiveness analysis of cetuximab in metastatic colorectal cancer: Evaluation of national cancer institute of canada clinical trials group CO.17 Trial. J Natl Cancer Inst. 2009;101(17):1182-92. EXC6

935. Miura N, Nakamura H, Sato R, et al. Clinical usefulness of serum telomerase reverse transcriptase (hTERT) mRNA and epidermal growth factor receptor (EGFR) mRNA as a novel tumor marker for lung cancer. Cancer Sci. 2006 Dec;97(12):1366-73. PMID: 17052260. EXC4

936. Miyazawa H, Tanaka T, Nagai Y, et al. Peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp-based detection test for gefitinib-refractory T790M epidermal growth factor receptor mutation. Cancer Sci. 2008 Mar;99(3):595-600. PMID: 18271876. EXC3

937. Mizota A, Shitara K, Kondo C, et al. Retrospective analysis of cetuximab monotherapy for patients with irinotecan-intolerant metastatic colorectal cancer. Int J Clin Oncol. 2011 Aug;16(4):416-20. PMID: 21437572. EXC5

938. Moatamed NA, Apple SK, Bennett CJ, et al. Exclusion of the uniform tetraploid cells significantly improves specificity of the urine FISH assay. Diagn Cytopathol. 2013 Mar;41(3):218-25. PMID: 21987521. Exc5

939. Modest DP, Jung A, Moosmann N, et al. The influence of KRAS and BRAF mutations on the efficacy of cetuximab-based first-line therapy of metastatic colorectal cancer: an analysis of the AIO KRK-0104-trial. Int J Cancer. 2012 Aug 15;131(4):980-6. PMID: 21960311. EXC3

940. Moghbeli M, Moaven O, Dadkhah E, et al. High frequency of microsatellite instability in sporadic colorectal cancer patients in Iran. Genet Mol Res. 2011;10(4):3520-9. PMID: 22194204. EXC6

941. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009 Sep 3;361(10):947-57. PMID: 19692680. EXC3

942. Molinari F, Felicioni L, Buscarino M, et al. Increased detection sensitivity for KRAS mutations enhances the prediction of anti-EGFR monoclonal antibody resistance in metastatic colorectal cancer. Clin Cancer Res. 2011 Jul 15;17(14):4901-14. PMID: 21632860. EXC6

943. Monaco SE, Nikiforova MN, Cieply K, et al. A comparison of EGFR and KRAS status in primary lung carcinoma and matched metastases. Hum Pathol. 2010 Jan;41(1):94-102. PMID: 19740513. EXC6

944. Montagut C, Iglesias M, Arumi M, et al. Mitogen-activated protein kinase phosphatase-1 (MKP-1) impairs the response to anti-epidermal growth factor receptor (EGFR) antibody cetuximab in metastatic colorectal cancer patients. Br J Cancer. 2010;102(7):1137-44. EXC6

945. Montomoli J, Jacques S, Hamilton D, et al. Retrospective analysis of KRAS status in metastatic colorectal cancer patients: A single-center feasibility study. Clinical and Experimental Gastroenterology. 2012;5(1):167-71. EXC3

946. Mook S, Bonnefoi H, Pruneri G, et al. Daily clinical practice of fresh tumour tissue freezing and gene expression profiling; logistics pilot study preceding the MINDACT trial. Eur J Cancer. 2009 May;45(7):1201-8. PMID: 19232484. EXC6

947. Mook S, Van't Veer LJ, Rutgers EJ, et al. Individualization of therapy using Mammaprint: from development to the MINDACT Trial. Cancer Genomics Proteomics. 2007 May-Jun;4(3):147-55. PMID: 17878518. EXC3

948. Moonen PM, Merkx GF, Peelen P, et al. UroVysion compared with cytology and quantitative cytology in the surveillance of non-muscle-invasive bladder cancer. Eur Urol. 2007 May;51(5):1275-80; discussion 80. PMID: 17084511. EXC6

949. Moore AM, Einhorn LH, Estes D, et al. Gefitinib in patients with chemo-sensitive and chemo-refractory relapsed small cell cancers: a Hoosier Oncology Group phase II trial. Lung Cancer. 2006 Apr;52(1):93-7. PMID: 16488055. EXC4

950. Moosmann N, von Weikersthal LF, Vehling-Kaiser U, et al. Cetuximab plus capecitabine and irinotecan compared with cetuximab plus capecitabine and oxaliplatin as first-line treatment for patients with metastatic colorectal cancer: AIO KRK-0104--a randomized trial of the German AIO CRC study group. J Clin Oncol. 2011 Mar 10;29(8):1050-8. PMID: 21300933. EXC5

951. Moran T, Sequist LV. Timing of epidermal growth factor receptor tyrosine kinase inhibitor therapy in patients with lung cancer with EGFR mutations. J Clin Oncol. 2012;30(27):3330-6. EXC6

952. Morandi L, De Biase D, Visani M, et al. T[20] repeat in the 3'-untranslated region of the MT1X gene: A marker with high sensitivity and specificity to detect microsatellite instability in colorectal cancer. Int J Colorectal Dis. 2012;27(5):647-56. EXC4

953. Mori Y, Selaru FM, Sato F, et al. The impact of microsatellite instability on the molecular phenotype of colorectal tumors. Cancer Res. 2003 Aug 1;63(15):4577-82. PMID: 12907634. EXC4

954. Morifuji M, Hiyama E, Murakami Y, et al. Fluorescent-based BAT-26 analysis for distinct screening of microsatellite instability in colorectal cancers. Int J Oncol. 2003 Apr;22(4):807-13. PMID: 12632072. EXC4

955. Morikawa T, Kuchiba A, Qian ZR, et al. Prognostic significance and molecular associations of tumor growth pattern in colorectal cancer. Ann Surg Oncol. 2012 Jun;19(6):1944-53. PMID: 22189472. EXC4

956. Morikawa T, Kuchiba A, Yamauchi M, et al. Association of CTNNB1 (beta-catenin) alterations, body mass index, and physical activity with survival in patients with colorectal cancer. JAMA. 2011 Apr 27;305(16):1685-94. PMID: 21521850. EXC4

957. Morinaga R, Okamoto I, Fujita Y, et al. Association of epidermal growth factor receptor (EGFR) gene mutations with EGFR amplification in advanced non-small cell lung cancer. Cancer Sci. 2008 Dec;99(12):2455-60. PMID: 18957054. EXC6

958. Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol. 2005 May;6(5):279-86. PMID: 15863375. EXC6

959. Morris PG, Reiner AS, Szenberg OR, et al. Leptomeningeal metastasis from non-small cell lung cancer: survival and the impact of whole brain radiotherapy. J Thorac Oncol. 2012 Feb;7(2):382-5. PMID: 22089116. EXC5

960. Mostert B, Jiang Y, Sieuwerts AM, et al. KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue. Int J Cancer. 2013 Jul;133(1):130-41. PMID: 23233388. EXC6

961. Motoi N, Szoke J, Riely GJ, et al. Lung adenocarcinoma: modification of the 2004 WHO mixed subtype to include the major histologic subtype suggests correlations between papillary and micropapillary adenocarcinoma subtypes, EGFR mutations and gene expression analysis. Am J Surg Pathol. 2008 Jun;32(6):810-27. PMID: 18391747. EXC6

962. Mrkonjic M, Roslin NM, Greenwood CM, et al. Specific variants in the MLH1 gene region may drive DNA methylation, loss of protein expression, and MSI-H colorectal cancer. PLoS One. 2010;5(10):e13314. PMID: 20967208. EXC4

963. Mu XL, Li LY, Zhang XT, et al. Gefitinib-sensitive mutations of the epidermal growth factor receptor tyrosine kinase domain in chinese patients with non-small cell lung cancer. Clin Cancer Res. 2005 Jun 15;11(12):4289-94. PMID: 15958609. EXC6

964. Mueller J, Gazzoli I, Bandipalliam P, et al. Comprehensive molecular analysis of mismatch repair gene defects in suspected Lynch syndrome (hereditary nonpolyposis colorectal cancer) cases. Cancer Res. 2009 Sep 1;69(17):7053-61. PMID: 19690142. EXC5

965. Mukohara T, Engelman JA, Hanna NH, et al. Differential effects of gefitinib and cetuximab on non-small-cell lung cancers bearing epidermal growth factor receptor mutations. J Natl Cancer Inst. 2005;97(16):1185-94. EXC4

966. Mukohara T, Kudoh S, Yamauchi S, et al. Expression of epidermal growth factor receptor (EGFR) and downstream-activated peptides in surgically excised non-small-cell lung cancer (NSCLC). Lung Cancer. 2003 Aug;41(2):123-30. PMID: 12871775. EXC4

967. Müller CI, Schulmann K, Reinacher-Schick A, et al. Predictive and prognostic value of microsatellite instability in patients with advanced colorectal cancer treated with a fluoropyrimidine and oxaliplatin containing first-line chemotherapy. A report of the AIO Colorectal Study Group. Int J Colorectal Dis. 2008(11):1033-9. PMID: CN-00668170. EXC3

968. Mumby PB, Lo SS, Norton J, et al. Prospective multi-center study of the impact of the 21-gene recurrence score assay on patient satisfaction, anxiety arid decisional conflict for adjuvant breast cancer treatment selection. Breast Cancer Res Treat. 2007 Dec;106(1 suppl):S73-S4. PMID: ISI:000251398500200. EXC6

969. Munfus-McCray D, Cui M, Zhang Z, et al. Comparison of EGFR and KRAS mutations in primary and unpaired metastatic lung adenocarcinoma with potential chemotherapy effect. Hum Pathol. 2013 Jul;44(7):1286-92. PMID: 23337026. EXC6

970. Munfus-Mccray D, Harada S, Adams C, et al. EGFR and KRAS mutations in metastatic lung adenocarcinomas. Hum Pathol. 2011;42(10):1447-53. EXC5

971. Murakami H, Tamura T, Takahashi T, et al. Phase I study of continuous afatinib (BIBW 2992) in patients with advanced non-small cell lung cancer after prior chemotherapy/erlotinib/gefitinib (LUX-Lung 4). Cancer Chemother Pharmacol. 2012 Apr;69(4):891-9. PMID: 22071596. EXC6

972. Murata S, Iseki M, Kinjo M, et al. Molecular and immunohistologic analyses cannot reliably solve diagnostic variation of flat intraepithelial lesions of the urinary bladder. Am J Clin Pathol. 2010 Dec;134(6):862-72. PMID: 21088148. EXC5

973. Murphy KM, Zhang S, Geiger T, et al. Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. J Mol Diagn. 2006 Jul;8(3):305-11. PMID: 16825502. EXC5

974. Murray S, Karavasilis V, Bobos M, et al. Molecular predictors of response to tyrosine kinase inhibitors in patients with Non-Small-Cell Lung Cancer. J Exp Clin Cancer Res. 2012;31:77. PMID: 22992338. EXC3

975. Murray S, Timotheadou E, Linardou H, et al. Mutations of the epidermal growth factor receptor tyrosine kinase domain and associations with clinicopathological features in non-small cell lung cancer patients. Lung Cancer. 2006 May;52(2):225-33. PMID: 16567021. EXC6

976. Mvundura M, Grosse SD, Hampel H, et al. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer (Structured abstract). Genetics in Medicine. 2010(2):93-104. PMID: NHSEED-22010000625. EXC6

977. Na, II, Rho JK, Choi YJ, et al. Clinical features reflect exon sites of EGFR mutations in patients with resected non-small-cell lung cancer. J Korean Med Sci. 2007 Jun;22(3):393-9. PMID: 17596643. EXC6

978. Na, II, Rho JK, Choi YJ, et al. The survival outcomes of patients with resected non-small cell lung cancer differ according to EGFR mutations and the P21 expression. Lung Cancer. 2007 Jul;57(1):96-102. PMID: 17337084. EXC5

979. Na KY, Kim KS, Lee JE, et al. The 70-gene prognostic signature for korean breast cancer patients. Journal of Breast Cancer. 2011;14(1):33-8. EXC5

980. Nagai Y, Miyazawa H, Huqun, et al. Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res. 2005 Aug 15;65(16):7276-82. PMID: 16105816. EXC6

981. Nagasaka T, Koi M, Kloor M, et al. Mutations in both KRAS and BRAF may contribute to the methylator phenotype in colon cancer. Gastroenterology. 2008 Jun;134(7):1950-60, 60 e1. PMID: 18435933. EXC4

982. Nagasaka T, Sasamoto H, Notohara K, et al. Colorectal cancer with mutation in BRAF, KRAS, and wild-type with respect to both oncogenes showing different patterns of DNA methylation. J Clin Oncol. 2004 Nov 15;22(22):4584-94. PMID: 15542810. EXC4

983. Naghibalhossaini F, Mokarram P, Khalili I, et al. MTHFR C677T and A1298C variant genotypes and the risk of microsatellite instability among Iranian colorectal cancer patients. Cancer Genet Cytogenet. 2010 Mar;197(2):142-51. PMID: 20193847. EXC6

984. Naguib A, Mitrou PN, Gay LJ, et al. Dietary, lifestyle and clinicopathological factors associated with BRAF and K-ras mutations arising in distinct subsets of colorectal cancers in the EPIC Norfolk study. BMC Cancer. 2010;10:99. PMID: 20233436. EXC4

985. Naidoo R, Tarin M, Chetty R. A comparative microsatellite analysis of colorectal cancer in patients <35 years and >50 years of age. Am J Gastroenterol. 2000 Nov;95(11):3266-75. PMID: 11095352. EXC4

986. Nakagawa H, Nagasaka T, Cullings HM, et al. Efficient molecular screening of Lynch syndrome by specific 3' promoter methylation of the MLH1 or BRAF mutation in colorectal cancer with high-frequency microsatellite instability. Oncol Rep. 2009;21(6):1577-83. EXC4

987. Nakagawa H, Nuovo GJ, Zervos EE, et al. Age-related hypermethylation of the 5' region of MLH1 in normal colonic mucosa is associated with microsatellite-unstable colorectal cancer development. Cancer Res. 2001 Oct 1;61(19):6991-5. PMID: 11585722. EXC6

988. Nakagawa M, Uramoto H, Shimokawa H, et al. Insulin-like growth factor receptor-1 expression predicts postoperative recurrence in adenocarcinoma of the lung. Experimental and Therapeutic Medicine. 2011;2(4):585-90. EXC4

989. Nakajima T, Kimura H, Takeuchi K, et al. Treatment of lung cancer with an ALK inhibitor after EML4-ALK fusion gene detection using endobronchial ultrasound-guided transbronchial needle aspiration. J Thorac Oncol. 2010;5(12):2041-2. EXC2

990. Nakajima T, Yasufuku K, Nakagawara A, et al. Multigene mutation analysis of metastatic lymph nodes in non-small cell lung cancer diagnosed by endobronchial ultrasound-guided transbronchial needle aspiration. Chest. 2011 Nov;140(5):1319-24. PMID: 21527506. EXC4

991. Nakamura T, Sueoka-Aragane N, Iwanaga K, et al. Application of a highly sensitive detection system for epidermal growth factor receptor mutations in plasma DNA. J Thorac Oncol. 2012 Sep;7(9):1369-81. PMID: 22858585. EXC4

992. Nash GM, Gimbel M, Cohen AM, et al. KRAS mutation and microsatellite instability: two genetic markers of early tumor development that influence the prognosis of colorectal cancer. Ann Surg Oncol. 2010 Feb;17(2):416-24. PMID: 19813061. EXC7

993. Nash GM, Gimbel M, Shia J, et al. Automated, multiplex assay for high-frequency microsatellite instability in colorectal cancer. J Clin Oncol. 2003 Aug 15;21(16):3105-12. PMID: 12915601. EXC4

994. Nash GM, Gimbel M, Shia J, et al. KRAS mutation correlates with accelerated metastatic progression in patients with colorectal liver metastases. Ann Surg Oncol. 2010 Feb;17(2):572-8. PMID: 19727962. EXC7

995. Natale RB, Bodkin D, Govindan R, et al. Vandetanib versus gefitinib in patients with advanced non-small-cell lung cancer: results from a two-part, double-blind, randomized phase ii study. J Clin Oncol. 2009 May 20;27(15):2523-9. PMID: 19332730. EXC4

996. National Cancer Institute at the National Institutes of Health. Cancer Staging. Bethesda, MD: National Cancer Institute. www.cancer.gov/cancertopics/factsheet/detection/staging. EXC8

997. National Institute for Health and Care Excellence. Gene expression profiling and expanded immunohistochemistry tests for guiding adjuvant chemotherapy decisions in early breast cancer management: MammaPrint, Oncotype DX, IHC4 and Mammostrat. NICE diagnostics guidance 10 London: NICE; 2013. www.nice.org.uk/dg10. Accessed on January 8, 2014. EXC1

998. Negri FV, Campanini N, Camisa R, et al. Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy. Br J Cancer. 2008 Jan 15;98(1):143-7. PMID: 18087284. EXC7

999. Nehls O, Hass HG, Okech T, et al. Prognostic implications of BAX protein expression and microsatellite instability in all non-metastatic stages of primary colon cancer treated by surgery alone. Int J Colorectal Dis. 2009 Jun;24(6):655-63. PMID: 19221769. EXC5

1000. Nehls O, Okech T, Hsieh CJ, et al. Studies on p53, BAX and Bcl-2 protein expression and microsatellite instability in stage III (UICC) colon cancer treated by adjuvant chemotherapy: major prognostic impact of proapoptotic BAX. Br J Cancer. 2007 May 7;96(9):1409-18. PMID: 17426704. EXC4

1001. Nemunaitis J, Klemow S, Tong A, et al. Prognostic value of K-ras mutations, ras oncoprotein, and c-erb B-2- oncoprotein expression in adenocarcinoma of the lung. American Journal of Clinical Oncology: Cancer Clinical Trials. 1998;21(2):155-60. EXC6

1002. Neninger E, Verdecia BG, Crombet T, et al. Combining an EGF-based cancer vaccine with chemotherapy in advanced nonsmall cell lung cancer. J Immunother. 2009 Jan;32(1):92-9. PMID: 19307998. EXC4

1003. Neumann J, Horst D, Kriegl L, et al. A simple immunohistochemical algorithm predicts the risk of distant metastases in right-sided colon cancer. Histopathology. 2012 Feb;60(3):416-26. PMID: 22276605. EXC4

1004. Neumann J, Zeindl-Eberhart E, Kirchner T, et al. Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer. Pathol Res Pract. 2009;205(12):858-62. PMID: 19679400. EXC6

1005. Newman TB, Kohn MA. Evidence-Based Diagnosis. New York: Cambridge University Press; 2009. EXC2

1006. Newnham GM, Conron M, McLachlan S, et al. Integrated mutation, copy number and expression profiling in resectable non-small cell lung cancer. BMC Cancer. 2011;11:93. PMID: 21385341. EXC6

1007. Ng K, Ogino S, Meyerhardt JA, et al. Relationship between statin use and colon cancer recurrence and survival: results from CALGB 89803. J Natl Cancer Inst. 2011 Oct 19;103(20):1540-51. PMID: 21849660. EXC4

1008. Nguyen B, Cusumano PG, Deck K, et al. Comparison of molecular subtyping with BluePrint, MammaPrint, and TargetPrint to local clinical subtyping in breast cancer patients. Ann Surg Oncol. 2012 Oct;19(10):3257-63. PMID: 22965266. EXC 6

1009. Nguyen CT, Litt DB, Dolar SE, et al. Prognostic significance of nondiagnostic molecular changes in urine detected by UroVysion fluorescence in situ hybridization during surveillance for bladder cancer. Urology. 2009 Feb;73(2):347-50. PMID: 19022486. EXC6

1010. Nie Q, Yang XN, An SJ, et al. CYP1A1*2A polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI and its combined effects with EGFR intron 1 (CA)n polymorphism. Eur J Cancer. 2011 Sep;47(13):1962-70. PMID: 21616658. EXC4

1011. Niemiec J, Kolodziejski L, Dyczek S. EGFR LI and Ki-67 LI are independent prognostic parameters influencing survivals of surgically treated squamous cell lung cancer patients. Neoplasma. 2005;52(3):231-7. PMID: 15875085. EXC4

1012. Niemiec J, Kolodziejski L, Dyczek S, et al. Prognostic significance of epidermal growth factor receptor in surgically treated squamous cell lung cancer patients. Folia Histochem Cytobiol. 2004;42(2):111-8. PMID: 15253134. EXC4

1013. Nilbert M, Planck M, Fernebro E, et al. Microsatellite instability is rare in rectal carcinomas and signifies hereditary cancer. Eur J Cancer. 1999 Jun;35(6):942-5. PMID: 10533476. EXC4

1014. Ninomiya H, Hiramatsu M, Inamura K, et al. Correlation between morphology and EGFR mutations in lung adenocarcinomas Significance of the micropapillary pattern and the hobnail cell type. Lung Cancer. 2009 Feb;63(2):235-40. PMID: 18571764. EXC6

1015. Nishino M, Cardarella S, Jackman DM, et al. RECIST 1.1 in NSCLC patients with EGFR mutations treated with EGFR tyrosine kinase inhibitors: comparison with RECIST 1.0. AJR Am J Roentgenol. 2013 Jul;201(1):W64-71. PMID: 23789698. EXC3

1016. Nishino M, Klepeis VE, Yeap BY, et al. Histologic and cytomorphologic features of ALK-rearranged lung adenocarcinomas. Mod Pathol. 2012 Nov;25(11):1462-72. PMID: 22743652. EXC6

1017. Nishio M, Yamanaka T, Matsumoto K, et al. Serum heparan sulfate concentration is correlated with the failure of epidermal growth factor receptor tyrosine kinase inhibitor treatment in patients with lung adenocarcinoma. J Thorac Oncol. 2011 Nov;6(11):1889-94. PMID: 21964526. EXC4

1018. Nitsche U, Rosenberg R, Balmert A, et al. Integrative marker analysis allows risk assessment for metastasis in stage II colon cancer. Ann Surg. 2012 Nov;256(5):763-71; discussion 71. PMID: 23095620. EXC6

1019. Niwinska A, Olszewski W, Murawska M, et al. Triple-negative breast cancer with brain metastases: A comparison between basal-like and non-basal-like biological subtypes. J Neurooncol. 2011;105(3):547-53. EXC4

1020. Noda H, Kato Y, Yoshikawa H, et al. Frequent involvement of ras-signalling pathways in both polypoid-type and flat-type early-stage colorectal cancers. J Exp Clin Cancer Res. 2006 Jun;25(2):235-42. PMID: 16918136. EXC6

1021. Noguchi M, Suzuki T, Kabayama K, et al. GM3 synthase gene is a novel biomarker for histological classification and drug sensitivity against epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Cancer Sci. 2007 Oct;98(10):1625-32. PMID: 17711504. EXC4

1022. Norrie MW, Hawkins NJ, Todd AV, et al. The role of hMLH1 methylation in the development of synchronous sporadic colorectal carcinomas. Dis Colon Rectum. 2002 May;45(5):674-80. PMID: 12004219. EXC6

1023. Norris S, Atkins D, Bruening W, et al. Selecting Observational Studies for Comparing Medical Interventions. Methods Guide for Effectiveness and Comparative Effectiveness Reviews. Rockville, MD: Agency for Healthcare Research and Quality; 2010. EXC2

1024. Nose N, Sugio K, Oyama T, et al. Association between estrogen receptor-beta expression and epidermal growth factor receptor mutation in the postoperative prognosis of adenocarcinoma of the lung. J Clin Oncol. 2009 Jan 20;27(3):411-7. PMID: 19064969. EXC6

1025. Nose N, Uramoto H, Iwata T, et al. Expression of estrogen receptor beta predicts a clinical response and longer progression-free survival after treatment with EGFR-TKI for adenocarcinoma of the lung. Lung Cancer. 2011 Mar;71(3):350-5. PMID: 20615575. EXC4

1026. Nosho K, Baba Y, Tanaka N, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol. 2010 Dec;222(4):350-66. PMID: 20927778. EXC5

1027. Nosho K, Kure S, Irahara N, et al. A Prospective Cohort Study Shows Unique Epigenetic, Genetic, and Prognostic Features of Synchronous Colorectal Cancers. Gastroenterology. 2009;137(5):1609-20.e3. EXC4

1028. Nosho K, Shima K, Irahara N, et al. DNMT3B expression might contribute to CpG island methylator phenotype in colorectal cancer. Clin Cancer Res. 2009 Jun 1;15(11):3663-71. PMID: 19470733. EXC4

1029. Nosho K, Yamamoto H, Hamamoto Y, et al. A case of multiple protruding and flat colorectal tumors analyzed by a cDNA array. Int J Colorectal Dis. 2007 Jun;22(6):723-4. PMID: 16075236. EXC2

1030. Nosho K, Yamamoto H, Takahashi T, et al. Correlation of laterally spreading type and JC virus with methylator phenotype status in colorectal adenoma. Hum Pathol. 2008 May;39(5):767-75. PMID: 18284934. EXC4

1031. Notarnicola M, Gristina R, Messa C, et al. Oestrogen receptors and microsatellite instability in colorectal carcinoma patients. Cancer Lett. 2001 Jul 10;168(1):65-70. PMID: 11368879. EXC4

1032. Nygaard AD, Garm Spindler KL, Pallisgaard N, et al. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. Lung Cancer. 2013 Mar;79(3):312-7. PMID: 23238036. EXC6

1033. Obermair A, Youlden DR, Young JP, et al. Risk of endometrial cancer for women diagnosed with HNPCC-related colorectal carcinoma. Int J Cancer. 2010;127(11):2678-84. EXC3

1034. O'Brien MJ, Yang S, Mack C, et al. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. Am J Surg Pathol. 2006 Dec;30(12):1491-501. PMID: 17122504. EXC5

1035. O'Byrne KJ, Cox G, Swinson D, et al. Towards a biological staging model for operable non-small cell lung cancer. Lung Cancer. 2001 Dec;34 Suppl 2:S83-9. PMID: 11720747. EXC4

1036. O'Byrne KJ, Gatzemeier U, Bondarenko I, et al. Molecular biomarkers in non-small-cell lung cancer: a retrospective analysis of data from the phase 3 FLEX study. Lancet Oncol. 2011 Aug;12(8):795-805. PMID: 21782507. EXC3

1037. Ocvirk J, Brodowicz T, Wrba F, et al. Cetuximab plus FOLFOX6 or FOLFIRI in metastatic colorectal cancer: CECOG trial. World J Gastroenterol. 2010 Jul 7;16(25):3133-43. PMID: 20593498. EXC3

1038. Ogino S, Brahmandam M, Kawasaki T, et al. Epigenetic profiling of synchronous colorectal neoplasias by quantitative DNA methylation analysis. Mod Pathol. 2006 Aug;19(8):1083-90. PMID: 16699497. EXC6

1039. Ogino S, Nosho K, Irahara N, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res. 2009 Oct 15;15(20):6412-20. PMID: 19825961. EXC6

1040. Ogino S, Nosho K, Irahara N, et al. Prognostic significance and molecular associations of 18q loss of heterozygosity: a cohort study of microsatellite stable colorectal cancers. J Clin Oncol. 2009 Sep 20;27(27):4591-8. PMID: 19704056. EXC4

1041. Ogino S, Nosho K, Irahara N, et al. Negative lymph node count is associated with survival of colorectal cancer patients, independent of tumoral molecular alterations and lymphocytic reaction. Am J Gastroenterol. 2010 Feb;105(2):420-33. PMID: 19809407. EXC4

1042. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009;58(1):90-6. EXC4

1043. Oh IJ, Ban HJ, Kim KS, et al. Retreatment of gefitinib in patients with non-small-cell lung cancer who previously controlled to gefitinib: a single-arm, open-label, phase II study. Lung Cancer. 2012 Jul;77(1):121-7. PMID: 22333554. EXC6

1044. Oh JE, An CH, Yoo NJ, et al. Detection of low-level EGFR T790M mutation in lung cancer tissues. APMIS. 2011 Jul;119(7):403-11. PMID: 21635547. EXC6

1045. Ohsaki Y, Tanno S, Fujita Y, et al. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. Oncol Rep. 2000 May-Jun;7(3):603-7. PMID: 10767376. EXC4

1046. Ohta M, Seto M, Ijichi H, et al. Decreased expression of the RAS-GTPase activating protein RASAL1 is associated with colorectal tumor progression. Gastroenterology. 2009 Jan;136(1):206-16. PMID: 18992247. EXC7

1047. Ohtsuka K, Ohnishi H, Furuyashiki G, et al. Clinico-pathological and biological significance of tyrosine kinase domain gene mutations and overexpression of epidermal growth factor receptor for lung adenocarcinoma. J Thorac Oncol. 2006 Oct;1(8):787-95. PMID: 17409961. EXC6

1048. Ohtsuka K, Ohnishi H, Kurai D, et al. Familial lung adenocarcinoma caused by the EGFR V843I germ-line mutation. J Clin Oncol. 2011 Mar 10;29(8):e191-2. PMID: 21172876. EXC2

1049. Okami J, Taniguchi K, Higashiyama M, et al. Prognostic factors for gefitinib-treated postoperative recurrence in non-small cell lung cancer. Oncology. 2007;72(3-4):234-42. PMID: 18176089. EXC6

1050. Okayama H, Kohno T, Ishii Y, et al. Identification of genes upregulated in ALK-positive and EGFR/KRAS/ALK-negative lung adenocarcinomas. Cancer Res. 2012;72(1):100-11. EXC5

1051. Okon K, Demczuk S, Klimkowska A, et al. Correlation of microsatellite status, proliferation, apoptotic and selected immunohistochemical markers in colorectal carcinoma studied with tissue microarray. Pol J Pathol. 2006;57(2):105-11. PMID: 17019973. EXC6

1052. Okon K, Klimkowska A, Wojcik P, et al. High thymidylate synthase expression is typical for sporadic MSI-H colorectal carcinoma. Pol J Pathol. 2006;57(1):29-33. PMID: 16739880. EXC6

1053. Okon K, Sinczak-Kuta A, Klimkowska A, et al. Tissue microarray FISH applied to colorectal carcinomas with various microsatellite status. Pol J Pathol. 2006;57(2):99-103. PMID: 17019972. EXC4

1054. Okon K, Zazula M, Rudzki Z, et al. CDX-2 expression is reduced in colorectal carcinomas with solid growth pattern and proximal location, but is largely independent of MSI status. Pol J Pathol. 2004;55(3):9-14. PMID: 15619975. EXC4

1055. Okuda K, Sasaki H, Dumontet C, et al. Expression of excision repair cross-complementation group 1 and class III beta-tubulin predict survival after chemotherapy for completely resected non-small cell lung cancer. Lung Cancer. 2008 Oct;62(1):105-12. PMID: 18395930. EXC6

1056. Okuda T, Kawakami K, Ishiguro K, et al. The profile of hMLH1 methylation and microsatellite instability in colorectal and non-small cell lung cancer. Int J Mol Med. 2005 Jan;15(1):85-90. PMID: 15583832. EXC6

1057. Okudela K, Woo T, Yazawa T, et al. Significant association between EGFR-mutated lung adenocarcinoma and past illness from gastric cancer or uterine myoma: Its implication in carcinogenesis. Lung Cancer. 2009;66(3):287-91. EXC6

1058. Olaru AV, Cheng Y, Agarwal R, et al. Unique patterns of CpG island methylation in inflammatory bowel disease-associated colorectal cancers. Inflamm Bowel Dis. 2012 Apr;18(4):641-8. PMID: 21830278. EXC6

1059. . Cost-effectiveness of the Oncotype DX assay in Australia: an exploratory analysis. International Society for Pharmoeconomics and Outcomes Research; 2010 May; Atlanta, GA. EXC6

1060. Oliart S, Martinez-Santos C, Moreno-Azcoita M, et al. Do MSI-L sporadic colorectal tumors develop through "mild mutator pathway"? Am J Clin Oncol. 2006 Aug;29(4):364-70. PMID: 16891863. EXC6

1061. Oliner K, Juan T, Suggs S, et al. A comparability study of 5 commercial KRAS tests. Diagn Pathol. 2010;5:23. PMID: 20398393. EXC4

1062. Oltedal S, Aasprong OG, Moller JH, et al. Heterogeneous distribution of K-ras mutations in primary colon carcinomas: implications for EGFR-directed therapy. Int J Colorectal Dis. 2011 Oct;26(10):1271-7. PMID: 21573767. EXC6

1063. Onitsuka T, Uramoto H, Ono K, et al. Comprehensive molecular analyses of lung adenocarcinoma with regard to the epidermal growth factor receptor, K-ras, MET, and hepatocyte growth factor status. J Thorac Oncol. 2010 May;5(5):591-6. PMID: 20150826. EXC6

1064. Onn A, Choe DH, Herbst RS, et al. Tumor cavitation in stage I non-small cell lung cancer: epidermal growth factor receptor expression and prediction of poor outcome. Radiology. 2005 Oct;237(1):342-7. PMID: 16183941. EXC4

1065. Onn A, Correa AM, Gilcrease M, et al. Synchronous overexpression of epidermal growth factor receptor and HER2-neu protein is a predictor of poor outcome in patients with stage I non-small cell lung cancer. Clin Cancer Res. 2004 Jan 1;10(1 Pt 1):136-43. PMID: 14734462. EXC4

1066. Oratz R, Kim B, Chao C, et al. Physician survey of the effect of the 21-gene recurrence score assay results on treatment recommendations for patients with lymph node-positive, estrogen receptor-positive breast cancer. J Oncol Pract. 2011 Mar;7(2):94-9. PMID: 21731516. Exc7

1067. Oratz R, Paul D, Cohn A, et al. Impact of Oncotype DX (TM) on decision making in breast cancer clinical practice. Breast Cancer Res Treat. 2005;94(suppl 1):S100-S. PMID: ISI:000233407100269. EXC2

1068. Oratz R, Paul D, Cohn AL, et al. Impact of a commercial reference laboratory test recurrence score on decision making in early-stage breast cancer. Journal of Oncology Practice. 2007;3(4):182-6. EXC5

1069. Orta L, Klimstra DS, Qin J, et al. Towards identification of hereditary DNA mismatch repair deficiency: sebaceous neoplasm warrants routine immunohistochemical screening regardless of patient's age or other clinical characteristics. Am J Surg Pathol. 2009 Jun;33(6):934-44. PMID: 19342947. EXC3

1070. Ortiz TM, Cohen DW, Kent MS, et al. KRAS mutation analysis helps to differentiate between pulmonary metastasis from colon adenocarcinoma in situ and primary lung adenocarcinoma. J Thorac Oncol. 2011 Jan;6(1):220-2. PMID: 21178719. EXC3

1071. Oshita F, Matsukuma S, Yoshihara M, et al. Novel heteroduplex method using small cytology specimens with a remarkably high success rate for analysing EGFR gene mutations with a significant correlation to gefitinib efficacy in non-small-cell lung cancer. Br J Cancer. 2006 Oct 23;95(8):1070-5. PMID: 17047654. EXC3

1072. Oster B, Thorsen K, Lamy P, et al. Identification and validation of highly frequent CpG island hypermethylation in colorectal adenomas and carcinomas. Int J Cancer. 2011 Dec 15;129(12):2855-66. PMID: 21400501. EXC4

1073. Ostwald C, Linnebacher M, Weirich V, et al. Chromosomally and microsatellite stable colorectal carcinomas without the CpG island methylator phenotype in a molecular classification. Int J Oncol. 2009 Aug;35(2):321-7. PMID: 19578746. EXC6

1074. Otani H, Toyooka S, Soh J, et al. Detection of EGFR gene mutations using the wash fluid of CT-guided biopsy needle in NSCLC patients. J Thorac Oncol. 2008 May;3(5):472-6. PMID: 18448998. EXC4

1075. Ottini L, Palli D, Falchetti M, et al. Microsatellite instability in gastric cancer is associated with tumor location and family history in a high-risk population from Tuscany. Cancer Res. 1997 Oct 15;57(20):4523-9. PMID: 9377564. EXC3

1076. Overbeek LI, Kets CM, Hebeda KM, et al. Patients with an unexplained microsatellite instable tumour have a low risk of familial cancer. Br J Cancer. 2007 May 21;96(10):1605-12. PMID: 17453009. EXC3

1077. Owens SR, Chiosea SI, Kuan SF. Selective expression of gastric mucin MUC6 in colonic sessile serrated adenoma but not in hyperplastic polyp aids in morphological diagnosis of serrated polyps. Mod Pathol. 2008 Jun;21(6):660-9. PMID: 18360351. EXC6

1078. Oxnard GR, Janjigian YY, Arcila ME, et al. Maintained sensitivity to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer recurring after adjuvant erlotinib or gefitinib. Clin Cancer Res. 2011 Oct 1;17(19):6322-8. PMID: 21831955. EXC5

1079. Oxnard GR, Miller VA, Robson ME, et al. Screening for germline EGFR T790M mutations through lung cancer genotyping. J Thorac Oncol. 2012 Jun;7(6):1049-52. PMID: 22588155. EXC4

1080. Oyama K, Kawakami K, Maeda K, et al. The association between methylenetetrahydrofolate reductase polymorphism and promoter methylation in proximal colon cancer. Anticancer Res. 2004 Mar-Apr;24(2B):649-54. PMID: 15161007. EXC4

1081. Ozer E, Yuksel E, Kizildag S, et al. Microsatellite instability in early-onset breast cancer. Pathology Research and Practice. 2002;198(8):525-30. EXC7

1082. Paez D, Pare L, Espinosa I, et al. Immunoglobulin G fragment C receptor polymorphisms and KRAS mutations: are they useful biomarkers of clinical outcome in advanced colorectal cancer treated with anti-EGFR-based therapy? Cancer Sci. 2010 Sep;101(9):2048-53. PMID: 20550522. EXC7

1083. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004 Jun 4;304(5676):1497-500. PMID: 15118125. EXC6

1084. Paik JH, Choe G, Kim H, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. J Thorac Oncol. 2011 Mar;6(3):466-72. PMID: 21258247. EXC4

1085. Paik JH, Choi CM, Kim H, et al. Clinicopathologic implication of ALK rearrangement in surgically resected lung cancer: a proposal of diagnostic algorithm for ALK-rearranged adenocarcinoma. Lung Cancer. 2012 Jun;76(3):403-9. PMID: 22129856. EXC6

1086. Paik PK, Johnson ML, D'Angelo SP, et al. Driver mutations determine survival in smokers and never-smokers with stage IIIB/IV lung adenocarcinomas. Cancer. 2012 Dec 1;118(23):5840-7. PMID: 22605530. EXC6

1087. Paik S, Shak S, Tang G, et al. Multi-gene RT-PCR assay for predicting recurrence in node negative breast cancer patients - NSABP studies B-20 and B-14. Breast Cancer Res Treat. 2003;82(Suppl 1):S10-S1. PMID: ISI:000186783100029. EXC2

1088. Paik S, Shak S, Tang G, et al. Expression of the 21 genes in the Recurrence Score assay and tamoxifen clinical benefit in the NSABP study B-14 of node negative, estrogen receptor positive breast cancer. J Clin Oncol. 2005 Jun 1;23(16):6S-S. PMID: ISI:000230326600022. EXC2

1089. Paik S, Shak S, Tang G, et al. Expression of the 21 genes in the Recurrence Score assay and prediction of clinical benefit from tamoxifen in NSABP study B-14 and chemotherapy in NSABP study B-20. Breast Cancer Res Treat. 2004;88(suppl 1):S15-S. PMID: ISI:000225589600040. EXC2

1090. Paik S, Shak S, Tang G, et al. Risk classification of breast cancer patients by the Recurrence Score assay: comparison to guidelines based on patient age, tumor size, and tumor grade. Breast Cancer Res Treat. 2004;88(Suppl 1):S21-S. PMID: ISI:000225589600056. EXC2

1091. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol. 2006 Aug 10;24(23):3726-34. PMID: 16720680. EXC5

1092. Pallis AG, Voutsina A, Kalikaki A, et al. 'Classical' but not 'other' mutations of EGFR kinase domain are associated with clinical outcome in gefitinib-treated patients with non-small cell lung cancer. Br J Cancer. 2007 Dec 3;97(11):1560-6. PMID: 18000506. EXC6

1093. Pallis AG, Voutsina A, Kentepozidis N, et al. A phase II trial of erlotinib as front-line treatment in clinically selected patients with non-small-cell lung cancer. Clin Lung Cancer. 2012 Mar;13(2):129-35. PMID: 22000696. EXC3

1094. Palmirotta R, Ludovici G, De Marchis ML, et al. A comprehensive procedural approach to genotyping KRAS and BRAF from paraffin embedded tissues for diagnostic purposes. In Vivo. 2012 Jul;26(4):537-47. PMID: 22773565. EXC6

1095. Palmirotta R, Matera S, Curia MC, et al. Correlations between phenotype and microsatellite instability in HNPCC: implications for genetic testing. Fam Cancer. 2004;3(2):117-21. PMID: 15340262. EXC4

1096. Palomaki GE, McClain MR, Melillo S, et al. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med. 2009 Jan;11(1):42-65. PMID: 19125127. EXC3

1097. Pancione M, Forte N, Fucci A, et al. Prognostic role of beta-catenin and p53 expression in the metastatic progression of sporadic colorectal cancer. Hum Pathol. 2010 Jun;41(6):867-76. PMID: 20129645. EXC4

1098. Pandey V, Prabhu JS, Payal K, et al. Assessment of microsatellite instability in colorectal carcinoma at an Indian center. Int J Colorectal Dis. 2007 Jul;22(7):777-82. PMID: 17160686. EXC6

1099. Pang B, Matthias D, Ong CW, et al. The positive impact of cytological specimens for EGFR mutation testing in non-small cell lung cancer: A single South East Asian laboratory's analysis of 670 cases. Cytopathology. 2012;23(4):229-36. EXC4

1100. Paradiso A, Tommasi S, Pinto R, et al. Exhaled breath condensate is not suitable to detect EGFR somatic mutations. Eur Respir J. 2008;32(4):1126-7. EXC2

1101. Paraf F, Gilquin M, Longy M, et al. MLH1 and MSH2 protein immunohistochemistry is useful for detection of hereditary non-polyposis colorectal cancer in young patients. Histopathology. 2001 Sep;39(3):250-8. PMID: 11532035. EXC4

1102. Parc Y, Gueroult S, Mourra N, et al. Prognostic significance of microsatellite instability determined by immunohistochemical staining of MSH2 and MLH1 in sporadic T3N0M0 colon cancer. Gut. 2004 Mar;53(3):371-5. PMID: 14960518. EXC4

1103. Park HS, Lee JK, Kim DW, et al. Immunohistochemical screening for anaplastic lymphoma kinase (ALK) rearrangement in advanced non-small cell lung cancer patients. Lung Cancer. 2012;77(2):288-92. EXC4

1104. Park JH, Han SW, Oh DY, et al. Analysis of KRAS, BRAF, PTEN, IGF1R, EGFR intron 1 CA status in both primary tumors and paired metastases in determining benefit from cetuximab therapy in colon cancer. Cancer Chemother Pharmacol. 2011 Oct;68(4):1045-55. PMID: 21340604. EXC5

1105. Park S, Choi YL, Sung CO, et al. High MET copy number and MET overexpression: poor outcome in non-small cell lung cancer patients. Histol Histopathol. 2012 Feb;27(2):197-207. PMID: 22207554. EXC4

1106. Park S, Ha SY, Cho HY, et al. Prognostic implications of hypoxia-inducible factor-1alpha in epidermal growth factor receptor-negative non-small cell lung cancer. Lung Cancer. 2011 Apr;72(1):100-7. PMID: 20822827. EXC4

1107. Park S, Holmes-Tisch AJ, Cho EY, et al. Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer. J Thorac Oncol. 2009 Jul;4(7):809-15. PMID: 19487967. EXC6

1108. Park SH, Ha SY, Lee JI, et al. Epidermal growth factor receptor mutations and the clinical outcome in male smokers with squamous cell carcinoma of lung. J Korean Med Sci. 2009 Jun;24(3):448-52. PMID: 19543508. EXC3

1109. Park SJ, Hong YS, Lee JL, et al. Genetic polymorphisms of Fc(gamma)RIIa and Fc(gamma)RIIIa are not predictive of clinical outcomes after cetuximab plus irinotecan chemotherapy in patients with metastatic colorectal cancer. Oncology. 2012;82(2):83-9. EXC5

1110. Park SY, Choe G, Lee HS, et al. Tumor budding as an indicator of isolated tumor cells in lymph nodes from patients with node-negative colorectal cancer. Dis Colon Rectum. 2005 Feb;48(2):292-302. PMID: 15616755. EXC4

1111. Park SY, Lee HS, Choe G, et al. Clinicopathological characteristics, microsatellite instability, and expression of mucin core proteins and p53 in colorectal mucinous adenocarcinomas in relation to location. Virchows Arch. 2006 Jul;449(1):40-7. PMID: 16645863. EXC6

1112. Parra HS, Cavina R, Latteri F, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. Br J Cancer. 2004 Jul 19;91(2):208-12. PMID: 15187994. EXC4

1113. Partanen R, Hemminki K, Koskinen H, et al. The detection of increased amounts of the extracellular domain of the epidermal growth factor receptor in serum during carcinogenesis in asbestosis patients. J Occup Med. 1994;36(12):1324-8. EXC4

1114. Partin JF, Mamounas EP. Impact of the 21-gene recurrence score assay compared with standard clinicopathologic guidelines in adjuvant therapy selection for node-negative, estrogen receptor-positive breast cancer. Ann Surg Oncol. 2011 Nov;18(12):3399-406. PMID: 21537874. EXC5

1115. Paule B, Castagne V, Picard V, et al. MDR1 polymorphism role in patients treated with cetuximab and irinotecan in irinotecan refractory colorectal cancer. Med Oncol. 2010 Dec;27(4):1066-72. PMID: 19862647. EXC4

1116. Pe PA, Azcoita MM, Alonso A, et al. Prognostic significancce of high microsatellite instability in a Spanish series of gastric adenocarcinomas. Anticancer Res. 2000;20(5 C):4009-14. EXC4

1117. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol. 2010 Nov 1;28(31):4706-13. PMID: 20921462. EXC6

1118. Peeters M, Siena S, Cutsem E, et al. Association of progression-free survival, overall survival, and patient-reported outcomes by skin toxicity and KRAS status in patients receiving panitumumab monotherapy. Cancer. 2009(7):1544-54. PMID: CN-00701635. EXC7

1119. Pelosi G, Gasparini P, Cavazza A, et al. Multiparametric molecular characterization of pulmonary sarcomatoid carcinoma reveals a nonrandom amplification of anaplastic lymphoma kinase (ALK) gene. Lung Cancer. 2012;77(3):507-14. EXC6

1120. Peng J, Xiao-ming M, Jian-qiu S, et al. Clinicopathological features of non-familial colorectal cancer with high-frequency microsatellite instability. Chin Med Sci J. 2010 Dec;25(4):228-32. PMID: 21232183. EXC6

1121. Penzel R, Schirmacher P, Warth A. A novel EML4-ALK variant: exon 6 of EML4 fused to exon 19 of ALK. J Thorac Oncol. 2012 Jul;7(7):1198-9. PMID: 22706607. EXC7

1122. Perea J, Alvaro E, Rodriguez Y, et al. Approach to early-onset colorectal cancer: Clinicopathological, familial, molecular and immunohistochemical characteristics. World Journal of Gastroenterology. 2010;16(29):3697-703. EXC6

1123. Perez-Ruiz E, Rueda A, Pereda T, et al. Involvement of K-RAS mutations and amino acid substitutions in the survival of metastatic colorectal cancer patients. Tumour Biol. 2012 Dec;33(6):1829-35. PMID: 22791568. EXC3

1124. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. J Clin Oncol. 2004 Aug 15;22(16):3238-47. PMID: 15310767. EXC5

1125. Perez-Villamil B, Romera-Lopez A, Hernandez-Prieto S, et al. Colon cancer molecular subtypes identified by expression profiling and associated to stroma, mucinous type and different clinical behavior. BMC Cancer. 2012;12:260. PMID: 22712570. EXC4

1126. Perkins G, Lievre A, Ramacci C, et al. Additional value of EGFR downstream signaling phosphoprotein expression to KRAS status for response to anti-EGFR antibodies in colorectal cancer. Int J Cancer. 2010 Sep 1;127(6):1321-31. PMID: 20049837. EXC4

1127. Perng RP, Yang CH, Chen YM, et al. High efficacy of erlotinib in Taiwanese NSCLC patients in an expanded access program study previously treated with chemotherapy. Lung Cancer. 2008 Oct;62(1):78-84. PMID: 18423781. EXC4

1128. Perrin J, Gouvernet J, Parriaux D, et al. MSH2 and MLH1 immunodetection and the prognosis of colon cancer. Int J Oncol. 2001 Nov;19(5):891-5. PMID: 11604984. EXC4

1129. Perrone F, Lampis A, Orsenigo M, et al. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. Ann Oncol. 2009 Jan;20(1):84-90. PMID: 18669866. EXC6

1130. Personeni N, Fieuws S, Piessevaux H, et al. Clinical usefulness of EGFR gene copy number as a predictive marker in colorectal cancer patients treated with cetuximab: a fluorescent in situ hybridization study. Clin Cancer Res. 2008 Sep 15;14(18):5869-76. PMID: 18794099. EXC4

1131. Pesch B, Nasterlack M, Eberle F, et al. The role of haematuria in bladder cancer screening among men with former occupational exposure to aromatic amines. BJU Int. 2011 Aug;108(4):546-52. PMID: 21223477. EXC6

1132. Pesek M, Benesova L, Belsanova B, et al. Dominance of EGFR and insignificant KRAS mutations in prediction of tyrosine-kinase therapy for NSCLC patients stratified by tumor subtype and smoking status. Anticancer Res. 2009;29(7):2767-73. EXC3

1133. Pesek M, Kopeckova M, Benesova L, et al. Clinical significance of hypermethylation status in NSCLC: evaluation of a 30-gene panel in patients with advanced disease. Anticancer Res. 2011 Dec;31(12):4647-52. PMID: 22199344. EXC4

1134. Petras ML, Lefferts JA, Ward BP, et al. KRAS detection in colonic tumors by DNA extraction from FTA paper: the molecular touch-prep. Diagn Mol Pathol. 2011 Dec;20(4):189-93. PMID: 22089345. EXC5

1135. Pfeiffer P, Clausen PP, Andersen K, et al. Lack of prognostic significance of epidermal growth factor receptor and the oncoprotein p185HER-2 in patients with systemically untreated non-small-cell lung cancer: an immunohistochemical study on cryosections. Br J Cancer. 1996 Jul;74(1):86-91. PMID: 8679464. EXC4

1136. Pfeiffer P, Nexo E, Bentzen SM, et al. Enzyme-linked immunosorbent assay of epidermal growth factor receptor in lung cancer: comparisons with immunohistochemistry, clinicopathological features and prognosis. Br J Cancer. 1998 Jul;78(1):96-9. PMID: 9662257. EXC4

1137. Phillips SM, Banerjea A, Feakins R, et al. Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic. Br J Surg. 2004 Apr;91(4):469-75. PMID: 15048750. EXC4

1138. Phipps AI, Baron J, Newcomb PA. Prediagnostic smoking history, alcohol consumption, and colorectal cancer survival: the Seattle Colon Cancer Family Registry. Cancer. 2011 Nov 1;117(21):4948-57. PMID: 21495019. EXC6

1139. Pichler M, Balic M, Stadelmeyer E, et al. Evaluation of high-resolution melting analysis as a diagnostic tool to detect the BRAF V600E mutation in colorectal tumors. J Mol Diagn. 2009 Mar;11(2):140-7. PMID: 19213871. EXC6

1140. Pichler M, Winter E, Stotz M, et al. Down-regulation of KRAS-interacting miRNA-143 predicts poor prognosis but not response to EGFR-targeted agents in colorectal cancer. Br J Cancer. 2012 May 22;106(11):1826-32. PMID: 22549179. EXC4

1141. Pino MS, Kikuchi H, Zeng M, et al. Epithelial to mesenchymal transition is impaired in colon cancer cells with microsatellite instability. Gastroenterology. 2010 Apr;138(4):1406-17. PMID: 20026115. EXC7

1142. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet. 2009 May 2;373(9674):1525-31. PMID: 19410716. EXC5

1143. Pirker R, Pereira JR, von Pawel J, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol. 2012 Jan;13(1):33-42. PMID: 22056021. EXC4

1144. Pistorius SR, Kruppa C, Haas S, et al. Clinical consequences of molecular diagnosis in families with mismatch repair gene germline mutations. Int J Colorectal Dis. 2000;15(5-6):255-63. EXC6

1145. Plaschke J, Schwanebeck U, Pistorius S, et al. Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. Cancer Lett. 2003 Mar 10;191(2):179-85. PMID: 12618331. EXC4

1146. Plummer JM, Chin SN, Aronson M, et al. Lynch syndrome in a predominantly Afrocentric population: a clinicopathological and genetic study. Can J Surg. 2012 Oct;55(5):294-300. PMID: 22854115. EXC3

1147. Pluquet E, Cadranel J, Legendre A, et al. Osteoblastic reaction in non-small cell lung carcinoma and its association to epidermal growth factor receptor tyrosine kinase inhibitors response and prolonged survival. J Thorac Oncol. 2010 Apr;5(4):491-6. PMID: 20195171. EXC3

1148. Poole EM, Curtin K, Hsu L, et al. Genetic variability in EGFR, Src and HER2 and risk of colorectal adenoma and cancer. International Journal of Molecular Epidemiology and Genetics. 2011;2(4):300-15. EXC6

1149. Popat S, Vieira de Araujo A, Min T, et al. Lung adenocarcinoma with concurrent exon 19 EGFR mutation and ALK rearrangement responding to erlotinib. J Thorac Oncol. 2011 Nov;6(11):1962-3. PMID: 22005476. EXC2

1150. Popovici V, Budinska E, Tejpar S, et al. Identification of a poor-prognosis BRAF-mutant-like population of patients with colon cancer. J Clin Oncol. 2012 Apr 20;30(12):1288-95. PMID: 22393095. EXC4

1151. Porta R, Sanchez-Torres JM, Paz-Ares L, et al. Brain metastases from lung cancer responding to erlotinib: the importance of EGFR mutation. Eur Respir J. 2011 Mar;37(3):624-31. PMID: 20595147. EXC5

1152. Poulogiannis G, Ichimura K, Hamoudi RA, et al. Prognostic relevance of DNA copy number changes in colorectal cancer. J Pathol. 2010 Feb;220(3):338-47. PMID: 19911421. EXC4

1153. Power DG, Shah MA, Asmis TR, et al. Safety and efficacy of panitumumab following cetuximab: retrospective review of the Memorial Sloan-Kettering experience. Invest New Drugs. 2010 Jun;28(3):353-60. PMID: 19468688. EXC4

1154. Poynter JN, Siegmund KD, Weisenberger DJ, et al. Molecular characterization of MSI-H colorectal cancer by MLHI promoter methylation, immunohistochemistry, and mismatch repair germline mutation screening. Cancer Epidemiol Biomarkers Prev. 2008 Nov;17(11):3208-15. PMID: 18990764. EXC4

1155. Prall F, Duhrkop T, Weirich V, et al. Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. Hum Pathol. 2004 Jul;35(7):808-16. PMID: 15257543. EXC4

1156. Prat A, Parker JS, Fan C, et al. Concordance among gene expression-based predictors for ER-positive breast cancer treated with adjuvant tamoxifen. Ann Oncol. 2012 Nov;23(11):2866-73. PMID: 22532584. EXC5

1157. Price KA, Azzoli CG, Krug LM, et al. Phase II trial of gefitinib and everolimus in advanced non-small cell lung cancer. J Thorac Oncol. 2010 Oct;5(10):1623-9. PMID: 20871262. EXC6

1158. Price TJ, Hardingham JE, Lee CK, et al. Impact of KRAS and BRAF Gene Mutation Status on Outcomes From the Phase III AGITG MAX Trial of Capecitabine Alone or in Combination With Bevacizumab and Mitomycin in Advanced Colorectal Cancer. J Clin Oncol. 2011 Jul 1;29(19):2675-82. PMID: 21646616. EXC3

1159. Psofaki V, Kalogera C, Tzambouras N, et al. Promoter methylation status of hMLH1, MGMT, and CDKN2A/p16 in colorectal adenomas. World J Gastroenterol. 2010 Jul 28;16(28):3553-60. PMID: 20653064. EXC6

1160. Pucciarelli S, Agostini M, Viel A, et al. Early-age-at-onset colorectal cancer and microsatellite instability as markers of hereditary nonpolyposis colorectal cancer. Dis Colon Rectum. 2003 Mar;46(3):305-12. PMID: 12626904. EXC6

1161. Pycha A, Lodde M, Comploj E, et al. Intermediate-risk urothelial carcinoma: an unresolved problem? Urology. 2004 Mar;63(3):472-5. PMID: 15028440. EXC7

1162. Qin L, Zhong W, Zhang L, et al. Comparison of three methods for detecting epidermal growth factor receptor mutations in plasma DNA samples of Chinese patients with advanced non-small cell lung cancer. Chin Med J (Engl). 2011 Mar;124(6):887-91. PMID: 21518597. EXC6

1163. Qiu H, Sirivongs P, Rothenberger M, et al. Molecular prognostic factors in rectal cancer treated by radiation and surgery. Dis Colon Rectum. 2000 Apr;43(4):451-9. PMID: 10789738. EXC6

1164. Qiu J, Compagnone M, Laibe S, et al. BRAF p.Val600Glu (V600E) somatic mutation is mainly associated with MSS phenotype in metastatic colorectal cancer. Cancer Genomics Proteomics. 2011 Jan-Feb;8(1):15-8. PMID: 21289333. EXC6

1165. Quasar Collaborative G, Gray R, Barnwell J, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 2007 Dec 15;370(9604):2020-9. PMID: 18083404. EXC6

1166. Querings S, Altmuller J, Ansen S, et al. Benchmarking of mutation diagnostics in clinical lung cancer specimens. PLoS One. 2011;6(5):e19601. PMID: 21573178. EXC6

1167. Quinn E, Hawkins N, Yip YL, et al. CD103+ intraepithelial lymphocytes--a unique population in microsatellite unstable sporadic colorectal cancer. Eur J Cancer. 2003 Mar;39(4):469-75. PMID: 12751377. EXC6

1168. Raedle J, Brieger A, Trojan J, et al. Evaluation of rapid microsatellite analysis of paraffin-embedded specimens in screening for hereditary nonpolyposis colorectal cancer. Mod Pathol. 1999 May;12(5):485-91. PMID: 10349986. EXC4

1169. Raedle J, Schaffner M, Esser N, et al. Frequency of the Amsterdam criteria in a regional German cohort of patients with colorectal cancer. Z Gastroenterol. 2002 Aug;40(8):561-8. PMID: 12297979. EXC3

1170. Raedle J, Trojan J, Brieger A, et al. Bethesda guidelines: relation to microsatellite instability and MLH1 promoter methylation in patients with colorectal cancer. Ann Intern Med. 2001 Oct 16;135(8 Pt 1):566-76. PMID: 11601928. EXC6

1171. Raevaara TE, Korhonen MK, Lohi H, et al. Functional significance and clinical phenotype of nontruncating mismatch repair variants of MLH1. Gastroenterology. 2005 Aug;129(2):537-49. PMID: 16083711. EXC3

1172. Rajkumar T, Soumittra N, Pandey D, et al. Mutation analysis of hMSH2 and hMLH1 in colorectal cancer patients in India. Genet Test. 2004 Summer;8(2):157-62. PMID: 15345113. EXC3

1173. Rako I, Jakic-Razumovic J, Katalinic D, et al. Mutation pattern of KRAS and BRAF oncogenes in colorectal cancer patients. Neoplasma. 2012;59(4):376-83. PMID: 22489692. EXC6

1174. Ramalingam S, Forster J, Naret C, et al. Dual inhibition of the epidermal growth factor receptor with cetuximab, an IgG1 monoclonal antibody, and gefitinib, a tyrosine kinase inhibitor, in patients with refractory non-small cell lung cancer (NSCLC): a phase I study. J Thorac Oncol. 2008 Mar;3(3):258-64. PMID: 18317068. EXC6

1175. Ramalingam SS, Blackhall F, Krzakowski M, et al. Randomized phase II study of dacomitinib (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol. 2012;30(27):3337-44. EXC6

1176. Ramalingam SS, Spigel DR, Chen D, et al. Randomized phase II study of erlotinib in combination with placebo or R1507, a monoclonal antibody to insulin-like growth factor-1 receptor, for advanced-stage non-small-cell lung cancer. J Clin Oncol. 2011(34):4574-80. PMID: CN-00805089. EXC6

1177. Ramirez N, Bandres E, Navarro A, et al. Epigenetic events in normal colonic mucosa surrounding colorectal cancer lesions. Eur J Cancer. 2008 Nov;44(17):2689-95. PMID: 18938072. EXC6

1178. Ramsey SD, Burke W, Clarke L. An economic viewpoint on alternative strategies for identifying persons with hereditary nonpolyposis colorectal cancer (Structured abstract). Genetics in Medicine. 2003(5):353-36. PMID: NHSEED-22003009942. EXC4

1179. Ramsey SD, Clarke L, Etzioni R, et al. Cost-effectiveness of microsatellite instability screening as a method for detecting hereditary nonpolyposis colorectal cancer (Structured abstract). Ann Intern Med. 2001(8 Part 1):577-88. PMID: NHSEED-22001008256. EXC6

1180. Rao B, Gao Y, Huang J, et al. Mutations of p53 and K-ras correlate TF expression in human colorectal carcinomas: TF downregulation as a marker of poor prognosis. Int J Colorectal Dis. 2011 May;26(5):593-601. PMID: 21404058. EXC5

1181. Rao C, Hu Q, Ma J, et al. Comparison of the epidermal growth factor receptor protein expression between primary non-small cell lung cancer and paired lymph node metastases: implications for targeted nuclide radiotherapy. J Exp Clin Cancer Res. 2010;29:7. PMID: 20096104. EXC4

1182. Raptis S, Mrkonjic M, Green RC, et al. MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. J Natl Cancer Inst. 2007 Mar 21;99(6):463-74. PMID: 17374836. EXC6

1183. Rasuck CG, Leite SMO, Komatsuzaki F, et al. Association between methylation in mismatch repair genes, V600E BRAF mutation and microsatellite instability in colorectal cancer patients. Mol Biol Rep. 2012;39(3):2553-60. EXC6

1184. Rasul KI, Kerr DJ. QUASAR results: The prognostic validity of a colon cancer recurrence score and the role of multigene profiles in determining risk. Curr Colorectal Cancer Rep. 2010;6:144-7. EXC2

1185. Rawson JB, Manno M, Mrkonjic M, et al. Promoter methylation of Wnt antagonists DKK1 and SFRP1 is associated with opposing tumor subtypes in two large populations of colorectal cancer patients. Carcinogenesis. 2011;32(5):741-7. EXC4

1186. Ready N, Janne PA, Bogart J, et al. Chemoradiotherapy and gefitinib in stage III non-small cell lung cancer with epidermal growth factor receptor and KRAS mutation analysis: cancer and leukemia group B (CALEB) 30106, a CALGB-stratified phase II trial. J Thorac Oncol. 2010 Sep;5(9):1382-90. PMID: 20686428. EXC6

1187. Rebersek M, Boc M, Cerkovnik P, et al. Efficacy of first-line systemic treatment in correlation with BRAF V600E and different KRAS mutations in metastatic colorectal cancer-a single institution retrospective analysis. Radiology and Oncology. 2011;45(4):285-91. EXC3

1188. Rector TS, Taylor BC, Wilt TJ. Systematic review of prognostic tests Chapter 12 of Methods Guide for Medical Test Reviews. AHRQ Publication No. 12-EHC017. Rockville, MD: Agency for Healthcare Research and Quality; June 2012. www.effectivehealthcare.ahrq.gov/reports/final.cfm. Also published in a special supplement to the Journal of General Internal Medicine, July 2012. EXC2

1189. Reed SD, Dinan MA, Schulman KA, et al. Cost-effectiveness of the 21-gene recurrence score assay in the context of multifactorial decision making to guide chemotherapy for early-stage breast cancer. Genet Med. 2013 Mar;15(3):203-11. PMID: 22975761. EXC2

1190. Reid JF, Gariboldi M, Sokolova V, et al. Integrative approach for prioritizing cancer genes in sporadic colon cancer. Genes Chromosomes Cancer. 2009 Nov;48(11):953-62. PMID: 19672874. EXC4

1191. Reidy DL, Vakiani E, Fakih MG, et al. Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. J Clin Oncol. 2010 Sep 20;28(27):4240-6. PMID: 20713879. EXC4

1192. Reissmann PT, Koga H, Figlin RA, et al. Amplification and overexpression of the cyclin D1 and epidermal growth factor receptor genes in non-small-cell lung cancer. Lung Cancer Study Group. J Cancer Res Clin Oncol. 1999;125(2):61-70. PMID: 10190311. EXC4

1193. Rekhtman N, Brandt SM, Sigel CS, et al. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol. 2011 Mar;6(3):451-8. PMID: 21266922. EXC6

1194. Rekhtman N, Paik PK, Arcila ME, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. Clin Cancer Res. 2012 Feb 15;18(4):1167-76. PMID: 22228640. EXC6

1195. Ren S, Chen X, Kuang P, et al. Association of EGFR mutation or ALK rearrangement with expression of DNA repair and synthesis genes in never-smoker women with pulmonary adenocarcinoma. Cancer. 2012 Nov 15;118(22):5588-94. PMID: 22569898. EXC6

1196. Ren S, Kuang P, Zheng L, et al. Analysis of driver mutations in female non-smoker Asian patients with pulmonary adenocarcinoma. Cell Biochem Biophys. 2012 Nov;64(2):155-60. PMID: 22707299. EXC6

1197. Renouf DJ, Wood-Baker R, Ionescu DN, et al. BCL-2 expression is prognostic for improved survival in non-small cell lung cancer. J Thorac Oncol. 2009;4(4):486-91. EXC4

1198. Retel VP, Joore MA, Harten WH. Head-to-head comparison of the 70-gene signature versus the 21-gene assay: cost-effectiveness and the effect of compliance (Provisional abstract). Breast Cancer Res Treat. 2012(2):627-36. PMID: NHSEED-22012005553. EXC5

1199. Revuelta I, Moya-Rull D, Garcia-Herrera A, et al. Role of oncogenic pathways and KRAS/BRAF mutations in the behavior of colon adenocarcinoma in renal transplant patients. Transplantation. 2012 Mar 15;93(5):509-17. PMID: 22245873. EXC3

1200. Reyes CM, Allen BA, Terdiman JP, et al. Comparison of selection strategies for genetic testing of patients with hereditary nonpolyposis colorectal carcinoma: effectiveness and cost-effectiveness (Structured abstract). Cancer. 2002(9):1848-56. PMID: NHSEED-22002001891. EXC3

1201. . Impact of the Recurrence Score on adjuvant decision-making in ER-positive early breast cancer - results of a large prospective multicentre decision impact study in node negative and node positive disease. SABCS; 2011. Exc2

1202. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. The New England journal of medicine. 2003(3):247-57. PMID: CN-00439389. EXC3

1203. Ricciardiello L, Ceccarelli C, Angiolini G, et al. High thymidylate synthase expression in colorectal cancer with microsatellite instability: implications for chemotherapeutic strategies. Clin Cancer Res. 2005 Jun 1;11(11):4234-40. PMID: 15930362. EXC6

1204. Richardson CM, Richardson D, Swinson DE, et al. Cyclooxygenase-2 protein levels are independent of epidermal growth factor receptor expression or activation in operable non-small cell lung cancer. Lung Cancer. 2005 Apr;48(1):47-57. PMID: 15777970. EXC4

1205. Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol. 2009(35):5931-7. PMID: CN-00732427. EXC3

1206. Rickman OB, Vohra PK, Sanyal B, et al. Analysis of ErbB receptors in pulmonary carcinoid tumors. Clin Cancer Res. 2009 May 15;15(10):3315-24. PMID: 19447869. EXC6

1207. Riegert-Johnson DL, Johnson RA, Rabe KG, et al. The value of MUTYH testing in patients with early onset microsatellite stable colorectal cancer referred for hereditary nonpolyposis colon cancer syndrome testing. Genet Test. 2007 Winter;11(4):361-5. PMID: 18294051. EXC4

1208. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res. 2008 Sep 15;14(18):5731-4. PMID: 18794081. EXC4

1209. Riely GJ, Pao W, Pham D, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res. 2006 Feb 1;12(3 Pt 1):839-44. PMID: 16467097. EXC4

1210. Rigau V, Sebbagh N, Olschwang S, et al. Microsatellite instability in colorectal carcinoma. The comparison of immunohistochemistry and molecular biology suggests a role for hMSH6 [correction of hMLH6] immunostaining. Arch Pathol Lab Med. 2003 Jun;127(6):694-700. PMID: 12741892. EXC6

1211. Rijcken FE, van der Sluis T, Hollema H, et al. Hyperplastic polyps in hereditary nonpolyposis colorectal cancer. Am J Gastroenterol. 2003 Oct;98(10):2306-11. PMID: 14572584. EXC3

1212. Risinger JI, Barrett JC, Watson P, et al. Molecular genetic evidence of the occurrence of breast cancer as an integral tumor in patients with the hereditary nonpolyposis colorectal carcinoma syndrome. Cancer. 1996 May 1;77(9):1836-43. PMID: 8646682. EXC3

1213. Risio M, Malacarne D, Giaretti W. KRAS transitions and villous growth in colorectal adenomas. Cell Oncol. 2005;27(5-6):363-6. PMID: 16373972. EXC3

1214. Risques RA, Moreno V, Ribas M, et al. Genetic pathways and genome-wide determinants of clinical outcome in colorectal cancer. Cancer Res. 2003 Nov 1;63(21):7206-14. PMID: 14612515. EXC6

1215. Rizvi NA, Rusch V, Pao W, et al. Molecular characteristics predict clinical outcomes: prospective trial correlating response to the EGFR tyrosine kinase inhibitor gefitinib with the presence of sensitizing mutations in the tyrosine binding domain of the EGFR gene. Clin Cancer Res. 2011 May 15;17(10):3500-6. PMID: 21558399. EXC6

1216. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res. 2009 Aug 15;15(16):5216-23. PMID: 19671850. EXC6

1217. Rodriguez J, Zarate R, Bandres E, et al. Fc gamma receptor polymorphisms as predictive markers of Cetuximab efficacy in epidermal growth factor receptor downstream-mutated metastatic colorectal cancer. Eur J Cancer. 2012 Aug;48(12):1774-80. PMID: 22305465. EXC3

1218. Rohde F, Rimkus C, Friederichs J, et al. Expression of osteopontin, a target gene of de-regulated Wnt signaling, predicts survival in colon cancer. Int J Cancer. 2007 Oct 15;121(8):1717-23. PMID: 17565744. EXC4

1219. Roman R, Verdu M, Calvo M, et al. Microsatellite instability of the colorectal carcinoma can be predicted in the conventional pathologic examination. A prospective multicentric study and the statistical analysis of 615 cases consolidate our previously proposed logistic regression model. Virchows Arch. 2010 May;456(5):533-41. PMID: 20393748. EXC4

1220. Romanowicz-Makowska H, Smolarz B, Langner E, et al. Analysis of microsatellite instability and BRCA1 mutations in patients from hereditary nonpolyposis colorectal cancer (HNPCC) family. Pol J Pathol. 2005;56(1):21-6. PMID: 15921010. EXC3

1221. Roncucci L, Mora E, Mariani F, et al. Myeloperoxidase-positive cell infiltration in colorectal carcinogenesis as indicator of colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2008 Sep;17(9):2291-7. PMID: 18768495. EXC4

1222. Rose JS, Serna DS, Martin LK, et al. Influence of KRAS mutation status in metachronous and synchronous metastatic colorectal adenocarcinoma. Cancer. 2012 Dec 15;118(24):6243-52. PMID: 22674181. EXC3

1223. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012 Mar;13(3):239-46. PMID: 22285168. EXC4

1224. Rosell R, Molina MA, Costa C, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. Clin Cancer Res. 2011 Mar 1;17(5):1160-8. PMID: 21233402. EXC6

1225. Rosell R, Moran T, Cardenal F, et al. Predictive biomarkers in the management of EGFR mutant lung cancer. Ann N Y Acad Sci. 2010 Oct;1210:45-52. PMID: 20973798. EXC2

1226. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009 Sep 3;361(10):958-67. PMID: 19692684. EXC6

1227. Rosell R, Perez-Roca L, Sanchez JJ, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. PLoS One. 2009;4(5):e5133. PMID: 19415121. EXC5

1228. Rosell R, Robinet G, Szczesna A, et al. Randomized phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in EGFR-expressing advanced non-small-cell lung cancer. Ann Oncol. 2008 Feb;19(2):362-9. PMID: 17947225. EXC5

1229. Ross HJ, Blumenschein GR, Jr., Aisner J, et al. Randomized phase II multicenter trial of two schedules of lapatinib as first- or second-line monotherapy in patients with advanced or metastatic non-small cell lung cancer. Clin Cancer Res. 2010 Mar 15;16(6):1938-49. PMID: 20215545. EXC6

1230. Rosty C, Buchanan DD, Walsh MD, et al. Phenotype and polyp landscape in serrated polyposis syndrome: a series of 100 patients from genetics clinics. Am J Surg Pathol. 2012 Jun;36(6):876-82. PMID: 22510757. EXC3

1231. Rosty C, Chazal M, Etienne MC, et al. Determination of microsatellite instability, p53 and K-ras mutations in hepatic metastases from patients with colorectal cancer: Relationship with response to 5-fluorouracil and survival. Int J Cancer. 2001;95(3):162-7. EXC6

1232. Roupret M, Azzouzi AR, Cussenot O. Microsatellite instability and transitional cell carcinoma of the upper urinary tract. BJU Int. 2005 Sep;96(4):489-92. PMID: 16104897. EXC4

1233. Rovella V, Carrara S, Crucitti SC, et al. Familial microsatellite-stable non-polyposis colorectal cancer: incidence and characteristics in a clinic-based population. Ann Oncol. 2001 Jun;12(6):813-8. PMID: 11484957. EXC4

1234. Rozek LS, Lipkin SM, Fearon ER, et al. CDX2 polymorphisms, RNA expression, and risk of colorectal cancer. Cancer Res. 2005 Jul 1;65(13):5488-92. PMID: 15994917. EXC4

1235. Rudzki Z, Zazula M, Okon K, et al. Low-level microsatellite instability colorectal carcinomas: do they really belong to a "gray zone" between high-level microsatellite instability and microsatellite-stable cancers? Int J Colorectal Dis. 2003 May;18(3):216-21. PMID: 12673486. EXC6

1236. Ruibal A, Nunez MI, Rodriguez J, et al. Cytosolic levels of neuron-specific enolase in squamous cell carcinomas of the lung. Int J Biol Markers. 2003 Jul-Sep;18(3):188-94. PMID: 14535589. EXC4

1237. Ruiz MIG, Floor K, Steinberg SM, et al. Combined assessment of EGFR pathway-related molecular markers and prognosis of NSCLC patients. Br J Cancer. 2009;100(1):145-52. Exc6

1238. Ruiz MIG, Van Cruijsen H, Smit EF, et al. Genetic heterogeneity in patients with multiple neoplastic lung lesions: A report of three cases. J Thorac Oncol. 2007;2(1):12-21. EXC7

1239. Rumilla K, Schowalter KV, Lindor NM, et al. Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated Lynch syndrome cases. J Mol Diagn. 2011 Jan;13(1):93-9. PMID: 21227399. EXC3

1240. Rusch V, Baselga J, Cordon-Cardo C, et al. Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res. 1993 May 15;53(10 Suppl):2379-85. PMID: 7683573. EXC4

1241. Russell PA, Barnett SA, Walkiewicz M, et al. Correlation of mutation status and survival with predominant histologic subtype according to the new IASLC/ATS/ERS lung adenocarcinoma classification in stage III (N2) patients. J Thorac Oncol. 2013 Apr;8(4):461-8. PMID: 23486266. EXC7

1242. Russo A, Sala P, Alberici P, et al. Prognostic relevance of MLH1 and MSH2 mutations in hereditary non-polyposis colorectal cancer patients. Tumori. 2009 Nov-Dec;95(6):731-8. PMID: 20210238. EXC4

1243. Rutgers E, Piccart-Gebhart MJ, Bogaerts J, et al. Baseline results of the EORTC 10041/MINDACT TRIAL (Microarray In Node 0-3 positive Disease may Avoid ChemoTherapy). ASCO Gastrointestinal Symposium; 2013 San Francisco, CA. EXC6

1244. Rutgers E, Piccart-Gebhart MJ, Bogaerts J, et al. The EORTC 10041/BIG 03-04 MINDACT trial is feasible: results of the pilot phase. Eur J Cancer. 2011 Dec;47(18):2742-9. PMID: 22051734. EXC 6

1245. Rutten A, Burgdorf W, Hugel H, et al. Cystic sebaceous tumors as marker lesions for the Muir-Torre syndrome: a histopathologic and molecular genetic study. Am J Dermatopathol. 1999 Oct;21(5):405-13. PMID: 10535567. EXC4

1246. Ryan BM, Lefort F, McManus R, et al. A prospective study of circulating mutant KRAS2 in the serum of patients with colorectal neoplasia: strong prognostic indicator in postoperative follow up. Gut. 2003 Jan;52(1):101-8. PMID: 12477769. EXC4

1247. Ryan P, Mulligan AM, Aronson M, et al. Comparison of clinical schemas and morphologic features in predicting Lynch syndrome in mutation-positive patients with endometrial cancer encountered in the context of familial gastrointestinal cancer registries. Cancer. 2012 Feb 1;118(3):681-8. PMID: 21721000. EXC3

1248. Sahoo R, Harini VV, Babu VC, et al. Screening for EGFR mutations in lung cancer, a report from India. Lung Cancer. 2011 Sep;73(3):316-9. PMID: 21315473. EXC6

1249. Saif MW, Kaley K, Penney R, et al. The efficacy of gemcitabine as salvage treatment in patients with refractory advanced colorectal cancer (CRC): a single institution experience. Anticancer Res. 2011 Sep;31(9):2971-4. PMID: 21868546. EXC4

1250. Saisho S, Yasuda K, Maeda A, et al. Post-recurrence survival of patients with non-small-cell lung cancer after curative resection with or without induction/adjuvant chemotherapy. Interact Cardiovasc Thorac Surg. 2013 Feb;16(2):166-72. PMID: 23143203. EXC3

1251. Sakuma Y, Matsukuma S, Yoshihara M, et al. Epidermal growth factor receptor gene mutations in atypical adenomatous hyperplasias of the lung. Mod Pathol. 2007 Sep;20(9):967-73. PMID: 17618248. EXC3

1252. Sakuma Y, Matsukuma S, Yoshihara M, et al. Distinctive evaluation of nonmucinous and mucinous subtypes of bronchioloalveolar carcinomas in EGFR and K-ras gene-mutation analyses for Japanese lung adenocarcinomas: confirmation of the correlations with histologic subtypes and gene mutations. Am J Clin Pathol. 2007 Jul;128(1):100-8. PMID: 17580276. EXC6

1253. Salahshor S, Kressner U, Fischer H, et al. Microsatellite instability in sporadic colorectal cancer is not an independent prognostic factor. Br J Cancer. 1999 Sep;81(2):190-3. PMID: 10496341. EXC4

1254. Salam MA, Elnahhas T, Azim HA, et al. The effect of EGFR overexpression in inoperable non-small cell lung cancer (NSCLC) patients receiving cisplatin-vinorelbine combination. Turkish Journal of Cancer. 2006;36(1):11-8. EXC5

1255. Salazar F, Molina MA, Sanchez-Ronco M, et al. First-line therapy and methylation status of CHFR in serum influence outcome to chemotherapy versus EGFR tyrosine kinase inhibitors as second-line therapy in stage IV non-small-cell lung cancer patients. Lung Cancer. 2011 Apr;72(1):84-91. PMID: 20705357. EXC4

1256. Salazar R, Roepman P, Capella G, et al. Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. J Clin Oncol. 2011 Jan 1;29(1):17-24. PMID: 21098318. EXC4

1257. Salido M, Pijuan L, Martinez-Aviles L, et al. Increased ALK gene copy number and amplification are frequent in non-small cell lung cancer. J Thorac Oncol. 2011 Jan;6(1):21-7. PMID: 21107285. EXC6

1258. Salovaara R, Loukola A, Kristo P, et al. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. J Clin Oncol. 2000 Jun;18(11):2193-200. PMID: 10829038. EXC4

1259. Salto-Tellez M, Lee SC, Chiu LL, et al. Microsatellite instability in colorectal cancer: considerations for molecular diagnosis and high-throughput screening of archival tissues. Clin Chem. 2004 Jun;50(6):1082-6. PMID: 15161730. EXC4

1260. Salto-Tellez M, Tan SY, Chiu LL, et al. Dinucleotide microsatellite repeats are essential for the diagnosis of microsatellite instability in colorectal cancer in Asian patients. World J Gastroenterol. 2005 May 14;11(18):2781-3. PMID: 15884122. EXC6

1261. Saltz L, Badarinath S, Dakhil S, et al. Phase III trial of cetuximab, bevacizumab, and 5-fluorouracil/leucovorin vs. FOLFOX-bevacizumab in colorectal cancer. Clin Colorectal Cancer. 2012 Jun;11(2):101-11. PMID: 22055112. EXC4

1262. Salvesen HB, Macdonald N, Ryan A, et al. Methylation of hMLH1 in a population-based series of endometrial carcinomas. Clin Cancer Res. 2000;6(9):3607-13. EXC6

1263. Sameer AS, Chowdri NA, Syeed N, et al. SMAD4--molecular gladiator of the TGF-beta signaling is trampled upon by mutational insufficiency in colorectal carcinoma of Kashmiri population: an analysis with relation to KRAS proto-oncogene. BMC Cancer. 2010;10:300. PMID: 20565773. EXC4

1264. Sammoud S, Khiari M, Semeh A, et al. Relationship between expression of ras p21 oncoprotein and mutation status of the K-ras gene in sporadic colorectal cancer patients in Tunisia. Appl Immunohistochem Mol Morphol. 2012 Mar;20(2):146-52. PMID: 21768877. EXC6

1265. Samowitz WS, Albertsen H, Herrick J, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology. 2005 Sep;129(3):837-45. PMID: 16143123. EXC4

1266. Samowitz WS, Curtin K, Ma KN, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001 Sep;10(9):917-23. PMID: 11535541. EXC4

1267. Samowitz WS, Curtin K, Wolff RK, et al. The MLH1 -93 G>A promoter polymorphism and genetic and epigenetic alterations in colon cancer. Genes Chromosomes Cancer. 2008 Oct;47(10):835-44. PMID: 18615680. EXC4

1268. Samowitz WS, Curtin K, Wolff RK, et al. Microsatellite instability and survival in rectal cancer. Cancer Causes Control. 2009 Nov;20(9):1763-8. PMID: 19669908. EXC4

1269. Samowitz WS, Slattery ML. Transforming growth factor-beta receptor type 2 mutations and microsatellite instability in sporadic colorectal adenomas and carcinomas. Am J Pathol. 1997 Jul;151(1):33-5. PMID: 9212728. EXC6

1270. Samowitz WS, Slattery ML. Regional reproducibility of microsatellite instability in sporadic colorectal cancer. Genes Chromosomes Cancer. 1999 Oct;26(2):106-14. PMID: 10469448. EXC4

1271. Samowitz WS, Slattery ML, Kerber RA. Microsatellite instability in human colonic cancer is not a useful clinical indicator of familial colorectal cancer. Gastroenterology. 1995 Dec;109(6):1765-71. PMID: 7498640. EXC4

1272. Samowitz WS, Slattery ML, Sweeney C, et al. APC mutations and other genetic and epigenetic changes in colon cancer. Mol Cancer Res. 2007 Feb;5(2):165-70. PMID: 17293392. EXC6

1273. Samowitz WS, Wolff RK, Ma KN, et al. Polymorphisms in insulin-related genes predispose to specific KRAS2 and TP53 mutations in colon cancer. Mutat Res. 2006 Mar 20;595(1-2):117-24. PMID: 16448675. EXC6

1274. Sanchez JA, Krumroy L, Plummer S, et al. Genetic and epigenetic classifications define clinical phenotypes and determine patient outcomes in colorectal cancer. Br J Surg. 2009 Oct;96(10):1196-204. PMID: 19787768. EXC4

1275. Sanchez JA, Vogel JD, Kalady MF, et al. Identifying Lynch syndrome: we are all responsible. Dis Colon Rectum. 2008 Dec;51(12):1750-6. PMID: 18682882. EXC4

1276. Sanchez-de-Abajo A, de la Hoya M, van Puijenbroek M, et al. Molecular analysis of colorectal cancer tumors from patients with mismatch repair proficient hereditary nonpolyposis colorectal cancer suggests novel carcinogenic pathways. Clin Cancer Res. 2007 Oct 1;13(19):5729-35. PMID: 17908962. EXC6

1277. Sanchez-Navarro I, Gamez-Pozo A, Pinto A, et al. An 8-gene qRT-PCR-based gene expression score that has prognostic value in early breast cancer. BMC Cancer. 2010;10:336. PMID: 20584321. EXC5

1278. Sanders HR, Li HR, Bruey JM, et al. Exon scanning by reverse transcriptase-polymerase chain reaction for detection of known and novel EML4-ALK fusion variants in non-small cell lung cancer. Cancer Genet. 2011 Jan;204(1):45-52. PMID: 21356191. EXC5

1279. Sanford M, Scott LJ. Gefitinib: a review of its use in the treatment of locally advanced/metastatic non-small cell lung cancer. Drugs. 2009 Nov 12;69(16):2303-28. PMID: 19852530. EXC2

1280. Santarpia M, Magri I, Sanchez-Ronco M, et al. mRNA expression levels and genetic status of genes involved in the EGFR and NF-kappaB pathways in metastatic non-small-cell lung cancer patients. J Transl Med. 2011;9:163. PMID: 21951562. EXC6

1281. Santini D, Loupakis F, Vincenzi B, et al. High concordance of KRAS status between primary colorectal tumors and related metastatic sites: implications for clinical practice. Oncologist. 2008 Dec;13(12):1270-5. PMID: 19056857. EXC6

1282. Santis G, Angell R, Nickless G, et al. Screening for EGFR and KRAS mutations in endobronchial ultrasound derived transbronchial needle aspirates in non-small cell lung cancer using COLD-PCR. PLoS One. 2011;6(9):e25191. PMID: 21949883. EXC6

1283. Santoro A, Cavina R, Latteri F, et al. Activity of a specific inhibitor, gefitinib (Iressa, ZD1839), of epidermal growth factor receptor in refractory non-small-cell lung cancer. Ann Oncol. 2004 Jan;15(1):33-7. PMID: 14679116. EXC4

1284. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010 Jul 10;28(20):3219-26. PMID: 20498393. EXC4

1285. Saridaki Z, Papadatos-Pastos D, Tzardi M, et al. BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients outcome. Br J Cancer. 2010;102(12):1762-8. EXC3

1286. Saridaki Z, Tzardi M, Papadaki C, et al. Impact of KRAS, BRAF, PIK3CA mutations, PTEN, AREG, EREG expression and skin rash in >/= 2 line cetuximab-based therapy of colorectal cancer patients. PLoS One. 2011;6(1):e15980. PMID: 21283802. EXC3

1287. Sarkaria IS, Zakowski MF, Pham D, et al. Epidermal growth factor receptor signaling in adenocarcinomas with bronchioloalveolar components. Ann Thorac Surg. 2008 Jan;85(1):216-23. PMID: 18154814. EXC4

1288. Sarli L, Bottarelli L, Azzoni C, et al. Loss of p27 expression and microsatellite instability in sporadic colorectal cancer. Surg Oncol. 2006 Aug;15(2):97-106. PMID: 17123889. EXC4

1289. Sarli L, Bottarelli L, Bader G, et al. Association between recurrence of sporadic colorectal cancer, high level of microsatellite instability, and loss of heterozygosity at chromosome 18q. Dis Colon Rectum. 2004 Sep;47(9):1467-82. PMID: 15486743. EXC4

1290. Sarosdy MF, Kahn PR, Ziffer MD, et al. Use of a multitarget fluorescence in situ hybridization assay to diagnose bladder cancer in patients with hematuria. J Urol. 2006 Jul;176(1):44-7. PMID: 16753364. EXC3

1291. Sarosdy MF, Schellhammer P, Bokinsky G, et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol. 2002 Nov;168(5):1950-4. PMID: 12394683. EXC6

1292. Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. PLoS One. 2009;4(10):e7287. PMID: 19806185. EXC3

1293. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res. 2009 Mar 1;69(5):1851-7. PMID: 19223544. EXC3

1294. Sartori G, Bettelli S, Schirosi L, et al. Microsatellite and EGFR, HER2 and K-RAS analyses in sclerosing hemangioma of the lung. Am J Surg Pathol. 2007 Oct;31(10):1512-20. PMID: 17895751. EXC3

1295. Sartori G, Cavazza A, Sgambato A, et al. EGFR and K-ras mutations along the spectrum of pulmonary epithelial tumors of the lung and elaboration of a combined clinicopathologic and molecular scoring system to predict clinical responsiveness to EGFR inhibitors. Am J Clin Pathol. 2009 Apr;131(4):478-89. PMID: 19289583. EXC6

1296. Sasaki H, Endo K, Konishi A, et al. EGFR Mutation status in Japanese lung cancer patients: genotyping analysis using LightCycler. Clin Cancer Res. 2005 Apr 15;11(8):2924-9. PMID: 15837743. EXC4

1297. Sasaki H, Endo K, Okuda K, et al. Epidermal growth factor receptor gene amplification and gefitinib sensitivity in patients with recurrent lung cancer. J Cancer Res Clin Oncol. 2008 May;134(5):569-77. PMID: 17932690. EXC3

1298. Sasaki H, Hikosaka Y, Kawano O, et al. Evaluation of Kras gene mutation and copy number gain in non-small cell lung cancer. J Thorac Oncol. 2011 Jan;6(1):15-20. PMID: 21150464. EXC4

1299. Sasaki H, Kawano O, Endo K, et al. EGFRvIII mutation in lung cancer correlates with increased EGFR copy number. Oncol Rep. 2007 Feb;17(2):319-23. PMID: 17203167. EXC6

1300. Sasaki H, Okuda K, Endo K, et al. CCND1 messenger RNA expression is correlated with EGFR mutation status in lung cancer. Clin Lung Cancer. 2007 Sep;8(8):493-6. PMID: 17922974. EXC4

1301. Sasaki H, Okuda K, Kawano O, et al. Fibroblast growth factor receptor 4 mutation and polymorphism in Japanese lung cancer. Oncol Rep. 2008;20(5):1125-30. EXC4

1302. Sasaki H, Okuda K, Takada M, et al. A novel EGFR mutation D1012H and polymorphism at exon 25 in Japanese lung cancer. J Cancer Res Clin Oncol. 2008;134(12):1371-6. EXC3

1303. Sasaki H, Shimizu S, Endo K, et al. EGFR and erbB2 mutation status in Japanese lung cancer patients. Int J Cancer. 2006 Jan 1;118(1):180-4. PMID: 16003726. EXC7

1304. Sasaki H, Shitara M, Yokota K, et al. Overexpression of GLUT1 correlates with Kras mutations in lung carcinomas. Mol Med Report. 2012 Mar;5(3):599-602. PMID: 22200795. EXC4

1305. Sasaki H, Yukiue H, Mizuno K, et al. Elevated serum epidermal growth factor receptor level is correlated with lymph node metastasis in lung cancer. Int J Clin Oncol. 2003 Apr;8(2):79-82. PMID: 12720099. EXC4

1306. Sastre J, Gravalos C, Rivera F, et al. First-line cetuximab plus capecitabine in elderly patients with advanced colorectal cancer: clinical outcome and subgroup analysis according to KRAS status from a Spanish TTD Group Study. Oncologist. 2012;17(3):339-45. PMID: 22363067. EXC3

1307. Sato R, Suzuki T, Katayose Y, et al. Steroid sulfatase and estrogen sulfotransferase in colon carcinoma: regulators of intratumoral estrogen concentrations and potent prognostic factors. Cancer Res. 2009 Feb 1;69(3):914-22. PMID: 19141651. EXC4

1308. Savic S, Zlobec I, Thalmann GN, et al. The prognostic value of cytology and fluorescence in situ hybridization in the follow-up of nonmuscle-invasive bladder cancer after intravesical Bacillus Calmette-Guerin therapy. Int J Cancer. 2009 Jun 15;124(12):2899-904. PMID: 19230026. EXC7

1309. Sawhney MS, Farrar WD, Gudiseva S, et al. Microsatellite instability in interval colon cancers. Gastroenterology. 2006 Dec;131(6):1700-5. PMID: 17087932. EXC4

1310. Scagliotti GV, Novello S, Schiller JH, et al. Rationale and design of MARQUEE: a phase III, randomized, double-blind study of tivantinib plus erlotinib versus placebo plus erlotinib in previously treated patients with locally advanced or metastatic, nonsquamous, non-small-cell lung cancer. Clinical Lung Cancer. 2012(5):391-5. PMID: CN-00860018. EXC3

1311. Scartozzi M, Bianchi F, Rosati S, et al. Mutations of hMLH1 and hMSH2 in patients with suspected hereditary nonpolyposis colorectal cancer: correlation with microsatellite instability and abnormalities of mismatch repair protein expression. J Clin Oncol. 2002 Mar 1;20(5):1203-8. PMID: 11870161. EXC4

1312. Scartozzi M, Giampieri R, Maccaroni E, et al. Analysis of HER-3, insulin growth factor-1, nuclear factor-kB and epidermal growth factor receptor gene copy number in the prediction of clinical outcome for K-RAS wild-type colorectal cancer patients receiving irinotecan-cetuximab. Ann Oncol. 2012 Jul;23(7):1706-12. PMID: 22112971. EXC3

1313. Scartozzi M, Mandolesi A, Giampieri R, et al. Insulin-like growth factor 1 expression correlates with clinical outcome in K-RAS wild type colorectal cancer patients treated with cetuximab and irinotecan. Int J Cancer. 2010 Oct 15;127(8):1941-7. PMID: 20099280. EXC3

1314. Schafmayer C, Buch S, Volzke H, et al. Investigation of the colorectal cancer susceptibility region on chromosome 8q24.21 in a large German case-control sample. Int J Cancer. 2009 Jan 1;124(1):75-80. PMID: 18839428. EXC4

1315. Scheel SK, Porzner M, Pfeiffer S, et al. Mutations in the WTX-gene are found in some high-grade microsatellite instable (MSI-H) colorectal cancers. BMC Cancer. 2010;10:413. PMID: 20696052. EXC4

1316. Schiemann U, Gunther S, Gross M, et al. Preoperative serum levels of the carcinoembryonic antigen in hereditary non-polyposis colorectal cancer compared to levels in sporadic colorectal cancer. Cancer Detect Prev. 2005;29(4):356-60. PMID: 16122885. EXC4

1317. Schiller JH, von Pawel J, Schutt P, et al. Pemetrexed with or without matuzumab as second-line treatment for patients with stage IIIB/IV non-small cell lung cancer. J Thorac Oncol. 2010 Dec;5(12):1977-85. PMID: 20978446. EXC4

1318. Schimanski CC, Moehler M, Gockel I, et al. Expression of chemokine receptor CCR5 correlates with the presence of hepatic molecular metastases in K-ras positive human colorectal cancer. J Cancer Res Clin Oncol. 2011 Jul;137(7):1139-45. PMID: 21468700. EXC4

1319. Schimanski CC, Zimmermann T, Schmidtmann I, et al. K-ras mutation status correlates with the expression of VEGFR1, VEGFR2, and PDGFRalpha in colorectal cancer. Int J Colorectal Dis. 2010 Feb;25(2):181-6. PMID: 19936766. EXC6

1320. Schirosi L, Lantuejoul S, Cavazza A, et al. Pleuro-pulmonary solitary fibrous tumors: a clinicopathologic, immunohistochemical, and molecular study of 88 cases confirming the prognostic value of de Perrot staging system and p53 expression, and evaluating the role of c-kit, BRAF, PDGFRs (alpha/beta), c-met, and EGFR. Am J Surg Pathol. 2008 Nov;32(11):1627-42. PMID: 18753943. EXC3

1321. Schittenhelm MM, Kollmannsberger C, Oechsle K, et al. Molecular determinants of response to matuzumab in combination with paclitaxel for patients with advanced non-small cell lung cancer. Mol Cancer Ther. 2009 Mar;8(3):481-9. PMID: 19276157. EXC6

1322. Schlegel J, Bocker T, Zirngibl H, et al. Detection of microsatellite instability in human colorectal carcinomas using a non-radioactive PCR-based screening technique. Virchows Arch. 1995;426(3):223-7. PMID: 7773500. EXC4

1323. Schlomer BJ, Ho R, Sagalowsky A, et al. Prospective validation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. J Urol. 2010 Jan;183(1):62-7. PMID: 19913822. EXC3

1324. Schmid K, Oehl N, Wrba F, et al. EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res. 2009 Jul 15;15(14):4554-60. PMID: 19584155. EXC6

1325. Schnabel PA, Smit E, Carpeno Jde C, et al. Influence of histology and biomarkers on first-line treatment of advanced non-small cell lung cancer in routine care setting: baseline results of an observational study (FRAME). Lung Cancer. 2012 Dec;78(3):263-9. PMID: 23040326. EXC7

1326. Schneider R, Schneider C, Kloor M, et al. Lynch syndrome: clinical, pathological, and genetic insights. Langenbecks Arch Surg. 2012 Apr;397(4):513-25. PMID: 22362054. EXC7

1327. Schoen RE. Families at risk for colorectal cancer: risk assessment and genetic testing. J Clin Gastroenterol. 2000 Sep;31(2):114-20. PMID: 10993425. EXC7

1328. Schofield L, Goldblatt J, Iacopetta B. Challenges in the diagnosis and management of Lynch Syndrome in an Indigenous family living in a remote West Australian community. Rural Remote Health. 2011;11(4):1836. PMID: 22188021. EXC7

1329. Schofield L, Watson N, Grieu F, et al. Population-based detection of Lynch syndrome in young colorectal cancer patients using microsatellite instability as the initial test. Int J Cancer. 2009 Mar 1;124(5):1097-102. PMID: 19072991. EXC4

1330. Scholtka B, Schneider M, Melcher R, et al. A gene marker panel covering the Wnt and the Ras-Raf-MEK-MAPK signalling pathways allows to detect gene mutations in 80% of early (UICC I) colon cancer stages in humans. Cancer Epidemiol. 2009 Aug;33(2):123-9. PMID: 19679059. EXC4

1331. Schulmann K, Brasch FE, Kunstmann E, et al. HNPCC-associated small bowel cancer: Clinical and molecular characteristics. Gastroenterology. 2005;128(3):590-9. EXC3

1332. Schulmann K, Hahn SA, Brasch F, et al. Microsatellite instability and expression of MLH1 and MSH2 in carcinomas of the small intestine [2] (multiple letters). Cancer. 2003;98(8):1774-6. EXC3

1333. Schulmann K, Mori Y, Croog V, et al. Molecular phenotype of inflammatory bowel disease-associated neoplasms with microsatellite instability. Gastroenterology. 2005 Jul;129(1):74-85. PMID: 16012936. EXC4

1334. Scott LD. Ethical issues in genetic testing. Am J Gastroenterol. 2004;99(10):1871-3. EXC2

1335. Sedivy R, Wolf B, Kalipciyan M, et al. Genetic analysis of multiple synchronous lesions of the colon adenoma-carcinoma sequence. Br J Cancer. 2000 Apr;82(7):1276-82. PMID: 10755401. EXC2

1336. Segal G, Liebermann N, Klang S, et al. [Identification of K-RAS mutations in colorectal cancer patients in Israel]. Harefuah. 2011 May;150(5):447-50, 91. PMID: 21678640. EXC2

1337. Sehofield L, Watson N, Grieu F, et al. Population-based detection of lynch syndrome in young colorectal cancer patients using microsatellite instability as the initial test. Int J Cancer. 2009;124(5):1097-102. EXC6

1338. Sekine A, Kato T, Hagiwara E, et al. Metastatic brain tumors from non-small cell lung cancer with EGFR mutations: distinguishing influence of exon 19 deletion on radiographic features. Lung Cancer. 2012 Jul;77(1):64-9. PMID: 22335887. EXC6

1339. Selamat SA, Chung BS, Girard L, et al. Genome-scale analysis of DNA methylation in lung adenocarcinoma and integration with mRNA expression. Genome Res. 2012 Jul;22(7):1197-211. PMID: 22613842. EXC4

1340. Selvaggi G, Novello S, Torri V, et al. Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol. 2004 Jan;15(1):28-32. PMID: 14679115. EXC4

1341. Sengupta SB, Yiu CY, Boulos PB, et al. Genetic instability in patients with metachronous colorectal cancers. Br J Surg. 1997 Jul;84(7):996-1000. PMID: 9240146. EXC4

1342. Senkus E, Kyriakides S, Penault-Llorca F, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi7-23. PMID: 23970019. EXC7

1343. Sequist LV, Gettinger S, Senzer NN, et al. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J Clin Oncol. 2010 Nov 20;28(33):4953-60. PMID: 20940188. EXC6

1344. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol. 2008 May 20;26(15):2442-9. PMID: 18458038. EXC7

1345. Sequist LV, von Pawel J, Garmey EG, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol. 2011 Aug 20;29(24):3307-15. PMID: 21768463. EXC3

1346. Serrano M, Lage P, Belga S, et al. Bethesda criteria for microsatellite instability testing: impact on the detection of new cases of Lynch syndrome. Fam Cancer. 2012 Dec;11(4):571-8. PMID: 22776989. EXC3

1347. Seya T, Tanaka N, Shinji S, et al. Squamous cell carcinoma arising from recurrent anal fistula. J Nippon Med Sch. 2007 Aug;74(4):319-24. PMID: 17878704. EXC2

1348. Shah L, Walter KL, Borczuk AC, et al. Expression of syndecan-1 and expression of epidermal growth factor receptor are associated with survival in patients with nonsmall cell lung carcinoma. Cancer. 2004 Oct 1;101(7):1632-8. PMID: 15378500. EXC4

1349. Shannon B, Gnanasampanthan S, Beilby J, et al. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut. 2002;50(4):520-4. EXC4

1350. Shannon C, Kirk J, Barnetson R, et al. Incidence of microsatellite instability in synchronous tumors of the ovary and endometrium. Clin Cancer Res. 2003;9(4):1387-92. EXC6

1351. Shaozhang Z, Xiaomei L, Aiping Z, et al. Detection of EML4-ALK fusion genes in non-small cell lung cancer patients with clinical features associated with EGFR mutations. Genes Chromosomes Cancer. 2012 Oct;51(10):925-32. PMID: 22736493. EXC6

1352. Sharif S, O'Connell MJ. Gene Signatures in Stage II Colon Cancer: A Clinical Review. Curr Colorectal Cancer Rep. 2012 Sep;8(3):225-31. PMID: 23002390. EXC2

1353. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol. 2009 Sep 10;27(26):4247-53. PMID: 19667264. EXC7

1354. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol. 2011 Oct;12(11):1004-12. PMID: 21933749. EXC5

1355. Shemirani AI, Haghighi MM, Milanizadeh S, et al. The role of kras mutations and MSI status in diagnosis of colorectal cancer. Gastroenterology and Hepatology from Bed to Bench. 2011;4(2):70-5. EXC6

1356. Shen H, Yuan Y, Hu HG, et al. Clinical significance of K-ras and BRAF mutations in Chinese colorectal cancer patients. World J Gastroenterol. 2011 Feb 14;17(6):809-16. PMID: 21390154. EXC6

1357. Shen L, Kondo Y, Hamilton SR, et al. P14 methylation in human colon cancer is associated with microsatellite instability and wild-type p53. Gastroenterology. 2003 Mar;124(3):626-33. PMID: 12612901. EXC4

1358. Shen L, Toyota M, Kondo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. Proc Natl Acad Sci U S A. 2007 Nov 20;104(47):18654-9. PMID: 18003927. EXC6

1359. Sheng JQ, Chan TL, Chan YW, et al. Microsatellite instability and novel mismatch repair gene mutations in northern Chinese population with hereditary non-polyposis colorectal cancer. Chin J Dig Dis. 2006;7(4):197-205. PMID: 17054581. EXC4

1360. Shetty S, Thomas P, Ramanan B, et al. Kras mutations and p53 overexpression in pseudomyxoma peritonei: association with phenotype and prognosis. J Surg Res. 2013 Mar;180(1):97-103. PMID: 23199549. EXC6

1361. Shi X, Li J, Zhao C, et al. Methylation analysis of hMLH1 gene promoter by a bisulfite-sensitive single-strand conformation polymorphism-capillary electrophoresis method. Biomed Chromatogr. 2006 Aug;20(8):815-20. PMID: 16358356. EXC6

1362. Shia J, Black D, Hummer AJ, et al. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. Hum Pathol. 2008;39(1):116-25. EXC5

1363. Shia J, Ellis NA, Paty PB, et al. Value of histopathology in predicting microsatellite instability in hereditary nonpolyposis colorectal cancer and sporadic colorectal cancer. Am J Surg Pathol. 2003 Nov;27(11):1407-17. PMID: 14576473. EXC6

1364. Shia J, Klimstra DS, Nafa K, et al. Value of immunohistochemical detection of DNA mismatch repair proteins in predicting germline mutation in hereditary colorectal neoplasms. Am J Surg Pathol. 2005 Jan;29(1):96-104. PMID: 15613860. EXC4

1365. Shibata T, Hanada S, Kokubu A, et al. Gene expression profiling of epidermal growth factor receptor/KRAS pathway activation in lung adenocarcinoma. Cancer Science. 2007;98(7):985-91. EXC5

1366. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res. 2005 Mar 1;65(5):1642-6. PMID: 15753357. EXC6

1367. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res. 2005;65(5):1642-6. EXC6

1368. Shima K, Morikawa T, Baba Y, et al. MGMT promoter methylation, loss of expression and prognosis in 855 colorectal cancers. Cancer Causes Control. 2011 Feb;22(2):301-9. PMID: 21140203. EXC4

1369. Shima K, Morikawa T, Yamauchi M, et al. TGFBR2 and BAX mononucleotide tract mutations, microsatellite instability, and prognosis in 1072 colorectal cancers. PLoS One. 2011;6(9):e25062. PMID: 21949851. EXC4

1370. Shima K, Nosho K, Baba Y, et al. Prognostic significance of CDKN2A (p16) promoter methylation and loss of expression in 902 colorectal cancers: Cohort study and literature review. Int J Cancer. 2011 Mar 1;128(5):1080-94. PMID: 20473920. EXC6

1371. Shin HJC, Thorson P, Gu J, et al. Detection of a subset of CD30+ anaplastic large cell lymphoma by interphase fluorescence in situ hybridization. Diagn Cytopathol. 2003;29(2):61-6. EXC3

1372. Shin KH, Park YJ, Park JG. Mutational analysis of the transforming growth factor beta receptor type II gene in hereditary nonpolyposis colorectal cancer and early-onset colorectal cancer patients. Clin Cancer Res. 2000 Feb;6(2):536-40. PMID: 10690536. EXC4

1373. Shin Y, Kim IJ, Kang HC, et al. A functional polymorphism (-347 G-->GA) in the E-cadherin gene is associated with colorectal cancer. Carcinogenesis. 2004 Nov;25(11):2173-6. PMID: 15231691. EXC4

1374. Shiozawa T, Ishii G, Goto K, et al. Clinicopathological characteristics of EGFR mutated adenosquamous carcinoma of the lung. Pathol Int. 2013 Feb;63(2):77-84. PMID: 23464964. EXC7

1375. Shiroiwa T, Motoo Y, Tsutani K. Cost-effectiveness analysis of KRAS testing and cetuximab as last-line therapy for colorectal cancer (Structured abstract). Molecular Diagnosis and Therapy. 2010(6):375-84. PMID: NHSEED-22011000410. EXC6

1376. Shitara K, Ura T, Matsuo K, et al. Sensitivity to previous irinotecan treatment does not predict the efficacy of combination chemotherapy with cetuximab plus irinotecan for wild-type KRAS metastatic colorectal cancer. Eur J Cancer. 2011 Dec;47(18):2673-80. PMID: 21652203. EXC4

1377. Shitara K, Yokota T, Takahari D, et al. Phase II study of combination chemotherapy with irinotecan and cetuximab for pretreated metastatic colorectal cancer harboring wild-type KRAS. Invest New Drugs. 2011 Aug;29(4):688-93. PMID: 20072801. EXC4

1378. Shitara K, Yuki S, Yoshida M, et al. Phase II study of combination chemotherapy with biweekly cetuximab and irinotecan for wild-type KRAS metastatic colorectal cancer refractory to irinotecan, oxaliplatin, and fluoropyrimidines. Invest New Drugs. 2012 Apr;30(2):787-93. PMID: 21174225. EXC4

1379. Shitara M, Sasaki H, Yokota K, et al. Polymorphisms in intron 1 of the EGFR gene in non-small cell lung cancer patients. Experimental and Therapeutic Medicine. 2012;4(5):785-9. EXC5

1380. Shoji F, Yano T, Yoshino I, et al. The characteristics and failure pattern of gefitinib responders with postoperative recurrence of pulmonary adenocarcinoma. Eur J Surg Oncol. 2008 Jan;34(1):89-93. PMID: 17449217. EXC5

1381. Shoji F, Yoshino I, Yano T, et al. Serum carcinoembryonic antigen level is associated with epidermal growth factor receptor mutations in recurrent lung adenocarcinomas. Cancer. 2007 Dec 15;110(12):2793-8. PMID: 17941001. EXC4

1382. Sholl LM, Weremowicz S, Gray SW, et al. Combined use of ALK immunohistochemistry and FISH for optimal detection of ALK-rearranged lung adenocarcinomas. J Thorac Oncol. 2013 Mar;8(3):322-8. PMID: 23407557. EXC5

1383. Sholl LM, Xiao Y, Joshi V, et al. EGFR mutation is a better predictor of response to tyrosine kinase inhibitors in non-small cell lung carcinoma than FISH, CISH, and immunohistochemistry. Am J Clin Pathol. 2010 Jun;133(6):922-34. PMID: 20472851. EXC6

1384. Sholl LM, Yeap BY, Iafrate AJ, et al. Lung adenocarcinoma with EGFR amplification has distinct clinicopathologic and molecular features in never-smokers. Cancer Res. 2009 Nov 1;69(21):8341-8. PMID: 19826035. EXC4

1385. Shukuya T, Takahashi T, Naito T, et al. Continuous EGFR-TKI administration following radiotherapy for non-small cell lung cancer patients with isolated CNS failure. Lung Cancer. 2011 Dec;74(3):457-61. PMID: 21571388. EXC5

1386. Siah SP, Quinn DM, Bennett GD, et al. Microsatellite instability markers in breast cancer: a review and study showing MSI was not detected at 'BAT 25' and 'BAT 26' microsatellite markers in early-onset breast cancer. Breast Cancer Res Treat. 2000 Mar;60(2):135-42. PMID: 10845276. EXC4

1387. Silver A, Sengupta N, Propper D, et al. A distinct DNA methylation profile associated with microsatellite and chromosomal stable sporadic colorectal cancers. Int J Cancer. 2012 Mar 1;130(5):1082-92. PMID: 21455990. EXC4

1388. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst. 2009 Nov 4;101(21):1446-52. PMID: 19815849. EXC7

1389. Simone G, Mangia A, Malfettone A, et al. Chromogenic in situ hybridization to detect EGFR gene copy number in cell blocks from fine-needle aspirates of non small cell lung carcinomas and lung metastases from colo-rectal cancer. J Exp Clin Cancer Res. 2010;29:125. PMID: 20843314. EXC4

1390. Simonetti S, Molina MA, Queralt C, et al. Detection of EGFR mutations with mutation-specific antibodies in stage IV non-small-cell lung cancer. J Transl Med. 2010;8:135. PMID: 21167064. EXC6

1391. Sinicrope F, Foster NR, Sargent DJ, et al. Model-based prediction of defective DNA mismatch repair using clinicopathological variables in sporadic colon cancer patients. Cancer. 2010 Apr 1;116(7):1691-8. PMID: 20186699. EXC4

1392. Sinicrope FA, Foster NR, Thibodeau SN, et al. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst. 2011 Jun 8;103(11):863-75. PMID: 21597022. EXC4

1393. Sinicrope FA, Rego RL, Garrity-Park MM, et al. Alterations in cell proliferation and apoptosis in colon cancers with microsatellite instability. Int J Cancer. 2007 Mar 15;120(6):1232-8. PMID: 17187355. EXC4

1394. Sinn DH, Chang DK, Kim YH, et al. Effectiveness of each Bethesda marker in defining microsatellite instability when screening for Lynch syndrome. Hepatogastroenterology. 2009 May-Jun;56(91-92):672-6. PMID: 19621678. EXC4

1395. Slattery ML, Curtin K, Schaffer D, et al. Associations between family history of colorectal cancer and genetic alterations in tumors. Int J Cancer. 2002 Feb 20;97(6):823-7. PMID: 11857362. EXC6

1396. Slattery ML, Curtin K, Wolff R, et al. PPARgamma and colon and rectal cancer: associations with specific tumor mutations, aspirin, ibuprofen and insulin-related genes (United States). Cancer Causes Control. 2006 Apr;17(3):239-49. PMID: 16489531. EXC4

1397. Slattery ML, Curtin K, Wolff RK, et al. A comparison of colon and rectal somatic DNA alterations. Dis Colon Rectum. 2009 Jul;52(7):1304-11. PMID: 19571709. EXC4

1398. Slattery ML, Herrick J, Curtin K, et al. Increased risk of colon cancer associated with a genetic polymorphism of SMAD7. Cancer Res. 2010 Feb 15;70(4):1479-85. PMID: 20124488. EXC6

1399. Slattery ML, Herrick JS, Lundgreen A, et al. Genetic variation in a metabolic signaling pathway and colon and rectal cancer risk: mTOR, PTEN, STK11, RPKAA1, PRKAG2, TSC1, TSC2, PI3K and Akt1. Carcinogenesis. 2010 Sep;31(9):1604-11. PMID: 20622004. EXC6

1400. Slattery ML, Wolff RK, Herrick J, et al. Tumor markers and rectal cancer: support for an inflammation-related pathway. Int J Cancer. 2009 Oct 1;125(7):1698-704. PMID: 19452524. EXC6

1401. Slodkowska EA, Ross JS. MammaPrint 70-gene signature: another milestone in personalized medical care for breast cancer patients. Expert Rev Mol Diagn. 2009 Jul;9(5):417-22. PMID: 19580427. EXC2

1402. Smartt P. A comparison of gene expression profiling tests for breast cancer (Structured abstract). Health Technology Assessment Database. 2010(3)PMID: HTA-32010001703. EXC1

1403. Smetana GW, Umscheid CA, Chang S, et al. Methods guide for authors of systematic reviews of medical tests: A collaboration between the Agency for Healthcare Research and Quality (AHRQ) and the Journal of General Internal Medicine. J Gen Intern Med. 2012 Jun;27 Suppl 1:S1-3. PMID: 22648668. EXC2

1404. Smit EF, Dingemans AM, Thunnissen FB, et al. Sorafenib in patients with advanced non-small cell lung cancer that harbor K-ras mutations: a brief report. J Thorac Oncol. 2010 May;5(5):719-20. PMID: 20421765. EXC5

1405. Smith GD, Bentz JS. "FISHing" to detect urinary and other cancers: validation of an imaging system to aid in interpretation. Cancer Cytopathol. 2010 Feb 25;118(1):56-64. PMID: 20099312. EXC6

1406. Smits AJ, Kummer JA, Hinrichs JW, et al. EGFR and KRAS mutations in lung carcinomas in the Dutch population: increased EGFR mutation frequency in malignant pleural effusion of lung adenocarcinoma. Cell Oncol (Dordr). 2012 Jun;35(3):189-96. PMID: 22528563. EXC6

1407. Smits KM, Paranjape T, Nallur S, et al. A let-7 microRNA SNP in the KRAS 3'UTR is prognostic in early-stage colorectal cancer. Clin Cancer Res. 2011 Dec 15;17(24):7723-31. PMID: 21994416. EXC4

1408. Smouse JH, Cibas ES, Janne PA, et al. EGFR mutations are detected comparably in cytologic and surgical pathology specimens of nonsmall cell lung cancer. Cancer. 2009 Feb 25;117(1):67-72. PMID: 19347832. EXC4

1409. Soda M, Isobe K, Inoue A, et al. A prospective PCR-based screening for the EML4-ALK oncogene in non-small cell lung cancer. Clin Cancer Res. 2012 Oct 15;18(20):5682-9. PMID: 22908099. EXC5

1410. Soh J, Toyooka S, Aoe K, et al. Usefulness of EGFR mutation screening in pleural fluid to predict the clinical outcome of gefitinib treated patients with lung cancer. Int J Cancer. 2006 Nov 15;119(10):2353-8. PMID: 16921488. EXC4

1411. Soh J, Toyooka S, Ichihara S, et al. Sequential molecular changes during multistage pathogenesis of small peripheral adenocarcinomas of the lung. J Thorac Oncol. 2008 Apr;3(4):340-7. PMID: 18379350. EXC6

1412. Sohn BS, Kim TW, Lee JL, et al. The role of KRAS mutations in predicting the efficacy of cetuximab-plus-irinotecan therapy in irinotecan-refractory Korean metastatic colorectal cancer patients. Oncology. 2009;77(3-4):224-30. PMID: 19738388. EXC3

1413. Soliman AS, Bondy ML, El-Badawy SA, et al. Contrasting molecular pathology of colorectal carcinoma in Egyptian and Western patients. Br J Cancer. 2001 Sep 28;85(7):1037-46. PMID: 11592777. EXC4

1414. Soliman PT, Broaddus RR, Schmeler KM, et al. Women with synchronous primary cancers of the endometrium and ovary: do they have Lynch syndrome? J Clin Oncol. 2005 Dec 20;23(36):9344-50. PMID: 16361634. EXC3

1415. Solin LJ. The Practical Use of Molecular Profiling: In Situ Carcinoma of the Breast: DCIS; 2012. EXC6

1416. Solin LJ, Gray R, Baehner FL, et al. A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast. J Natl Cancer Inst. 2013 May 15;105(10):701-10. PMID: 23641039. EXC7

1417. Solomon SB, Zakowski MF, Pao W, et al. Core needle lung biopsy specimens: adequacy for EGFR and KRAS mutational analysis. AJR Am J Roentgenol. 2010 Jan;194(1):266-9. PMID: 20028932. EXC4

1418. Song MJ, Lee HM, Kim SH. Clinical usefulness of fluorescence in situ hybridization for diagnosis and surveillance of bladder cancer. Cancer Genet Cytogenet. 2010;198(2):144-50. EXC5

1419. Sonnweber B, Dlaska M, Skvortsov S, et al. High predictive value of epidermal growth factor receptor phosphorylation but not of EGFRvIII mutation in resected stage I non-small cell lung cancer (NSCLC). J Clin Pathol. 2006;59(3):255-9. EXC4

1420. Sonobe M, Date H, Wada H, et al. Prognostic factors after complete resection of pN2 non-small cell lung cancer. J Thorac Cardiovasc Surg. 2013 Oct;146(4):788-95. PMID: 23810113. EXC7

1421. Sonobe M, Kobayashi M, Ishikawa M, et al. Impact of KRAS and EGFR gene mutations on recurrence and survival in patients with surgically resected lung adenocarcinomas. Ann Surg Oncol. 2012 Jul;19 Suppl 3:S347-54. PMID: 21607772. EXC5

1422. Sood A, McClain D, Maitra R, et al. PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer. Clin Colorectal Cancer. 2012 Jun;11(2):143-50. PMID: 22285706. EXC3

1423. Soreide K, Nedrebo BS, Soreide JA, et al. Lymph node harvest in colon cancer: influence of microsatellite instability and proximal tumor location. World J Surg. 2009 Dec;33(12):2695-703. PMID: 19823901. EXC4

1424. Soreide K, Soreide JA, Korner H. Prognostic role of carcinoembryonic antigen is influenced by microsatellite instability genotype and stage in locally advanced colorectal cancers. World J Surg. 2011 Apr;35(4):888-94. PMID: 21301835. EXC4

1425. Soria JC, Baselga J, Hanna N, et al. Phase I-IIa study of BMS-690514, an EGFR, HER-2 and -4 and VEGFR-1 to -3 oral tyrosine kinase inhibitor, in patients with advanced or metastatic solid tumours. Eur J Cancer. 2013 May;49(8):1815-24. PMID: 23490650. EXC2

1426. Souder C, Leitzel K, Ali SM, et al. Serum epidermal growth factor receptor/HER-2 predicts poor survival in patients with metastatic breast cancer. Cancer. 2006;107(10):2337-45. EXC4

1427. Souglakos J, Philips J, Wang R, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer. 2009 Aug 4;101(3):465-72. PMID: 19603024. EXC3

1428. Soulie P, Fourme E, Hamelin R, et al. TP53 status and gene amplification in human colorectal carcinomas. Cancer Genet Cytogenet. 1999 Dec;115(2):118-22. PMID: 10598144. EXC4

1429. Soung YH, Lee JW, Kim SY, et al. Somatic mutations of the ERBB4 kinase domain in human cancers. Int J Cancer. 2006;118(6):1426-9. EXC6

1430. South CD, Yearsley M, Martin E, et al. Immunohistochemistry staining for the mismatch repair proteins in the clinical care of patients with colorectal cancer. Genet Med. 2009 Nov;11(11):812-7. PMID: 19752738. EXC4

1431. Southey MC, Jenkins MA, Mead L, et al. Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. J Clin Oncol. 2005 Sep 20;23(27):6524-32. PMID: 16116158. EXC4

1432. . 10-year update of E2197: Phase III doxorubicin/docetaxel (AT) versus doxorubicin/cyclophosphamide (AC) adjuvant treatment of LN+ and high-risk LN- breast cancer and the comparison of the prognostic utility of the 21-gene recurrence score (RS) with clinicopathologic features. ASCO; 2010. EXC7

1433. Spathis A, Georgoulakis J, Foukas P, et al. KRAS and BRAF mutation analysis from liquid-based cytology brushings of colorectal carcinoma in comparison with formalin-fixed, paraffin-embedded tissue. Anticancer Res. 2010 Jun;30(6):1969-75. PMID: 20651341. EXC4

1434. Spigel DR, Burris HA, 3rd, Greco FA, et al. Randomized, double-blind, placebo-controlled, phase II trial of sorafenib and erlotinib or erlotinib alone in previously treated advanced non-small-cell lung cancer. J Clin Oncol. 2011 Jun 20;29(18):2582-9. PMID: 21576636. EXC3

1435. Spindler KL, Pallisgaard N, Lindebjerg J, et al. EGFR related mutational status and association to clinical outcome of third-line cetuximab-irinotecan in metastatic colorectal cancer. BMC Cancer. 2011;11:107. PMID: 21439039. EXC3

1436. Spindler KL, Pallisgaard N, Vogelius I, et al. Quantitative cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. Clin Cancer Res. 2012 Feb 15;18(4):1177-85. PMID: 22228631. EXC4

1437. Srivastava A, Redston M, Farraye FA, et al. Hyperplastic/serrated polyposis in inflammatory bowel disease: a case series of a previously undescribed entity. Am J Surg Pathol. 2008 Feb;32(2):296-303. PMID: 18223333. EXC3

1438. Stabile LP, Rothstein ME, Keohavong P, et al. Targeting of both the c-Met and EGFR pathways results in additive inhibition of lung tumorigenesis in transgenic mice. Cancers. 2010;2(4):2153-70. EXC4

1439. Steiner H, Bergmeister M, Verdorfer I, et al. Early results of bladder-cancer screening in a high-risk population of heavy smokers. BJU Int. 2008 Aug;102(3):291-6. PMID: 18336612. EXC3

1440. Stella GM, Scabini R, Inghilleri S, et al. EGFR and KRAS mutational profiling in fresh non-small cell lung cancer (NSCLC) cells. J Cancer Res Clin Oncol. 2013 Aug;139(8):1327-35. PMID: 23644698. EXC6

1441. Stigliano V, Assisi D, Cosimelli M, et al. Survival of hereditary non-polyposis colorectal cancer patients compared with sporadic colorectal cancer patients. J Exp Clin Cancer Res. 2008;27:39. PMID: 18803843. EXC4

1442. Stintzing S, Fischer von Weikersthal L, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer-subgroup analysis of patients with KRAS: mutated tumours in the randomised German AIO study KRK-0306. Ann Oncol. 2012 Jul;23(7):1693-9. PMID: 22219013. EXC4

1443. Stone JG, Robertson D, Houlston RS. Immunohistochemistry for MSH2 and MHL1: a method for identifying mismatch repair deficient colorectal cancer. J Clin Pathol. 2001 Jun;54(6):484-7. PMID: 11376026. EXC4

1444. Storojeva I, Boulay JL, Heinimann K, et al. Prognostic and predictive relevance of microsatellite instability in colorectal cancer. Oncol Rep. 2005 Jul;14(1):241-9. PMID: 15944796. EXC4

1445. Straver ME, Glas AM, Hannemann J, et al. The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat. 2010 Feb;119(3):551-8. PMID: 19214742. EXC6

1446. Strul H, Liberman E, Kariv R, et al. Prospective assessment of microsatellite instability in gastrointestinal neoplasia in Ashkenazi and non-Ashkenazi Jews. J Med. 2003;34(1-6):139-48. PMID: 17682319. EXC3

1447. . Comparison of Oncotype DX Recurrence Scores between surgical and core biopsy specimens in breast cancer patients. SABCS; 2011. EXC5

1448. Suehara Y, Arcila M, Wang L, et al. Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. Clin Cancer Res. 2012 Dec 15;18(24):6599-608. PMID: 23052255. EXC4

1449. Suehiro Y, Wong CW, Chirieac LR, et al. Epigenetic-genetic interactions in the APC/WNT, RAS/RAF, and P53 pathways in colorectal carcinoma. Clin Cancer Res. 2008 May 1;14(9):2560-9. PMID: 18451217. EXC6

1450. Suehisa H, Toyooka S, Hotta K, et al. Epidermal growth factor receptor mutation status and adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. J Clin Oncol. 2007 Sep 1;25(25):3952-7. PMID: 17761979. EXC6

1451. Suenaga M, Matsusaka S, Ueno M, et al. Predictors of the efficacy of FOLFIRI plus bevacizumab as second-line treatment in metastatic colorectal cancer patients. Surg Today. 2011 Aug;41(8):1067-74. PMID: 21773895. EXC4

1452. Sueoka-Aragane N, Imai K, Komiya K, et al. Exon 19 of EGFR mutation in relation to the CA-repeat polymorphism in intron 1. Cancer Sci. 2008 Jun;99(6):1180-7. PMID: 18422739. EXC6

1453. Sugai T, Habano W, Jiao YF, et al. Analysis of allelic imbalances at multiple cancer-related chromosomal loci and microsatellite instability within the same tumor using a single tumor gland from colorectal carcinomas. Int J Cancer. 2005 Apr 10;114(3):337-45. PMID: 15578702. EXC4

1454. Sugai T, Habano W, Nakamura S, et al. Analysis of Ki-ras gene mutations associated with DNA diploid, aneuploid, and multiploid colorectal carcinomas using a crypt isolation technique. Cytometry. 2001 Dec 15;46(6):345-50. PMID: 11754204. EXC4

1455. Sugai T, Habano W, Nakamura S, et al. Genetic alterations in DNA diploid, aneuploid and multiploid colorectal carcinomas identified by the crypt isolation technique. Int J Cancer. 2000 Nov 15;88(4):614-9. PMID: 11058879. EXC4

1456. Sugai T, Habano W, Uesugi N, et al. Molecular validation of the modified Vienna classification of colorectal tumors. J Mol Diagn. 2002 Nov;4(4):191-200. PMID: 12411586. EXC4

1457. Sugai T, Takahashi H, Habano W, et al. Analysis of genetic alterations, classified according to their DNA ploidy pattern, in the progression of colorectal adenomas and early colorectal carcinomas. J Pathol. 2003 Jun;200(2):168-76. PMID: 12754737. EXC4

1458. Sugano M, Nagasaka T, Sasaki E, et al. HNF4alpha as a marker for invasive mucinous adenocarcinoma of the lung. Am J Surg Pathol. 2013 Feb;37(2):211-8. PMID: 23108025. EXC4

1459. Sugimoto T, Ohta M, Ikenoue T, et al. Macroscopic morphologic subtypes of laterally spreading colorectal tumors showing distinct molecular alterations. Int J Cancer. 2010 Oct 1;127(7):1562-9. PMID: 20091866. EXC4

1460. Sugio K, Uramoto H, Onitsuka T, et al. Prospective phase II study of gefitinib in non-small cell lung cancer with epidermal growth factor receptor gene mutations. Lung Cancer. 2009 Jun;64(3):314-8. PMID: 18992959. EXC6

1461. Sun JM, Rampal S, Lee G, et al. Real world impact of epidermal growth factor receptor mutation status on treatment patterns in patients with non-small cell lung cancer. Lung Cancer. 2013 May;80(2):191-6. PMID: 23384673. EXC6

1462. Sun JM, Won YW, Kim ST, et al. The different efficacy of gefitinib or erlotinib according to epidermal growth factor receptor exon 19 and exon 21 mutations in Korean non-small cell lung cancer patients. J Cancer Res Clin Oncol. 2011 Apr;137(4):687-94. PMID: 20552223. EXC3

1463. Sun PL, Seol H, Lee HJ, et al. High incidence of EGFR mutations in Korean men smokers with no intratumoral heterogeneity of lung adenocarcinomas: correlation with histologic subtypes, EGFR/TTF-1 expressions, and clinical features. J Thorac Oncol. 2012 Feb;7(2):323-30. PMID: 22237264. EXC6

1464. Sun Y, Goodison S, Li J, et al. Improved breast cancer prognosis through the combination of clinical and genetic markers. Bioinformatics. 2007;23(1):30-7. EXC7

1465. Sunde L, Bisgaard ML, Soll-Johanning H, et al. Familial colorectal cancer, can it be identified by microsatellite instability and chromosomal instability? - A case-control study. Cancer Biomark. 2009;5(4):197-205. PMID: 19729829. EXC3

1466. Sung MT, Zhang S, Lopez-Beltran A, et al. Urothelial carcinoma following augmentation cystoplasty: an aggressive variant with distinct clinicopathological characteristics and molecular genetic alterations. Histopathology. 2009 Aug;55(2):161-73. PMID: 19694823. EXC3

1467. Suspitsin EN, Levchenko EV, Moiseyenko FV, et al. Rapid symptomatic improvement in gefitinib-treated patients with EGFR-mutated lung cancer: possible role of downregulation of inflammatory molecules? Onkologie. 2011;34(10):559-60. PMID: 21985857. EXC7

1468. Sutter C, Dallenbach-Hellweg G, Schmidt D, et al. Molecular analysis of endometrial hyperplasia in HNPCC-suspicious patients may predict progression to endometrial carcinoma. Int J Gynecol Pathol. 2004 Jan;23(1):18-25. PMID: 14668545. EXC4

1469. Suwinski R, Klusek A, Tyszkiewicz T, et al. Gene expression from bronchoscopy obtained tumour samples as a predictor of outcome in advanced inoperable lung cancer. PLoS One. 2012;7(7). EXC4

1470. Suzuki M, Shigematsu H, Hiroshima K, et al. Epidermal growth factor receptor expression status in lung cancer correlates with its mutation. Hum Pathol. 2005 Oct;36(10):1127-34. PMID: 16226114. EXC6

1471. Suzuki M, Shigematsu H, Iizasa T, et al. Exclusive mutation in epidermal growth factor receptor gene, HER-2, and KRAS, and synchronous methylation of nonsmall cell lung cancer. Cancer. 2006 May 15;106(10):2200-7. PMID: 16598760. EXC6

1472. Suzuki M, Shigematsu H, Nakajima T, et al. Synchronous alterations of Wnt and epidermal growth factor receptor signaling pathways through aberrant methylation and mutation in non small cell lung cancer. Clin Cancer Res. 2007 Oct 15;13(20):6087-92. PMID: 17947472. EXC7

1473. Suzuki S, Dobashi Y, Sakurai H, et al. Protein overexpression and gene amplification of epidermal growth factor receptor in nonsmall cell lung carcinomas. An immunohistochemical and fluorescence in situ hybridization study. Cancer. 2005 Mar 15;103(6):1265-73. PMID: 15712203. EXC5

1474. Svatek RS, Sagalowsky AI, Lotan Y. Economic impact of screening for bladder cancer using bladder tumor markers: a decision analysis. Urol Oncol. 2006 Jul-Aug;24(4):338-43. PMID: 16818188. EXC2

1475. Svrcek M, El-Bchiri J, Chalastanis A, et al. Specific clinical and biological features characterize inflammatory bowel disease associated colorectal cancers showing microsatellite instability. J Clin Oncol. 2007 Sep 20;25(27):4231-8. PMID: 17878476. EXC3

1476. Sweeney C, Boucher KM, Samowitz WS, et al. Oncogenetic tree model of somatic mutations and DNA methylation in colon tumors. Genes Chromosomes Cancer. 2009 Jan;48(1):1-9. PMID: 18767147. EXC4

1477. Swinson DE, Cox G, O'Byrne KJ. Coexpression of epidermal growth factor receptor with related factors is associated with a poor prognosis in non-small-cell lung cancer. Br J Cancer. 2004 Oct 4;91(7):1301-7. PMID: 15365565. EXC4

1478. Sylvester BE, Huo D, Khramtsov A, et al. Molecular analysis of colorectal tumors within a diverse patient cohort at a single institution. Clin Cancer Res. 2012 Jan 15;18(2):350-9. PMID: 22114137. EXC6

1479. . Evaluation of variables that may impact the use of Oncotype DX testing. ASCO; 2012. EXC6

1480. Taga M, Mechanic LE, Hagiwara N, et al. EGFR somatic mutations in lung tumors: Radon exposure and passive smoking in former- and never-smoking U.S. women. Cancer Epidemiology Biomarkers and Prevention. 2012;21(6):988-92. EXC5

1481. Tahara T, Inoue N, Hisamatsu T, et al. Clinical significance of microsatellite instability in the inflamed mucosa for the prediction of colonic neoplasms in patients with ulcerative colitis. J Gastroenterol Hepatol. 2005 May;20(5):710-5. PMID: 15853983. EXC4

1482. Taillade L, Penault-Llorca F, Boulet T, et al. Immunohistochemichal expression of biomarkers: a comparative study between diagnostic bronchial biopsies and surgical specimens of non-small-cell lung cancer. Ann Oncol. 2007 Jun;18(6):1043-50. PMID: 17355950. EXC4

1483. Takagi S, Kinouchi Y, Hiwatashi N, et al. Relationship between microsatellite instability and telomere shortening in colorectal cancer. Dis Colon Rectum. 2000 Oct;43(10 Suppl):S12-7. PMID: 11052472. EXC5

1484. Takahashi T, Nosho K, Yamamoto H, et al. Flat-type colorectal advanced adenomas (laterally spreading tumors) have different genetic and epigenetic alterations from protruded-type advanced adenomas. Mod Pathol. 2007 Jan;20(1):139-47. PMID: 17143260. EXC3

1485. Takamochi K, Oh S, Matsuoka J, et al. Clonality status of multifocal lung adenocarcinomas based on the mutation patterns of EGFR and K-ras. Lung Cancer. 2012 Mar;75(3):313-20. PMID: 22209037. EXC6

1486. Takano K, Ichikawa Y, Ueno E, et al. Microsatellite instability and expression of mismatch repair genes in sporadic endometrial cancer coexisting with colorectal or breast cancer. Oncol Rep. 2005 Jan;13(1):11-6. PMID: 15583795. EXC4

1487. Takano T, Fukui T, Ohe Y, et al. EGFR mutations predict survival benefit from gefitinib in patients with advanced lung adenocarcinoma: a historical comparison of patients treated before and after gefitinib approval in Japan. J Clin Oncol. 2008 Dec 1;26(34):5589-95. PMID: 18794545. EXC3

1488. Takano T, Ohe Y, Sakamoto H, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol. 2005 Oct 1;23(28):6829-37. PMID: 15998907. EXC3

1489. Takano T, Ohe Y, Tsuta K, et al. Epidermal growth factor receptor mutation detection using high-resolution melting analysis predicts outcomes in patients with advanced non small cell lung cancer treated with gefitinib. Clin Cancer Res. 2007 Sep 15;13(18 Pt 1):5385-90. PMID: 17875767. EXC3

1490. Takeda K, Kinoshita I, Shimizu Y, et al. Expression of LGR5, an intestinal stem cell marker, during each stage of colorectal tumorigenesis. Anticancer Res. 2011 Jan;31(1):263-70. PMID: 21273608. EXC4

1491. Takeda M, Okamoto I, Sakai K, et al. Successful long-term treatment with pemetrexed of NSCLC associated with EML4-ALK and low thymidylate synthase expression. Clinical lung cancer. 2012;13(2):157-9. EXC7

1492. Takemoto N, Konishi F, Yamashita K, et al. The correlation of microsatellite instability and tumor-infiltrating lymphocytes in hereditary non-polyposis colorectal cancer (HNPCC) and sporadic colorectal cancers: the significance of different types of lymphocyte infiltration. Jpn J Clin Oncol. 2004 Feb;34(2):90-8. PMID: 15067103. EXC4

1493. Tam IY, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. Clin Cancer Res. 2006 Mar 1;12(5):1647-53. PMID: 16533793. EXC6

1494. Tam IY, Leung EL, Tin VP, et al. Double EGFR mutants containing rare EGFR mutant types show reduced in vitro response to gefitinib compared with common activating missense mutations. Mol Cancer Ther. 2009 Aug;8(8):2142-51. PMID: 19671738. EXC6

1495. Tanaka H, Deng G, Matsuzaki K, et al. BRAF mutation, CpG island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. Int J Cancer. 2006 Jun 1;118(11):2765-71. PMID: 16381005. EXC4

1496. Tanaka M, Chang P, Li Y, et al. Association of CHFR promoter methylation with disease recurrence in locally advanced colon cancer. Clin Cancer Res. 2011 Jul 1;17(13):4531-40. PMID: 21551253. EXC4

1497. Tanaka N, Huttenhower C, Nosho K, et al. Novel application of structural equation modeling to correlation structure analysis of CpG island methylation in colorectal cancer. Am J Pathol. 2010 Dec;177(6):2731-40. PMID: 21037082. EXC4

1498. Tanaka T, Watanabe T, Kitayama J, et al. Chromosome 18q deletion as a novel molecular predictor for colorectal cancer with simultaneous hepatic metastasis. Diagn Mol Pathol. 2009 Dec;18(4):219-25. PMID: 19861895. EXC4

1499. Tang R, Wang JY, Fan CW, et al. p53 is an independent pre-treatment markers for long-term survival in stage II and III colorectal cancers: an analysis of interaction between genetic markers and fluorouracil-based adjuvant therapy. Cancer Lett. 2004 Jul 8;210(1):101-9. PMID: 15172127. EXC6

1500. Tang X, Varella-Garcia M, Xavier AC, et al. Epidermal growth factor receptor abnormalities in the pathogenesis and progression of lung adenocarcinomas. Cancer Prev Res (Phila). 2008 Aug;1(3):192-200. PMID: 19138956. EXC6

1501. Taniguchi K, Uchida J, Nishino K, et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. Clin Cancer Res. 2011 Dec 15;17(24):7808-15. PMID: 21976538. EXC4

1502. Tapia C, Glatz K, Obermann EC, et al. Evaluation of chromosomal aberrations in patients with benign conditions and reactive changes in urinary cytology. Cancer Cytopathol. 2011 Dec 25;119(6):404-10. PMID: 21732550. EXC5

1503. Taron M, Ichinose Y, Rosell R, et al. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. Clin Cancer Res. 2005 Aug 15;11(16):5878-85. PMID: 16115929. EXC3

1504. Tejpar S, Celik I, Schlichting M, et al. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. J Clin Oncol. 2012 Oct 10;30(29):3570-7. PMID: 22734028. EXC6

1505. Teng HW, Huang YC, Lin JK, et al. BRAF mutation is a prognostic biomarker for colorectal liver metastasectomy. J Surg Oncol. 2012 Aug 1;106(2):123-9. PMID: 22331825. EXC3

1506. Terdiman JP, Levin TR, Allen BA, et al. Hereditary nonpolyposis colorectal cancer in young colorectal cancer patients: high-risk clinic versus population-based registry. Gastroenterology. 2002 Apr;122(4):940-7. PMID: 11910346. EXC4

1507. Tessem MB, Selnaes KM, Sjursen W, et al. Discrimination of patients with microsatellite instability colon cancer using 1H HR MAS MR spectroscopy and chemometric analysis. J Proteome Res. 2010 Jul 2;9(7):3664-70. PMID: 20507057. EXC4

1508. Thomassen M, Tan Q, Eiriksdottir F, et al. Prediction of metastasis from low-malignant breast cancer by gene expression profiling. Int J Cancer. 2007 Mar 1;120(5):1070-5. PMID: 17131339. EXC5

1509. Tian S, Roepman P, Popovici V, et al. A robust genomic signature for the detection of colorectal cancer patients with microsatellite instability phenotype and high mutation frequency. J Pathol. 2012 December;228(4):586-95. PMID: 2012737386 FULL TEXT LINK http://dx.doi.org/10.1002/path.4092. EXC4

1510. Tikidzhieva A, Benner A, Michel S, et al. Microsatellite instability and Beta2-Microglobulin mutations as prognostic markers in colon cancer: results of the FOGT-4 trial. Br J Cancer. 2012 Mar 13;106(6):1239-45. PMID: 22353804. EXC4

1511. Timmermann B, Kerick M, Roehr C, et al. Somatic mutation profiles of MSI and MSS colorectal cancer identified by whole exome next generation sequencing and bioinformatics analysis. PLoS One. 2010;5(12):e15661. PMID: 21203531. EXC4

1512. Tiseo M, Capelletti M, De Palma G, et al. Epidermal growth factor receptor intron-1 polymorphism predicts gefitinib outcome in advanced non-small cell lung cancer. J Thorac Oncol. 2008 Oct;3(10):1104-11. PMID: 18827605. EXC3

1513. Tiseo M, Rossi G, Capelletti M, et al. Predictors of gefitinib outcomes in advanced non-small cell lung cancer (NSCLC): study of a comprehensive panel of molecular markers. Lung Cancer. 2010 Mar;67(3):355-60. PMID: 19473722. EXC3

1514. Togashi Y, Masago K, Kubo T, et al. Association between vascular-poor area of primary tumors and epidermal growth factor receptor gene status in advanced lung adenocarcinoma. Med Oncol. 2012 Dec;29(5):3169-75. PMID: 22492281. EXC6

1515. Togashi Y, Masago K, Kubo T, et al. Association of diffuse, random pulmonary metastases, including miliary metastases, with epidermal growth factor receptor mutations in lung adenocarcinoma. Cancer. 2011;117(4):819-25. EXC3

1516. Togo G, Okamoto M, Shiratori Y, et al. Does mutation of transforming growth factor-beta type II receptor gene play an important role in colorectal polyps? Dig Dis Sci. 1999 Sep;44(9):1803-9. PMID: 10505718. EXC3

1517. . The first report of the association between the recurrence score and the risk of recurrence in a Japanese population (JBCRG-TR 003). Kyoto Breast Cancer Consensus Conference; 2009. EXC9

1518. Toi M, Iwata H, Yamanaka T, et al. Clinical significance of the 21-gene signature (Oncotype DX) in hormone receptor-positive early stage primary breast cancer in the Japanese population. Cancer. 2010 Jul 1;116(13):3112-8. PMID: 20564629. EXC7

1519. Tokumo M, Toyooka S, Kiura K, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res. 2005 Feb 1;11(3):1167-73. PMID: 15709185. EXC6

1520. Tol J, Dijkstra JR, Klomp M, et al. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. Eur J Cancer. 2010 Jul;46(11):1997-2009. PMID: 20413299. EXC6

1521. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med. 2009 Feb 5;360(6):563-72. PMID: 19196673. EXC6

1522. Tommasi S, Pinto R, Petriella D, et al. Oncosuppressor methylation: a possible key role in colon metastatic progression. J Cell Physiol. 2011 Jul;226(7):1934-9. PMID: 21506124. EXC4

1523. Tougeron D, Fauquembergue E, Rouquette A, et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol. 2009 Sep;22(9):1186-95. PMID: 19503063. EXC4

1524. Toyooka S, Matsuo K, Shigematsu H, et al. The impact of sex and smoking status on the mutational spectrum of epidermal growth factor receptor gene in non small cell lung cancer. Clin Cancer Res. 2007 Oct 1;13(19):5763-8. PMID: 17908966. EXC6

1525. Toyooka S, Takano T, Kosaka T, et al. Epidermal growth factor receptor mutation, but not sex and smoking, is independently associated with favorable prognosis of gefitinib-treated patients with lung adenocarcinoma. Cancer Science. 2008;99(2):303-8. EXC3

1526. Toyooka S, Tokumo M, Shigematsu H, et al. Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. Cancer Res. 2006;66(3):1371-5. EXC5

1527. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer. 2011;117(20):4623-32. EXC3

1528. Trano G, Sjursen W, Wasmuth HH, et al. Performance of clinical guidelines compared with molecular tumour screening methods in identifying possible Lynch syndrome among colorectal cancer patients: a Norwegian population-based study. Br J Cancer. 2010 Feb 2;102(3):482-8. PMID: 20051945. EXC7

1529. Trautmann K, Terdiman JP, French AJ, et al. Chromosomal instability in microsatellite-unstable and stable colon cancer. Clin Cancer Res. 2006 Nov 1;12(21):6379-85. PMID: 17085649. EXC6

1530. Tresallet C, Brouquet A, Julie C, et al. Evaluation of predictive models in daily practice for the identification of patients with Lynch syndrome. Int J Cancer. 2012 Mar 15;130(6):1367-77. PMID: 21520036. EXC7

1531. Trevisiol C, Di Fabio F, Nascimbeni R, et al. Prognostic value of circulating KRAS2 gene mutations in colorectal cancer with distant metastases. Int J Biol Markers. 2006 Oct-Dec;21(4):223-8. PMID: 17177160. EXC4

1532. Trojan J, Raedle J, Herrmann G, et al. Detection of microsatellite instability from archival, hematoxylin-eosin-stained colorectal cancer specimen. Arch Pathol Lab Med. 2002 Feb;126(2):202-4. PMID: 11825119. EXC2

1533. Truninger K, Menigatti M, Luz J, et al. Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterology. 2005 May;128(5):1160-71. PMID: 15887099. EXC6

1534. Truta B, Chen YY, Blanco AM, et al. Tumor histology helps to identify Lynch syndrome among colorectal cancer patients. Fam Cancer. 2008;7(3):267-74. PMID: 18283560. EXC4

1535. Tsai JR, Wang HM, Liu PL, et al. High expression of heme oxygenase-1 is associated with tumor invasiveness and poor clinical outcome in non-small cell lung cancer patients. Cell Oncol (Dordr). 2012 Dec;35(6):461-71. PMID: 23055342. EXC6

1536. Tsai TH, Su KY, Wu SG, et al. RNA is favourable for analysing EGFR mutations in malignant pleural effusion of lung cancer. Eur Respir J. 2012 Mar;39(3):677-84. PMID: 21719485. EXC6

1537. Tsai TH, Wu SG, Chang YL, et al. Effusion immunocytochemistry as an alternative approach for the selection of first-line targeted therapy in advanced lung adenocarcinoma. J Thorac Oncol. 2012 Jun;7(6):993-1000. PMID: 22525557. EXC4

1538. Tsakiridis T, Cutz JC, Singh G, et al. Association of phosphorylated epidermal growth factor receptor with survival in patients with locally advanced non-small cell lung cancer treated with radiotherapy. J Thorac Oncol. 2008 Jul;3(7):716-22. PMID: 18594316. EXC4

1539. Tsao AS, Tang XM, Sabloff B, et al. Clinicopathologic characteristics of the EGFR gene mutation in non-small cell lung cancer. J Thorac Oncol. 2006 Mar;1(3):231-9. PMID: 17409862. EXC6

1540. Tsiatis AC, Norris-Kirby A, Rich RG, et al. Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: Diagnostic and clinical implications. J Molec Diagnost. 2010;12(4):425-32. EXC5

1541. Tsoi DT, Inoue M, Kelly CM, et al. Cost-effectiveness analysis of recurrence score-guided treatment using a 21-gene assay in early breast cancer. Oncologist. 2010;15(5):457-65. PMID: 20421264. EXC6

1542. Tsunematsu Y, Yoshizawa Y, Miyauchi J, et al. A novel case of Wilms' tumor followed by colon cancer, both showing microsatellite instability. Oncology. 2000 Feb;58(2):159-60. PMID: 10705243. EXC2

1543. Tug E, Balaban YH, Sahin EK. Mapping of microsatellite instability in endoscopic normal colon. Genet Test Mol Biomarkers. 2012 May;16(5):388-95. PMID: 22224632. EXC6

1544. Tuononen K, Maki-Nevala S, Sarhadi VK, et al. Comparison of targeted next-generation sequencing (NGS) and real-time PCR in the detection of EGFR, KRAS, and BRAF mutations on formalin-fixed, paraffin-embedded tumor material of non-small cell lung carcinoma-superiority of NGS. Genes Chromosomes Cancer. 2013 May;52(5):503-11. PMID: 23362162. EXC6

1545. Tuononen K, Sarhadi VK, Wirtanen A, et al. Targeted resequencing reveals ALK fusions in non-small cell lung carcinomas detected by FISH, immunohistochemistry, and real-time RT-PCR: a comparison of four methods. Biomed Res Int. 2013;2013:757490. PMID: 23484153. EXC4

1546. Tveit KM, Guren T, Glimelius B, et al. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. J Clin Oncol. 2012 May 20;30(15):1755-62. PMID: 22473155. EXC7

1547. Ueda E, Watanabe T, Ishigami H, et al. Microsatellite instability of colorectal cancer and adenoma in synchronous multiple colorectal cancer patients with associated extracolonic malignancies. Surg Today. 2001;31(5):405-9. PMID: 11381503. EXC4

1548. Ueda E, Watanabe T, Umetani N, et al. Microsatellite instability of cancers and concomitant adenomas in synchronous multiple colorectal cancer patients. J Exp Clin Cancer Res. 2002 Jun;21(2):149-54. PMID: 12148569. EXC4

1549. Ueno T, Toyooka S, Suda K, et al. Impact of age on epidermal growth factor receptor mutation in lung cancer. Lung Cancer. 2012 Dec;78(3):207-11. PMID: 23036155. EXC6

1550. Ulivi P, Capelli L, Valgiusti M, et al. Predictive role of multiple gene alterations in response to cetuximab in metastatic colorectal cancer: A single center study. Journal of Translational Medicine. 2012;10(1). EXC3

1551. Ulivi P, Romagnoli M, Chiadini E, et al. Assessment of EGFR and K-ras mutations in fixed and fresh specimens from transesophageal ultrasound-guided fine needle aspiration in non-small cell lung cancer patients. Int J Oncol. 2012 Jul;41(1):147-52. PMID: 22504767. EXC7

1552. Ulrich CM, Curtin K, Samowitz W, et al. MTHFR variants reduce the risk of G:C->A:T transition mutations within the p53 tumor suppressor gene in colon tumors. J Nutr. 2005 Oct;135(10):2462-7. PMID: 16177213. EXC6

1553. Umeda Y, Nagasaka T, Mori Y, et al. Poor prognosis of KRAS or BRAF mutant colorectal liver metastasis without microsatellite instability. J Hepatobiliary Pancreat Sci. 2013 Feb;20(2):223-33. PMID: 23010994. EXC3

1554. Umemura S, Tsubouchi K, Yoshioka H, et al. Clinical outcome in patients with leptomeningeal metastasis from non-small cell lung cancer: Okayama Lung Cancer Study Group. Lung Cancer. 2012 Jul;77(1):134-9. PMID: 22487432. EXC7

1555. Uramoto H, Iwata T, Onitsuka T, et al. Epithelial-mesenchymal transition in EGFR-TKI acquired resistant lung adenocarcinoma. Anticancer Res. 2010 Jul;30(7):2513-7. PMID: 20682976. EXC6

1556. Uramoto H, So T, Nagata Y, et al. Correlation between HLA alleles and EGFR mutation in Japanese patients with adenocarcinoma of the lung. J Thorac Oncol. 2010 Aug;5(8):1136-42. PMID: 20548248. EXC6

1557. Uramoto H, Yamada S, Tanaka F. Angiogenesis of lung cancer utilizes existing blood vessels rather than developing new vessels using signals from carcinogenesis. Anticancer Res. 2013 May;33(5):1913-6. PMID: 23645738. EXC6

1558. Uramoto H, Yamada T, Yano S, et al. Prognostic value of aquired resistance-related molecules in Japanese patients with NSCLC treated with an EGFR-TKI. Anticancer Res. 2012;32(9):3785-90. EXC4

1559. Uramoto H, Yamada T, Yano S, et al. Prognostic value of acquired resistance-related molecules in Japanese patients with NSCLC treated with an EGFR-TKI. Anticancer Res. 2012 Sep;32(9):3785-90. PMID: 22993320. EXC6

1560. Vacirca J, Tsai MC, Brufsky AM, et al. Initial results from the 21-gene Breast Cancer Assay Registry: A prospective observational study in patients (pts) with ER+, early stage invasive breast cancer (EBC). EXC9

1561. Vaguliene N, Zemaitis M, Sarauskas V, et al. The role of mutation status of the epidermal growth factor receptor gene in advanced non-small cell lung cancer. Medicina (Kaunas). 2012;48(4):175-81. PMID: 22836289. EXC6

1562. Valentini AM, Renna L, Armentano R, et al. Mismatch repair, p53 and (beta)-catenin proteins in colorectal cancer. Anticancer Res. 2002;22(4):2083-8. EXC4

1563. Valle L, Carbonell P, Fernandez V, et al. MLH1 germline epimutations in selected patients with early-onset non-polyposis colorectal cancer. Clin Genet. 2007;71(3):232-7. EXC6

1564. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 2009;360(14):1408-17. EXC6

1565. Van Cutsem E, Kohne CH, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011 May 20;29(15):2011-9. PMID: 21502544. EXC7

1566. Van Cutsem E, Tejpar S, Vanbeckevoort D, et al. Intrapatient cetuximab dose escalation in metastatic colorectal cancer according to the grade of early skin reactions: the randomized EVEREST study. J Clin Oncol. 2012 Aug 10;30(23):2861-8. PMID: 22753904. EXC7

1567. van der Drift MA, Prinsen CF, Knuiman GJ, et al. Diagnosing peripheral lung cancer: the additional value of the Ras-association domain family 1A gene methylation and Kirsten rat sarcoma 2 viral oncogene homolog mutation analyses in washings in nondiagnostic bronchoscopy. Chest. 2012 Jan;141(1):169-75. PMID: 21700687. EXC6

1568. Van Rijnsoever M, Elsaleh H, Joseph D, et al. CpG island methylator phenotype is an independent predictor of survival benefit from 5-fluorouracil in stage III colorectal cancer. Clin Cancer Res. 2003 Aug 1;9(8):2898-903. PMID: 12912934. EXC3

1569. Vanderlaan BF, Broder MS, Chang EY, et al. Cost-effectiveness of 21-gene assay in node-positive, early-stage breast cancer (Structured abstract). Am J Manag Care. 2011(7):455-64. PMID: NHSEED-22011001362. EXC4

1570. Varella-Garcia M, Akduman B, Sunpaweravong P, et al. The UroVysion fluorescence in situ hybridization assay is an effective tool for monitoring recurrence of bladder cancer. Urol Oncol. 2004 Jan-Feb;22(1):16-9. PMID: 14969798. EXC6

1571. Varella-Garcia M, Mitsudomi T, Yatabe Y, et al. EGFR and HER2 genomic gain in recurrent non-small cell lung cancer after surgery: impact on outcome to treatment with gefitinib and association with EGFR and KRAS mutations in a Japanese cohort. J Thorac Oncol. 2009 Mar;4(3):318-25. PMID: 19247083. EXC3

1572. Varghese AM, Sima CS, Chaft JE, et al. Lungs don't forget: Comparison of the KRAS and EGFR mutation profile and survival of collegiate smokers and never smokers with advanced lung cancers. J Thorac Oncol. 2013 Jan;8(1):123-5. PMID: 23242442. EXC6

1573. Vasen HF, Hendriks Y, de Jong AE, et al. Identification of HNPCC by molecular analysis of colorectal and endometrial tumors. Dis Markers. 2004;20(4-5):207-13. PMID: 15528786. EXC3

1574. Vataire AL, Laas E, Aballea S, et al. [Cost-effectiveness of a chemotherapy predictive test]. Bull Cancer. 2012 Oct;99(10):907-14. PMID: 23041366. EXC2

1575. Veeramachaneni R, Nordberg ML, Shi R, et al. Evaluation of fluorescence in situ hybridization as an ancillary tool to urine cytology in diagnosing urothelial carcinoma. Diagn Cytopathol. 2003 Jun;28(6):301-7. PMID: 12768634. EXC7

1576. Viana Lde S, Affonso RJ, Jr., Silva SR, et al. Relationship between the expression of the extracellular matrix genes SPARC, SPP1, FN1, ITGA5 and ITGAV and clinicopathological parameters of tumor progression and colorectal cancer dissemination. Oncology. 2013;84(2):81-91. PMID: 23128103. EXC4

1577. Vickers MM, Bar J, Gorn-Hondermann I, et al. Stage-dependent differential expression of microRNAs in colorectal cancer: potential role as markers of metastatic disease. Clin Exp Metastasis. 2012 Feb;29(2):123-32. PMID: 22120473. EXC4

1578. Vidaurreta M, Maestro ML, Rafael S, et al. Telomerase activity in colorectal cancer, prognostic factor and implications in the microsatellite instability pathway. World J Gastroenterol. 2007 Jul 28;13(28):3868-72. PMID: 17657844. EXC6

1579. Villaruz LC, Socinski MA, Cunningham DE, et al. The prognostic and predictive value of KRAS oncogene substitutions in lung adenocarcinoma. Cancer. 2013 Jun 15;119(12):2268-74. PMID: 23526491. EXC6

1580. Vincenten J, Smit EF, Vos W, et al. Negative NKX2-1 (TTF-1) as temporary surrogate marker for treatment selection during EGFR-mutation analysis in patients with non-small-cell lung cancer. J Thorac Oncol. 2012;7(10):1522-7. EXC4

1581. Vlajnic T, Andreozzi MC, Schneider S, et al. VEGFA gene locus (6p12) amplification identifies a small but highly aggressive subgroup of colorectal cancer [corrected] patients. Mod Pathol. 2011 Oct;24(10):1404-12. PMID: 21743435. EXC4

1582. Vlaykova T, Mitkova A, Stancheva G, et al. Microsatellite instability and promoter hypermethylation of MLH1 and MSH2 in patients with sporadic colorectal cancer. J BUON. 2011 Apr-Jun;16(2):265-73. PMID: 21766496. EXC4

1583. Vollebergh MA, Kappers I, Klomp HM, et al. Ligands of epidermal growth factor receptor and the insulin-like growth factor family as serum biomarkers for response to epidermal growth factor receptor inhibitors in patients with advanced non-small cell lung cancer. J Thorac Oncol. 2010 Dec;5(12):1939-48. PMID: 21102259. EXC4

1584. Voortman J, Goto A, Mendiboure J, et al. MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma. Cancer Res. 2010(21):8288-98. PMID: CN-00772442. EXC4

1585. Voutsina A, Tzardi M, Kalikaki A, et al. Combined analysis of KRAS and PIK3CA mutations, MET and PTEN expression in primary tumors and corresponding metastases in colorectal cancer. Mod Pathol. 2013 Feb;26(2):302-13. PMID: 22936063. EXC3

1586. Wada M, Yamamoto M, Ryuge S, et al. Phase II study of S-1 monotherapy in patients with previously treated, advanced non-small-cell lung cancer. Cancer Chemother Pharmacol. 2012 Apr;69(4):1005-11. PMID: 22160350. EXC3

1587. Wagner A, Barrows A, Wijnen JT, et al. Molecular analysis of hereditary nonpolyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene. Am J Hum Genet. 2003 May;72(5):1088-100. PMID: 12658575. EXC7

1588. Wagner PL, Perner S, Rickman DS, et al. In situ evidence of KRAS amplification and association with increased p21 levels in non-small cell lung carcinoma. Am J Clin Pathol. 2009 Oct;132(4):500-5. PMID: 19762526. EXC4

1589. Wallander ML, Geiersbach KB, Tripp SR, et al. Comparison of reverse transcription-polymerase chain reaction, immunohistochemistry, and fluorescence in situ hybridization methodologies for detection of echinoderm microtubule-associated proteinlike 4-anaplastic lymphoma kinase fusion-positive non-small cell lung carcinoma: implications for optimal clinical testing. Arch Pathol Lab Med. 2012 Jul;136(7):796-803. PMID: 22742552. EXC5

1590. Wan YW, Raese RA, Fortney JE, et al. A smoking-associated 7-gene signature for lung cancer diagnosis and prognosis. Int J Oncol. 2012;41(4):1387-96. EXC4

1591. Wang C, van Rijnsoever M, Grieu F, et al. Prognostic significance of microsatellite instability and Ki-ras mutation type in stage II colorectal cancer. Oncology. 2003;64(3):259-65. PMID: 12697967. EXC4

1592. Wang F, Wang S, Wang Z, et al. Phosphorylated EGFR expression may predict outcome of EGFR-TKIs therapy for the advanced NSCLC patients with wild-type EGFR. J Exp Clin Cancer Res. 2012;31:65. PMID: 22901364. EXC6

1593. Wang J, Luo MH, Zhang ZX, et al. Clinical and molecular analysis of hereditary non-polyposis colorectal cancer in Chinese colorectal cancer patients. World Journal of Gastroenterology. 2007;13(10):1612-7. EXC6

1594. Wang S, An T, Wang J, et al. Potential clinical significance of a plasma-based KRAS mutation analysis in patients with advanced non-small cell lung cancer. Clin Cancer Res. 2010 Feb 15;16(4):1324-30. PMID: 20145159. EXC3

1595. Wang W, Wang GQ, Sun XW, et al. Prognostic values of chromosome 18q microsatellite alterations in stage II colonic carcinoma. World J Gastroenterol. 2010 Dec 21;16(47):6026-34. PMID: 21157981. EXC4

1596. Wang Y, Liu ZD, Zhao LM, et al. Individualized treatment of NSCLC: from research to clinical practice. Neoplasma. 2013;60(5):538-45. PMID: 23790173. EXC6

1597. Wang Z, Zhang X, Bai H, et al. EML4-ALK Rearrangement and its clinical significance in chinese patients with advanced non-small cell lung cancer. Oncology (Switzerland). 2012;83(5):248-56. EXC6

1598. Wang ZJ, An TT, Mok T, et al. Immediate versus delayed treatment with EGFR tyrosine kinase inhibitors after first-line therapy in advanced mon-small-cell lung cancer. Chinese Journal of Cancer Research. 2011;23(2):112-7. EXC5

1599. . Evaluation of the Oncotype Dx recurrence score (RS) in hormone-positive ipsilateral breast tumor recurrences. ASCO; 2012. EXC6

1600. Ward R, Meagher A, Tomlinson I, et al. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut. 2001 Jun;48(6):821-9. PMID: 11358903. EXC6

1601. Ward RL, Cheong K, Ku SL, et al. Adverse prognostic effect of methylation in colorectal cancer is reversed by microsatellite instability. J Clin Oncol. 2003 Oct 15;21(20):3729-36. PMID: 14551292. EXC6

1602. Ward RL, Turner J, Williams R, et al. Routine testing for mismatch repair deficiency in sporadic colorectal cancer is justified. J Pathol. 2005;207(4):377-84. EXC4

1603. Watanabe T, Kobunai T, Yamamoto Y, et al. Chromosomal instability (CIN) phenotype, CIN high or CIN low, predicts survival for colorectal cancer. J Clin Oncol. 2012 Jun 20;30(18):2256-64. PMID: 22547595. EXC4

1604. Watanabe T, Wu TT, Catalano PJ, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001 Apr 19;344(16):1196-206. PMID: 11309634. EXC6

1605. Watanabe T, Yoshino T, Uetake H, et al. KRAS mutational status in Japanese patients with colorectal cancer: results from a nationwide, multicenter, cross-sectional study. Jpn J Clin Oncol. 2013 Jul;43(7):706-12. PMID: 23657052. EXC6

1606. Webb S, Thomas M, Metcalf C, et al. EGFR mutation testing in NSCLC: Patterns of care and outcomes in Western Australia. Asia-Pacific Journal of Clinical Oncology. 2009;5(1):66-71. EXC6

1607. Webster J, Kauffman TL, Feigelson HS, et al. KRAS testing and epidermal growth factor receptor inhibitor treatment for colorectal cancer in community settings. Cancer Epidemiol Biomarkers Prev. 2013 Jan;22(1):91-101. PMID: 23155138. EXC3

1608. Weickhardt AJ, Price TJ, Chong G, et al. Dual targeting of the epidermal growth factor receptor using the combination of cetuximab and erlotinib: preclinical evaluation and results of the phase II DUX study in chemotherapy-refractory, advanced colorectal cancer. J Clin Oncol. 2012 May 1;30(13):1505-12. PMID: 22412142. EXC7

1609. Weinberg DS. Towards the better identification of mutation carriers: Has HNPCC met its match? Commentary. Evidence-Based Gastroenterology. 2007;8(1):18-9. EXC4

1610. Westra JL, Schaapveld M, Hollema H, et al. Determination of TP53 mutation is more relevant than microsatellite instability status for the prediction of disease-free survival in adjuvant-treated stage III colon cancer patients. J Clin Oncol. 2005 Aug 20;23(24):5635-43. PMID: 16110022. EXC3

1611. Whitson JM, Berry AB, Carroll PR, et al. UroVysion testing can lead to early identification of intravesical therapy failure in patients with high risk non-muscle invasive bladder cancer. Int Braz J Urol. 2009 Nov-Dec;35(6):664-70; discussion 71-2. PMID: 20028572. EXC5

1612. Wilkerson MD, Yin X, Walter V, et al. Differential pathogenesis of lung adenocarcinoma subtypes involving sequence mutations, copy number, chromosomal instability, and methylation. PLoS One. 2012;7(5):e36530. PMID: 22590557. EXC6

1613. Williams DJ, Cohen C, Darrow M, et al. Proliferation (Ki-67 and phosphohistone H3) and oncotype DX recurrence score in estrogen receptor-positive breast cancer. Applied Immunohistochemistry and Molecular Morphology. 2011;19(5):431-6. EXC6

1614. Williamson SR, Zhang S, Lopez-Beltran A, et al. Lymphoepithelioma-like carcinoma of the urinary bladder: clinicopathologic, immunohistochemical, and molecular features. Am J Surg Pathol. 2011 Apr;35(4):474-83. PMID: 21383609. EXC5

1615. Wittner BS, Sgroi DC, Ryan PD, et al. Analysis of the MammaPrint breast cancer assay in a predominantly postmenopausal cohort. Clin Cancer Res. 2008 May 15;14(10):2988-93. PMID: 18483364. EXC6

1616. Wolf I, Ben-Baruch N, Shapira-Frommer R, et al. Association between standard clinical and pathologic characteristics and the 21-gene recurrence score in breast cancer patients: a population-based study. Cancer. 2008 Feb 15;112(4):731-6. PMID: 18076012. EXC5

1617. Woll E, Greil R, Eisterer W, et al. Oxaliplatin, irinotecan and cetuximab in advanced gastric cancer. A multicenter phase ii trial (Gastric-2) of the Arbeitsgemeinschaft Medikamentose Tumortherapie (AGMT). Anticancer Res. 2011;31(12):4439-43. EXC3

1618. Won YW, Han JY, Lee GK, et al. Comparison of clinical outcome of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations. J Clin Pathol. 2011 Nov;64(11):947-52. PMID: 21725039. EXC3

1619. Wong AS, Teo C, Lim SW, et al. Targeted therapy at the end of life for patients with lung cancer. Journal of Palliative Medicine. 2010;13(8):945-8. EXC4

1620. Wong NS, Fernando NH, Nixon AB, et al. A phase II study of capecitabine, oxaliplatin, bevacizumab and cetuximab in the treatment of metastatic colorectal cancer. Anticancer Res. 2011 Jan;31(1):255-61. PMID: 21273607. EXC7

1621. Wong W, Cooper J, Richardson S, et al. Relationship between Oncotype DX testing and the use of chemotherapy in high-risk patients (pts). ASCO; 2012. EXC6

1622. Woods MO, Younghusband HB, Parfrey PS, et al. The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. Gut. 2010 Oct;59(10):1369-77. PMID: 20682701. EXC3

1623. Wright CM, Dent OF, Barker M, et al. Prognostic significance of extensive microsatellite instability in sporadic clinicopathological stage C colorectal cancer. Br J Surg. 2000 Sep;87(9):1197-202. PMID: 10971428. EXC3

1624. Wright CM, Dent OF, Newland RC, et al. Low level microsatellite instability may be associated with reduced cancer specific survival in sporadic stage C colorectal carcinoma. Gut. 2005 Jan;54(1):103-8. PMID: 15591513. EXC3

1625. Wu F, Yang LY, Li YF, et al. Novel role for epidermal growth factor-like domain 7 in metastasis of human hepatocellular carcinoma. Hepatology. 2009;50(6):1839-50. EXC3

1626. Wu JY, Shih JY, Yang CH, et al. Second-line treatments after first-line gefitinib therapy in advanced nonsmall cell lung cancer. Int J Cancer. 2010 Jan 1;126(1):247-55. PMID: 19536777. EXC3

1627. Wu JY, Wu SG, Yang CH, et al. Comparison of gefitinib and erlotinib in advanced NSCLC and the effect of EGFR mutations. Lung Cancer. 2011 May;72(2):205-12. PMID: 20832137. EXC3

1628. Wu JY, Wu SG, Yang CH, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res. 2008 Aug 1;14(15):4877-82. PMID: 18676761. EXC7

1629. Wu JY, Yang CH, Hsu YC, et al. Use of cetuximab after failure of gefitinib in patients with advanced non-small-cell lung cancer. Clin Lung Cancer. 2010 Jul 1;11(4):257-63. PMID: 20630828. EXC6

1630. Wu JY, Yu CJ, Yang CH, et al. First- or second-line therapy with gefitinib produces equal survival in non-small cell lung cancer. Am J Respir Crit Care Med. 2008;178(8):847-53. EXC5

1631. Wu M, Zhao J, Song SW, et al. EGFR mutations are associated with prognosis but not with the response to front-line chemotherapy in the Chinese patients with advanced non-small cell lung cancer. Lung Cancer. 2010 Mar;67(3):343-7. PMID: 19477549. EXC6

1632. Wu SG, Chang YL, Lin JW, et al. Including total EGFR staining in scoring improves EGFR mutations detection by mutation-specific antibodies and EGFR TKIs response prediction. PLoS One. 2011;6(8):e23303. PMID: 21858063. EXC6

1633. Wu SG, Gow CH, Yu CJ, et al. Frequent epidermal growth factor receptor gene mutations in malignant pleural effusion of lung adenocarcinoma. Eur Respir J. 2008 Oct;32(4):924-30. PMID: 18508816. EXC6

1634. Wu SG, Hu FC, Chang YL, et al. Frequent EGFR mutations in nonsmall cell lung cancer presenting with miliary intrapulmonary carcinomatosis. Eur Respir J. 2013 Feb;41(2):417-24. PMID: 22523351. EXC6

1635. Wu SG, Kuo YW, Chang YL, et al. EML4-ALK translocation predicts better outcome in lung adenocarcinoma patients with wild-type EGFR. J Thorac Oncol. 2012 Jan;7(1):98-104. PMID: 22124476. EXC4

1636. Wu SG, Yang CH, Yu CJ, et al. Good response to pemetrexed in patients of lung adenocarcinoma with epidermal growth factor receptor (EGFR) mutations. Lung Cancer. 2011 Jun;72(3):333-9. PMID: 21111508. EXC3

1637. Wulf MA, Bode B, Zimmermann D, et al. Silver-enhanced in situ hybridization for determination of EGFR copy number alterations in non-small cell lung cancer. Am J Surg Pathol. 2012 Dec;36(12):1801-8. PMID: 23154768. EXC6

1638. Xi JJ, Jiang W, Lu SH, et al. Primary pulmonary mucoepidermoid carcinoma: an analysis of 21 cases. World J Surg Oncol. 2012;10:232. PMID: 23114230. EXC4

1639. Xicola RM, Llor X, Pons E, et al. Performance of different microsatellite marker panels for detection of mismatch repair-deficient colorectal tumors. J Natl Cancer Inst. 2007 Feb 7;99(3):244-52. PMID: 17284719. EXC6

1640. Xu J, He J, Yang H, et al. Somatic mutation analysis of EGFR, KRAS, BRAF and PIK3CA in 861 patients with non-small cell lung cancer. Cancer Biomark. 2011;10(2):63-9. PMID: 22430133. EXC7

1641. Xu JM, Han Y, Duan HQ, et al. EGFR mutations and HER2/3 protein expression and clinical outcome in Chinese advanced non-small cell lung cancer patients treated with gefitinib. J Cancer Res Clin Oncol. 2009 Jun;135(6):771-82. PMID: 19020901. EXC3

1642. Yagi K, Akagi K, Hayashi H, et al. Three DNA methylation epigenotypes in human colorectal cancer. Clin Cancer Res. 2010;16(1):21-33. EXC6

1643. Yam I, Lam DC, Chan K, et al. EGFR array: uses in the detection of plasma EGFR mutations in non-small cell lung cancer patients. J Thorac Oncol. 2012 Jul;7(7):1131-40. PMID: 22610259. EXC6

1644. Yamada K, Kanazawa S, Koike J, et al. Microsatellite instability at tetranucleotide repeats in sporadic colorectal cancer in Japan. Oncol Rep. 2010;23(2):551-61. EXC4

1645. Yamada N, Oizumi S, Asahina H, et al. The peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp-based test for epidermal growth factor receptor mutations in bronchoscopic cytological specimens of non-small cell lung cancer. Oncology. 2012;82(6):341-6. PMID: 22677909. EXC7

1646. Yamaguchi F, Kugawa S, Tateno H, et al. Analysis of EGFR, KRAS and P53 mutations in lung cancer using cells in the curette lavage fluid obtained by bronchoscopy. Lung Cancer. 2012 Dec;78(3):201-6. PMID: 23026641. EXC6

1647. Yamamoto H, Hanafusa H, Ouchida M, et al. Single nucleotide polymorphisms in the EXO1 gene and risk of colorectal cancer in a Japanese population. Carcinogenesis. 2005 Feb;26(2):411-6. PMID: 15550454. EXC4

1648. Yamauchi H, Nakagawa C, Yamashige S, et al. Societal economics of the 21-gene Recurrence Score in estrogen receptor-positive early-stage breast cancer in Japan. SABCS; 2012. EXC6

1649. Yamazaki K, Sugio K, Yamanaka T, et al. Prognostic factors in non-small cell lung cancer patients with postoperative recurrence following third-generation chemotherapy. Anticancer Res. 2010 Apr;30(4):1311-5. PMID: 20530445. EXC4

1650. Yan B, Chin SY, Ismail TM, et al. KRAS mutation analysis as a diagnostic tool. Int J Colorectal Dis. 2011 Aug;26(8):1083-4. PMID: 21128077. EXC2

1651. Yan HL, Hao LQ, Jin HY, et al. Clinical features and mismatch repair genes analyses of Chinese suspected hereditary non-polyposis colorectal cancer: a cost-effective screening strategy proposal. Cancer Sci. 2008 Apr;99(4):770-80. PMID: 18307539. EXC3

1652. Yanagisawa S, Morikawa N, Kimura Y, et al. Large-cell neuroendocrine carcinoma with epidermal growth factor receptor mutation: possible transformation of lung adenocarcinoma. Respirology. 2012 Nov;17(8):1275-7. PMID: 22943430. EXC2

1653. Yang CH, Yu CJ, Shih JY, et al. Specific EGFR mutations predict treatment outcome of stage IIIB/IV patients with chemotherapy-naive non-small-cell lung cancer receiving first-line gefitinib monotherapy. J Clin Oncol. 2008 Jun 1;26(16):2745-53. PMID: 18509184. EXC3

1654. Yang JCH, Shih JY, Su WC, et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): A phase 2 trial. The lancet oncology. 2012;13(5):539-48. EXC5

1655. Yang JJ, Chen HJ, Yan HH, et al. Clinical modes of EGFR tyrosine kinase inhibitor failure and subsequent management in advanced non-small cell lung cancer. Lung Cancer. 2013 Jan;79(1):33-9. PMID: 23079155. EXC6

1656. Yang M, Tufail W, Patil DS, et al. Knowledge and preferences of breast and colorectal cancer survivors and patients for pharmacogenomic molecular diagnostics. Pharmacoepidemiology and Drug Safety. 2011;20:S330. EXC4

1657. Yang MJ, Chiu HH, Wang HM, et al. Enhancing detection of circulating tumor cells with activating KRAS oncogene in patients with colorectal cancer by weighted chemiluminescent membrane array method. Ann Surg Oncol. 2010 Feb;17(2):624-33. PMID: 19937133. EXC4

1658. Yang SY, Yang TY, Chen KC, et al. EGFR L858R mutation and polymorphisms of genes related to estrogen biosynthesis and metabolism in never-smoking female lung adenocarcinoma patients. Clin Cancer Res. 2011 Apr 15;17(8):2149-58. PMID: 21300759. EXC6

1659. Yang SY, Yang TY, Li YJ, et al. EGFR exon 19 in-frame deletion and polymorphisms of DNA repair genes in never-smoking female lung adenocarcinoma patients. Int J Cancer. 2013 Jan 15;132(2):449-58. PMID: 22573488. EXC6

1660. Yano M, Sasaki H, Kobayashi Y, et al. Epidermal growth factor receptor gene mutation and computed tomographic findings in peripheral pulmonary adenocarcinoma. J Thorac Oncol. 2006 Jun;1(5):413-6. PMID: 17409892. EXC6

1661. Yardley DA, Peacock NW, Hendricks C, et al. Correlation of Oncotype DX Recurrence Scores with pathologic response following neoadjuvant ixabepilone and cyclophosphamide in patients with HER2-negative breast cancer. San Antonio Breast Cancer Symposium; 2011 December; San Antonio, TX. EXC6

1662. Yardley DA, Peacock NW, Hendricks C, et al. Ixabepilone and Cyclophosphamide as Neoadjuvant Therapy in HER2-Negative Breast Cancer with Exploratory Oncotype DX® Assessments: A Sarah Cannon Research Institute Phase II Trial. EXC5

1663. Yasuda H, Soejima K, Nakayama S, et al. Bronchoscopic microsampling is a useful complementary diagnostic tool for detecting lung cancer. Lung Cancer. 2011 Apr;72(1):32-8. PMID: 20813423. EXC4

1664. Yearsley M, Hampel H, Lehman A, et al. Histologic features distinguish microsatellite-high from microsatellite-low and microsatellite-stable colorectal carcinomas, but do not differentiate germline mutations from methylation of the MLH1 promoter. Hum Pathol. 2006 Jul;37(7):831-8. PMID: 16784982. EXC6

1665. Yen LC, Uen YH, Wu DC, et al. Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. Ann Surg. 2010 Feb;251(2):254-60. PMID: 20010090. EXC7

1666. Yen LC, Yeh YS, Chen CW, et al. Detection of KRAS oncogene in peripheral blood as a predictor of the response to cetuximab plus chemotherapy in patients with metastatic colorectal cancer. Clin Cancer Res. 2009 Jul 1;15(13):4508-13. PMID: 19549774. EXC3

1667. Yeo WL, Riely GJ, Yeap BY, et al. Erlotinib at a dose of 25 mg daily for non-small cell lung cancers with EGFR mutations. J Thorac Oncol. 2010 Jul;5(7):1048-53. PMID: 20512075. EXC6

1668. Yip PY, Yu B, Cooper WA, et al. Patterns of DNA mutations and ALK rearrangement in resected node negative lung adenocarcinoma. J Thorac Oncol. 2013 Apr;8(4):408-14. PMID: 23392229. EXC6

1669. Yoder BJ, Skacel M, Hedgepeth R, et al. Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: a prospective study with focus on the natural history of anticipatory positive findings. Am J Clin Pathol. 2007 Feb;127(2):295-301. PMID: 17210520. EXC7

1670. Yokota K, Sasaki H, Okuda K, et al. KIF5B/RET fusion gene in surgically-treated adenocarcinoma of the lung. Oncol Rep. 2012;28(4):1187-92. EXC4

1671. Yokota T, Ura T, Shibata N, et al. BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. Br J Cancer. 2011 Mar 1;104(5):856-62. PMID: 21285991. EXC3

1672. Yoshida A, Kohno T, Tsuta K, et al. ROS1-rearranged lung cancer: a clinicopathologic and molecular study of 15 surgical cases. Am J Surg Pathol. 2013 Apr;37(4):554-62. PMID: 23426121. EXC4

1673. Yoshida K, Yatabe Y, Park J, et al. Clinical outcomes of advanced non-small cell lung cancer patients screened for epidermal growth factor receptor gene mutations. J Cancer Res Clin Oncol. 2010;136(4):527-35. EXC3

1674. Yoshida K, Yatabe Y, Park JY, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. J Thorac Oncol. 2007 Jan;2(1):22-8. PMID: 17410005. EXC3

1675. Yoshida Y, Shibata T, Kokubu A, et al. Mutations of the epidermal growth factor receptor gene in atypical adenomatous hyperplasia and bronchioloalveolar carcinoma of the lung. Lung Cancer. 2005 Oct;50(1):1-8. PMID: 15950315. EXC6

1676. Yoshimasu T, Ohta F, Oura S, et al. Histoculture drug response assay for gefitinib in non-small-cell lung cancer. Gen Thorac Cardiovasc Surg. 2009 Mar;57(3):138-43. PMID: 19280309. EXC6

1677. Yoshitake N, Fujii S, Mukawa K, et al. Mutational analysis of the BRAF gene in colorectal mucinous carcinoma in association with histological configuration. Oncol Rep. 2007 Jan;17(1):9-15. PMID: 17143472. EXC3

1678. Yoshizawa A, Sumiyoshi S, Sonobe M, et al. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol. 2013 Jan;8(1):52-61. PMID: 23242438. EXC4

1679. Yothers G, O'Connell MJ, Allegra CJ, et al. Oxaliplatin as adjuvant therapy for colon cancer: updated results of NSABP C-07 trial, including survival and subset analyses. J Clin Oncol. 2011 Oct 1;29(28):3768-74. PMID: 21859995. EXC6

1680. Yothers G, O'Connell MJ, Lee M, et al. Validation of the 12-Gene Colon Cancer Recurrence Score in NSABP C-07 As a Predictor of Recurrence in Patients With Stage II and III Colon Cancer Treated With Fluorouracil and Leucovorin (FU/LV) and FU/LV Plus Oxaliplatin. J Clin Oncol. 2013 Dec 20;31(36):4512-9. PMID: 24220557. EX8

1681. Youssef RF, Schlomer BJ, Ho R, et al. Role of fluorescence in situ hybridization in bladder cancer surveillance of patients with negative cytology. Urol Oncol. 2012 May-Jun;30(3):273-7. PMID: 20451422. EXC3

1682. Alberts SR, Yu T, Behrens RJ, et al. Real-world comparative economics of a 12-gene assay for prognosis in stage II colon cancer. American Society of Clinical Oncology; 2013 June; Chicago, IL. EXC6

1683. Yuan S, Yu SL, Chen HY, et al. Clustered genomic alterations in chromosome 7p dictate outcomes and targeted treatment responses of lung adenocarcinoma with EGFR-activating mutations. J Clin Oncol. 2011 Sep 1;29(25):3435-42. PMID: 21810691. EXC4

1684. Yuen ST, Chan TL, Ho JWC, et al. Germline, somatic and epigenetic events underlying mismatch repair deficiency in colorectal and HNPCC-related cancers. Oncogene. 2002;21(49):7585-92. EXC4

1685. Yun HR, Yi LJ, Cho YK, et al. Double primary malignancy in colorectal cancer patients--MSI is the useful marker for predicting double primary tumors. Int J Colorectal Dis. 2009 Apr;24(4):369-75. PMID: 18797888. EXC6

1686. Zaanan A, Flejou JF, Emile JF, et al. Defective mismatch repair status as a prognostic biomarker of disease-free survival in stage III colon cancer patients treated with adjuvant FOLFOX chemotherapy. Clin Cancer Res. 2011 Dec 1;17(23):7470-8. PMID: 21998335. EXC6

1687. Zavodna K, Konecny M, Krivulcik T, et al. Genetic analysis of KRAS mutation status in metastatic colorectal cancer patients. Neoplasma. 2009;56(3):275-8. PMID: 19309232. EXC3

1688. Zech VF, Dlaska M, Tzankov A, et al. Prognostic and diagnostic relevance of hnRNP A2/B1, hnRNP B1 and S100 A2 in non-small cell lung cancer. Cancer Detect Prev. 2006;30(5):395-402. PMID: 17067748. EXC4

1689. Zhang H, Song J, Ren H, et al. Detection of low-abundance KRAS mutations in colorectal cancer using microfluidic capillary electrophoresis-based restriction fragment length polymorphism method with optimized assay conditions. PLoS One. 2013;8(1):e54510. PMID: 23355875. EXC4

1690. Zhang J, Liang Z, Gao J, et al. Pulmonary adenocarcinoma with a micropapillary pattern: a clinicopathological, immunophenotypic and molecular analysis. Histopathology. 2011 Dec;59(6):1204-14. PMID: 22175900. EXC6

1691. Zhang L. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing. J Mol Diagn. 2008 Jul;10(4):301-7. PMID: 18556776. EXC2

1692. Zhang L, Xiao H, Zhou H, et al. Development of transcriptomic biomarker signature in human saliva to detect lung cancer. Cell Mol Life Sci. 2012;69(19):3341-50. EXC5

1693. Zhang L, Yang H, Zhao Y, et al. Detection of EGFR somatic mutations in non-small cell lung cancer (NSCLC) using a novel mutant-enriched liquidchip (MEL) technology. Current Drug Metabolism. 2012;13(7):1007-11. EXC6

1694. Zhang W, Azuma M, Lurje G, et al. Molecular predictors of combination targeted therapies (cetuximab, bevacizumab) in irinotecan-refractory colorectal cancer (BOND-2 study). Anticancer Res. 2010 Oct;30(10):4209-17. PMID: 21036743. EXC8

1695. Zhang W, Stabile LP, Keohavong P, et al. Mutation and polymorphism in the EGFR-TK domain associated with lung cancer. J Thorac Oncol. 2006 Sep;1(7):635-47. PMID: 17409930. EXC6

1696. Zhang W, Winder T, Ning Y, et al. A let-7 microRNA-binding site polymorphism in 3'-untranslated region of KRAS gene predicts response in wild-type KRAS patients with metastatic colorectal cancer treated with cetuximab monotherapy. Ann Oncol. 2011 Jan;22(1):104-9. PMID: 20603437. EXC8

1697. Zhang X, Liu G, Kang Y, et al. N-cadherin expression is associated with acquisition of EMT phenotype and with enhanced invasion in erlotinib-resistant lung cancer cell lines. PLoS One. 2013;8(3):e57692. PMID: 23520479. EXC4

1698. Zhang Y, Sun Y, Pan Y, et al. Frequency of driver mutations in lung adenocarcinoma from female never-smokers varies with histologic subtypes and age at diagnosis. Clin Cancer Res. 2012 Apr 1;18(7):1947-53. PMID: 22317764. EXC6

1699. Zhao Q, Shentu J, Xu N, et al. Phase I study of icotinib hydrochloride (BPI-2009H), an oral EGFR tyrosine kinase inhibitor, in patients with advanced NSCLC and other solid tumors. Lung Cancer. 2011 Aug;73(2):195-202. PMID: 21144613. EXC6

1700. Zhao X, Han RB, Zhao J, et al. Comparison of epidermal growth factor receptor mutation statuses in tissue and plasma in stage I-IV non-small cell lung cancer patients. Respiration. 2013;85(2):119-25. PMID: 22797485. EXC6

1701. Zhao Y, Oki E, Ando K, et al. The impact of a high-frequency microsatellite instability phenotype on the tumor location-related genetic differences in colorectal cancer. Cancer Genet Cytogenet. 2010 Jan 15;196(2):133-9. PMID: 20082848. EXC8

1702. Zhao YY, Li S, Zhang Y, et al. The relationship between drug exposure and clinical outcomes of non-small cell lung cancer patients treated with gefitinib. Med Oncol. 2011 Sep;28(3):697-702. PMID: 20458561. EXC3

1703. Zheng T, Bepler G, Cantor A, et al. Small tumor size and limited smoking history predicts activated epidermal growth factor receptor in early-stage non-small cell lung cancer. Chest. 2005;128(1):308-16. EXC4

1704. Zhou HH, Yan SY, Zhou XY, et al. MLH1 promoter germline-methylation in selected probands of Chinese hereditary non-polyposis colorectal cancer families. World J Gastroenterol. 2008 Dec 28;14(48):7329-34. PMID: 19109866. EXC8

1705. Zhou Q, Zhang XC, Chen ZH, et al. Relative abundance of EGFR mutations predicts benefit from gefitinib treatment for advanced non-small-cell lung cancer. J Clin Oncol. 2011;29(24):3316-21. EXC5

1706. Zhou ZW, Rieger N, Ruszkiewicz A, et al. Detection of lymph nodes micrometastases in Dukes' A and B colorectal cancer using anti-cytokeratin antibodies AE1/AE3. World Journal of Gastroenterology. 2005;11(23):3640-3. EXC4

1707. Zhu CQ, da Cunha Santos G, Ding K, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol. 2008 Sep 10;26(26):4268-75. PMID: 18626007. EXC3

1708. Zhu YJ, Xia Y, Ren GJ, et al. Efficacy and clinical/molecular predictors of erlotinib monotherapy for Chinese advanced non-small cell lung cancer. Chin Med J (Engl). 2010 Nov;123(22):3200-5. PMID: 21163115. EXC6

1709. Zhuo ML, Wu MN, Zhao J, et al. Epidermal growth factor receptor genotype in plasma DNA and outcome of chemotherapy in the Chinese patients with advanced non-small cell lung cancer. Chin Med J (Engl). 2011;124(21):3510-4. EXC4

1710. Zikria J, Krishnamoorthy S, Kaley K, et al. Sweeping KRAS generalizations: Are we depriving patients of an effective treatment? A novel KRAS mutation and dramatic response to panitumumab in a patient with metastatic colorectal cancer. Int J Colorectal Dis. 2011;26(10):1353-4. EXC4

1711. Zlobec I, Baker K, Terracciano LM, et al. RHAMM, p21 combined phenotype identifies microsatellite instability-high colorectal cancers with a highly adverse prognosis. Clin Cancer Res. 2008 Jun 15;14(12):3798-806. PMID: 18559599. EXC8

1712. Zlobec I, Bihl M, Foerster A, et al. Comprehensive analysis of CpG island methylator phenotype (CIMP)-high, -low, and -negative colorectal cancers based on protein marker expression and molecular features. J Pathol. 2011 Nov;225(3):336-43. PMID: 21660972. EXC8

1713. Zlobec I, Kovac M, Erzberger P, et al. Combined analysis of specific KRAS mutation, BRAF and microsatellite instability identifies prognostic subgroups of sporadic and hereditary colorectal cancer. Int J Cancer. 2010 Dec 1;127(11):2569-75. PMID: 20162668. EXC5

1714. Zlobec I, Molinari F, Kovac M, et al. Prognostic and predictive value of TOPK stratified by KRAS and BRAF gene alterations in sporadic, hereditary and metastatic colorectal cancer patients. Br J Cancer. 2010 Jan 5;102(1):151-61. PMID: 19935791. EXC4

1715. Zupa A, Improta G, Silvestri A, et al. A pilot characterization of human lung NSCLC by protein pathway activation mapping. J Thorac Oncol. 2012 Dec;7(12):1755-66. PMID: 23154546. EXC6

1716. Zwitter M, Rajer M, Kovac V, et al. Intermittent chemotherapy and erlotinib for nonsmokers or light smokers with advanced adenocarcinoma of the lung: a phase II clinical trial. J Biomed Biotechnol. 2011;2011:185646. PMID: 21541241. EXC3

Appendix C. Criteria for Evaluating Risk of Bias

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3396	Abbot	2011

ROB RATING: UNCLEAR

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	*	Medium	*	High	*	N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
2. For RCTs, were randomization and allocation concealment adequate?						
3. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
4. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
5. Were groups similar at baseline?						
6. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
7. Were outcomes assessed using valid and reliable measures, implemented consistently						

across all study participants?							
8. Was overall attrition less than 30%?							
9. Was differential attrition less than 15%?							
10. Does the analysis control for baseline differences between groups?							
11. Does the analysis account for differences in treatment received by the groups?							
12. Are the statistical methods used to assess the outcomes appropriate?							
13. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?							X
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?			X				
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?				X			
Were the reference standard results interpreted without knowledge of the results of the index test?				X			
Could the reference standard, its conduct, or its interpretation have introduced bias?							X
Domain 4: Flow and Timing							
Did all patients receive a reference standard?				X			
Did patients receive the same reference standard?				X			
Were all patients included in the analysis?				X			
Could the patient flow have introduced bias?							X
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
159	Ademuyiwa	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
14. What is the study design?				**x**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
15. For RCTs, were randomization and allocation concealment adequate?					x	
16. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
17. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
18. Were groups similar at baseline?					x	
19. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	x					
20. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?		x				
21. Was overall attrition less than 30%?					x	
22. Was differential attrition less than 15%?					x	
23. Does the analysis control for baseline	x					

differences between groups?						
24. Does the analysis account for differences in treatment received by the groups?					x	
25. Are the statistical methods used to assess the outcomes appropriate?	x					
26. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments: This analysis claims that the Oncotype test changes decision making based on the study of hypotherical treatment decisions made by only TWO medical oncologists who disagreed in 20% of the case on what their treatment recommendation would be, in which case the authors just threw out one opinion. The design of the study is fine, but the conclusions they drew from it are vastly over-inflated.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (X__)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
153	Albanell	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
27. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
28. For RCTs, were randomization and allocation concealment adequate?					x	
29. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
30. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					pre/post test design
31. Were groups similar at baseline?					x	
32. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	x					in pretest yes
33. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
34. Was overall attrition less than 30%?				x		
35. Was differential attrition less than 15%?				x		
36. Does the analysis control for baseline	x					

differences between groups?							
37. Does the analysis account for differences in treatment received by the groups?	x						
38. Are the statistical methods used to assess the outcomes appropriate?	x						
39. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments: I marked this as a prospective cohort rather than a case series (as in other similar pre/post test decision making studies) b/c patients were enrolled prospectively for this purpose rather than their cases being used as examples retrospectively for a group of physicians.							

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2772	An	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
40. What is the study design?				**x**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
41. For RCTs, were randomization and allocation concealment adequate?					x	
42. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
43. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
44. Were groups similar at baseline?				x		
45. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		x				
46. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
47. Was overall attrition less than 30%?					x	
48. Was differential attrition less than 15%?					x	
49. Does the analysis control for baseline	x					

differences between groups?						
50. Does the analysis account for differences in treatment received by the groups?		x				
51. Are the statistical methods used to assess the outcomes appropriate?	x					
52. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments: adjusted HRs for survival by EGFR status are reported ONLY for stage I patients, text reports that EGFR was not a significant predictor in the overall sample. Hard to know how to interpret.						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (_X_)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2774	Angulo	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	x	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
53. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
54. For RCTs, were randomization and allocation concealment adequate?						
55. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
56. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
57. Were groups similar at baseline?						
58. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
59. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
60. Was overall attrition less than 30%?						
61. Was differential attrition less than 15%?						
62. Does the analysis control for baseline						

differences between groups?							
63. Does the analysis account for differences in treatment received by the groups?							
64. Are the statistical methods used to assess the outcomes appropriate?							
65. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			x				
Was a case-control design avoided?	x						
Did the study avoid inappropriate exclusions?			x				
Could the selection of patients have introduced bias?							x
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			x				
If a threshold was used, was it pre-specified?	x						
Could the conduct or interpretation of the index test have introduced bias?					x		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	x						
Were the reference standard results interpreted without knowledge of the results of the index test?	x						
Could the reference standard, its conduct, or its interpretation have introduced bias?					x		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	x						
Did patients receive the same reference standard?	x						
Were all patients included in the analysis?		x					
Could the patient flow have introduced bias?					x		
Comments: 3 of 136 pts not included due to insufficient DNA material. How patient tissues were selected from those available at the institution (consecutive, random, other) was not reported.							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3435	Bando	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium	X	High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
66. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
67. For RCTs, were randomization and allocation concealment adequate?						
68. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
69. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
70. Were groups similar at baseline?						
71. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
72. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
73. Was overall attrition less than 30%?						

74. Was differential attrition less than 15%?						
75. Does the analysis control for baseline differences between groups?						
76. Does the analysis account for differences in treatment received by the groups?						
77. Are the statistical methods used to assess the outcomes appropriate?						
78. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?	X						
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?			X				
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
4021	Bargallo	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
79. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
80. For RCTs, were randomization and allocation concealment adequate?					X	
81. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					Consecutive sample
82. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
83. Were groups similar at baseline?				X		
84. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		But, not as important as some studies since the outcome is the questionnaire result
85. Were outcomes assessed using valid and reliable measures, implemented consistently				X		Multidisciplinary team completed

across all study participants?						questionnaires; no other information provided to determine validity and reliability of measures
86. Was overall attrition less than 30%?	X					
87. Was differential attrition less than 15%?	X					
88. Does the analysis control for baseline differences between groups?		X				
89. Does the analysis account for differences in treatment received by the groups?					X	
90. Are the statistical methods used to assess the outcomes appropriate?	X					
91. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
272	Bazan	2005

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
92. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
93. For RCTs, were randomization and allocation concealment adequate?					x	
94. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
95. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
96. Were groups similar at baseline?				x		
97. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				x		Outcome is survival, so blinding unlikely to matter
98. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					Medical records
99. Was overall attrition less than 30%?				x		
100. Was differential attrition less than 15%?				x		

101. Does the analysis control for baseline differences between groups?	x					
102. Does the analysis account for differences in treatment received by the groups?			x			Adjusts for surgery but not receipt of chemo
103. Are the statistical methods used to assess the outcomes appropriate?	x					
104. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_x_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1813	Beau-Faller	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low	X	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
105. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
106. For RCTs, were randomization and allocation concealment adequate?						
107. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
108. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
109. Were groups similar at baseline?						
110. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
111. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
112. Was overall attrition less than 30%?						
113. Was differential attrition less than 15%?						
114. Does the analysis control for baseline						

differences between groups?							
115. Does the analysis account for differences in treatment received by the groups?							
116. Are the statistical methods used to assess the outcomes appropriate?							
117. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?	X						
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?		X					
Could the patient flow have introduced bias?					X		
Comments: 6 frozen samples (out of 74) were excluded from portions of the analyses because they contained less than 30% tumor cells							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1814	Beau-Faller	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low	X	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
118. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
119. For RCTs, were randomization and allocation concealment adequate?						
120. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
121. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
122. Were groups similar at baseline?						
123. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
124. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
125. Was overall attrition less than 30%?						
126. Was differential attrition less than 15%?						
127. Does the analysis control for baseline						

differences between groups?							
128. Does the analysis account for differences in treatment received by the groups?							
129. Are the statistical methods used to assess the outcomes appropriate?							
130. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?	X						
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			X				
If a threshold was used, was it pre-specified?	X						
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
4027	Brenner	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
131. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
132. For RCTs, were randomization and allocation concealment adequate?					X	
133. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		Unclear, but seems to imply all patients during the time frame listed
134. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
135. Were groups similar at baseline?				X		
136. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		They don't report blinding of outcome assessors.
137. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		No description of how they

						were ascertained
138. Was overall attrition less than 30%?	X					
139. Was differential attrition less than 15%?	X					
140. Does the analysis control for baseline differences between groups?					X	
141. Does the analysis account for differences in treatment received by the groups?					X	
142. Are the statistical methods used to assess the outcomes appropriate?	X					
143. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: Very limited reporting of study design and methods. At least medium risk of ascertainment/measurement bias. There are multiple reasons why someone might receive a different treatment than initially recommended (e.g., patient preferences, other medical problems limiting ability to undergo treatment). This study assumes that the test result is the reason, without attempting to measure causality accurately. They did not assess treatment recommendation before vs. after; they assessed treatment recommendation before vs. what ultimately happened.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
165	Bueno-de-Mesquita	2007

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
144. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
145. For RCTs, were randomization and allocation concealment adequate?					X	
146. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
147. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
148. Were groups similar at baseline?		X				
149. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		Pathologists were masked, but the outcomes of relevance for this article were decisions about treatment (NR if the

						investigators handling that data were masked to MammaPrint results)
150. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
151. Was overall attrition less than 30%?	X					
152. Was differential attrition less than 15%?	X					
153. Does the analysis control for baseline differences between groups?		X				But, not essential to do so for the question of whether having the MammaPrint information changes decisions
154. Does the analysis account for differences in treatment received by the groups?		X				But, not essential for this question
155. Are the statistical methods used to assess the outcomes appropriate?	X					
156. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: Low risk of bias for assessing whether treatment decisions differ when using only the Dutch CBO guidelines (clinical information) and when also using MammaPrint results. Related to applicability of this study, the Dutch guidelines only include age, grade, and size, so whether adding MammaPrint result to decisions based on these 3 things doesn't quite get us the information of relevance to current practice in the US. We would need decisions based on all the current known prognostic factors, and then to determine whether they change with adding MammaPrint. In Table 2, they report a similar rate of discordance between Adjuvant! Online and MammaPrint as they report for the Dutch CBO guidelines and MammaPrint. This might suggest that adding MammaPrint to Adjuvant! Online would change a similar proportion of decisions as when adding it to the Dutch guidelines.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have							

introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3363	Bueno de Mesquita	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
157. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
158. For RCTs, were randomization and allocation concealment adequate?					X	
159. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
160. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
161. Were groups similar at baseline?				X		
162. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
163. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
164. Was overall attrition less than 30%?	X					
165. Was differential attrition less than 15%?	X					
166. Does the analysis control for baseline		X				

differences between groups?						
167. Does the analysis account for differences in treatment received by the groups?		X				
168. Are the statistical methods used to assess the outcomes appropriate?	X					
169. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
166	Buyse	2006

RATING: UNCLEAR

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:	unclear							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
170. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
171. For RCTs, were randomization and allocation concealment adequate?					X	
172. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
173. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
174. Were groups similar at baseline?		X				
175. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
176. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
177. Was overall attrition less than 30%?				X		

178. Was differential attrition less than 15%?				X		
179. Does the analysis control for baseline differences between groups?				X		
180. Does the analysis account for differences in treatment received by the groups?		X				
181. Are the statistical methods used to assess the outcomes appropriate?	X					
182. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments: Reference standard here was pyrosequencing							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3405	Cartwright	Unknown

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:	.Cross sectional survey of oncologists who had previously ordered oncotype DX							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	non-RCT	Prospective cohort study	Retrospective cohort study	Case-control study	Case Series
183. What is the study design?		**THIS STUDY WAS A CROSS SECTIONAL SURVEY OF ONCOLOGISTS SO DOES NOT FIT ANY OF THESE**				
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
184. For RCTs, were randomization and allocation concealment adequate?					X	
185. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		

186. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?					X	
187. Were groups similar at baseline?					X	
188. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
189. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
190. Was overall attrition less than 30%?			X			
191. Was differential attrition less than 15%?					X	
192. Does the analysis control for baseline differences between groups?					X	
193. Does the analysis account for differences in treatment received by the groups?					X	
194. Are the statistical methods used to assess the outcomes appropriate?	X					
195. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments: Abstract shows only preliminary analysis and says full data will be presented at meeting.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
385	Chang	2006

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	x
Risk of bias for KQ 2:	Low		Medium		High		N/A	x
Risk of bias for KQ 3:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	x
Risk of bias for KQ 4b:	Low		Medium		High		N/A	x
Risk of bias for KQ 5:	Low		Medium		High		N/A	x
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
196. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
197. For RCTs, were randomization and allocation concealment adequate?					x	
198. xDid the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
199. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
200. Were groups similar at baseline?		x				
201. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	x					
202. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
203. Was overall attrition less than 30%?	x					
204. Was differential attrition less than 15%?	x					
205. Does the analysis control for baseline		x				BRAF

differences between groups?						differences
206. Does the analysis account for differences in treatment received by the groups?		X				
207. Are the statistical methods used to assess the outcomes appropriate?	X					
208. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Treatment not controlled for despite it being a prospective study.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3350	Clark-Langone	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium	X	High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
209. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
210. For RCTs, were randomization and allocation concealment adequate?						
211. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
212. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
213. Were groups similar at baseline?						
214. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
215. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
216. Was overall attrition less than 30%?						
217. Was differential attrition less than 15%?						
218. Does the analysis control for baseline						

differences between groups?						
219. Does the analysis account for differences in treatment received by the groups?						
220. Are the statistical methods used to assess the outcomes appropriate?						
221. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?				X			
If a threshold was used, was it pre-specified?				X			
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?				X			
Did patients receive the same reference standard?				X			
Were all patients included in the analysis?				X			
Could the patient flow have introduced bias?					X		
Comments:This is a paper that is looking at LOD, reproducibility, cross lab comparisons etc. Many of these criteria are in applicable							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
4000	Cronin	2007

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High				N/A	X
Risk of bias for KQ 2:	Low		Medium		High		Unclear	X	N/A	
Risk of bias for KQ 3:	Low		Medium		High				N/A	X
Risk of bias for KQ 4a:	Low		Medium		High				N/A	X
Risk of bias for KQ 4b:	Low		Medium		High				N/A	X
Risk of bias for KQ 5:	Low		Medium		High				N/A	X
Explain any **High** ratings:										

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
222. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
223. For RCTs, were randomization and allocation concealment adequate?						
224. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
225. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
226. Were groups similar at baseline?						
227. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
228. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
229. Was overall attrition less than 30%?						
230. Was differential attrition less than 15%?						
231. Does the analysis control for baseline						

differences between groups?						
232. Does the analysis account for differences in treatment received by the groups?						
233. Are the statistical methods used to assess the outcomes appropriate?						
234. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?							X
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			X				
If a threshold was used, was it pre-specified?				X			
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?			X				
Were the reference standard results interpreted without knowledge of the results of the index test?			X				
Could the reference standard, its conduct, or its interpretation have introduced bias?							X
Domain 4: Flow and Timing							
Did all patients receive a reference standard?			X				
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?			X				
Could the patient flow have introduced bias?							X
Comments: Limited information about patient/sample selection process, flow and timing; regarding the reference standard, as the authors describe, universal standard reference RNAs are not available for the 21 genes (their approach seems possibly reasonable, given the lack of a standard, but still raises some concern for bias).							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3461	Davidson	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
235. What is the study design?		**Uncontrolled trial**				
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
236. For RCTs, were randomization and allocation concealment adequate?					X	
237. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
238. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					Pre-post for change in decisions
239. Were groups similar at baseline?					X	1 group
240. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
241. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
242. Was overall attrition less than 30%?	X					

243. Was differential attrition less than 15%?	X					
244. Does the analysis control for baseline differences between groups?					X	
245. Does the analysis account for differences in treatment received by the groups?					X	
246. Are the statistical methods used to assess the outcomes appropriate?	X					
247. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: No information reported on the number of physicians making the decisions; unclear how wide a range of decisions this represents. Article does not show what information was provided to physicians with the test results (giving just the test results vs. more information about interpretation or recommendations for treatment that could impact decisions)						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3504	De Boer	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
248. What is the study design?		**Uncontrolled trial**				
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
249. For RCTs, were randomization and allocation concealment adequate?					X	
250. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
251. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
252. Were groups similar at baseline?				X		
253. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
254. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
255. Was overall attrition less than 30%?	X					

256. Was differential attrition less than 15%?	X					
257. Does the analysis control for baseline differences between groups?		X				
258. Does the analysis account for differences in treatment received by the groups?	X					
259. Are the statistical methods used to assess the outcomes appropriate?	X					
260. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
4031	Delahaye	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High				N/A	X
Risk of bias for KQ 2:	Low		Medium	X	High				N/A	
Risk of bias for KQ 3:	Low		Medium		High				N/A	X
Risk of bias for KQ 4a:	Low		Medium		High				N/A	X
Risk of bias for KQ 4b:	Low		Medium		High				N/A	X
Risk of bias for KQ 5:	Low		Medium		High				N/A	X
Explain any **High** ratings:										

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
261. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
262. For RCTs, were randomization and allocation concealment adequate?						
263. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
264. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
265. Were groups similar at baseline?						
266. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
267. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
268. Was overall attrition less than 30%?						
269. Was differential attrition less than 15%?						
270. Does the analysis control for baseline						

differences between groups?							
271. Does the analysis account for differences in treatment received by the groups?							
272. Are the statistical methods used to assess the outcomes appropriate?							
273. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (_X_)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			X				
If a threshold was used, was it pre-specified?				X			
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?			X				
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?			X				
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?			X				
Could the patient flow have introduced bias?							X
Comments: Although some domains are unclear here, many methodological strengths described that may not be captured in this form.							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1584	Deschoolmeester	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low	*	Medium	*	High	*	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	Unclear risk of selection bias, ascertainment bias, and confounding							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
274. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
275. For RCTs, were randomization and allocation concealment adequate?					X	
276. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		None reported; unclear how the 164 tissue samples were selected other than that they were sporadic CRC
277. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					KRAS mutation vs wild-type
278. Were groups similar at baseline?				X		No data reported for the KRAS mutation vs. wild-type groups; only reports

						overall, and separate characteristic for colon and rectum cancers
279. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
280. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		No information reported about how recurrence and death were ascertained
281. Was overall attrition less than 30%?				X		Median f/u was 4.5 years or longer, but data NR about how many subjects were missing outcome data or lost to follow up
282. Was differential attrition less than 15%?				X		
283. Does the analysis control for baseline differences between groups?				X		No baseline information reported, so unable to determine
284. Does the analysis account for differences in treatment received by the groups?				X		See comments
285. Are the statistical methods used to assess the outcomes appropriate?	X					
286. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?				X		See comments
Comments: For the multivariate analysis, it is unclear what was included. The methods report that they used a stepwise backward binary logistic regression to identify which of the clinicopathological parameters had the strongest impact on survival, but which parameters were considers is NR (possibly the things they report baseline characteristics for in Table 2: age, sex, location, grade, stage, therapy/neo-adjuvant/adjuvant, and MSI status). In the results (p 1630) they report results for multiple Cox regression analyses with age and stage.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							

Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
477	Donada	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
287. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
288. For RCTs, were randomization and allocation concealment adequate?					x	
289. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
290. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
291. Were groups similar at baseline?					x	non-randomized study, MSI+ and MSI – naturally different
292. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		x				retrospective study, blinding not possible
293. Were outcomes assessed using valid and reliable measures, implemented consistently	x					

across all study participants?						
294. Was overall attrition less than 30%?					x	retrospective study limited to those with followup
295. Was differential attrition less than 15%?					x	
296. Does the analysis control for baseline differences between groups?	x					
297. Does the analysis account for differences in treatment received by the groups?					x	all treated with adjuvant 5FU
298. Are the statistical methods used to assess the outcomes appropriate?	x					
299. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
180	Dowsett	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
300. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
301. For RCTs, were randomization and allocation concealment adequate?					X	
302. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
303. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
304. Were groups similar at baseline?				X		
305. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	X					Described in discussion
306. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
307. Was overall attrition less than 30%?				X		
308. Was differential attrition less than 15%?				X		
309. Does the analysis control for baseline				X		

differences between groups?						
310. Does the analysis account for differences in treatment received by the groups?		X				
311. Are the statistical methods used to assess the outcomes appropriate?	X					
312. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Analysis adjusted for tumor size, central grade, age, and positive nodes (>=4 vs. 1-3 nodes) (essentially looked at added value of RS beyond using Adjuvant! online. Positive nodes variable was only included in analyses for N+ patients. All subjects were hormone receptor + from a trial of tamoxifen vs. anastrozole. In this study the HR for RS was similar for patients receiving either treatment and there was no significant interaction of RS with treatment arm, reducing concern that treatment differences account for findings.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3336	Eireman	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
313. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
314. For RCTs, were randomization and allocation concealment adequate?					X	
315. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
316. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					This is like a pre-post trst so the comparison group is the same group of pts & physicians
317. Were groups similar at baseline?					X	
318. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	X					
319. Were outcomes assessed using valid and reliable measures, implemented consistently	X					

across all study participants?						
320. Was overall attrition less than 30%?	X					
321. Was differential attrition less than 15%?	X					
322. Does the analysis control for baseline differences between groups?					X	
323. Does the analysis account for differences in treatment received by the groups?					X	
324. Are the statistical methods used to assess the outcomes appropriate?	X					
325. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (X__)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3548	Eklof	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
326. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
327. For RCTs, were randomization and allocation concealment adequate?					X	
328. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
329. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
330. Were groups similar at baseline?		X				Differences for age, sex, site
331. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
332. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Used Swedish population registry and patient records;

						otherwise, little information reported
333. Was overall attrition less than 30%?	X					
334. Was differential attrition less than 15%?	X					
335. Does the analysis control for baseline differences between groups?	X					
336. Does the analysis account for differences in treatment received by the groups?		X				
337. Are the statistical methods used to assess the outcomes appropriate?	X					
338. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Final model adjusted for sex, age at diagnosis, stage, tumor site
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
501	Farina-Sarasqueta	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
339. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
340. For RCTs, were randomization and allocation concealment adequate?					X	
341. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
342. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
343. Were groups similar at baseline?		X				observational study, patients with mutations likely naturally different
344. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
345. Were outcomes assessed using valid and reliable measures, implemented consistently	X					

across all study participants?						
346. Was overall attrition less than 30%?					X	restrospective cohort
347. Was differential attrition less than 15%?					X	retrospective cohort—limited to patients with complete records
348. Does the analysis control for baseline differences between groups?	X					
349. Does the analysis account for differences in treatment received by the groups?			X			controlled for stage, and onlystage III patients got chemotherapy
350. Are the statistical methods used to assess the outcomes appropriate?	X					
351. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3551	Feigelson	2012

Indicate the appropriate quality rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable **AND** for an Overall rating. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	X	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
352. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
353. For RCTs, were randomization and allocation concealment adequate?						
354. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
355. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
356. Were groups similar at baseline?						
357. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
358. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
359. Was overall attrition less than 30%?						
360. Was differential attrition less than 15%?						
361. Does the analysis control for baseline						

differences between groups?						
362. Does the analysis account for differences in treatment received by the groups?						
363. Are the statistical methods used to assess the outcomes appropriate?						
364. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?		X					
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?				X			
If a threshold was used, was it pre-specified?				X			
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: The study was looking at inter lab concordance. So not quite sure how the questions here apply. Some differences in techniques betweebn labs and LLD is different but still achieved 90% concordance in detection. Sample size is small.							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
534	Gao	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low	*	Medium	*	High	*	N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	Unclear risk of bias due to inadequate reporting of patient selection and other methods							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
365. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
366. For RCTs, were randomization and allocation concealment adequate?						
367. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
368. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
369. Were groups similar at baseline?						
370. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
371. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
372. Was overall attrition less than 30%?						
373. Was differential attrition less than 15%?						

374. Does the analysis control for baseline differences between groups?							
375. Does the analysis account for differences in treatment received by the groups?							
376. Are the statistical methods used to assess the outcomes appropriate?							
377. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?							X
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			X				
If a threshold was used, was it pre-specified?				X			
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?			X				
Could the reference standard, its conduct, or its interpretation have introduced bias?							X
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: Unclear risk of bias due to incomplete reporting							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
183	Geffen	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
378. What is the study design?				**xx**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
379. For RCTs, were randomization and allocation concealment adequate?					x	
380. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?			x			All women tested were examined but authors acknowledge that some eligible women were not tested
381. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?					x	Pre/post comparison same subjects
382. Were groups similar at baseline?					x	Pre/post comparison
383. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		x				

384. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?			x			Authors state they determined "pre test" recommendation on based on usual clinical practice but acknowledge this included clinical judgement, actual recommendation was not recorded before getting RS
385. Was overall attrition less than 30%?					x	
386. Was differential attrition less than 15%?					x	
387. Does the analysis control for baseline differences between groups?					x	
388. Does the analysis account for differences in treatment received by the groups?					x	
389. Are the statistical methods used to assess the outcomes appropriate?						
390. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (X__)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							

Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
548	Geido	2002

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
391. What is the study design?				**x**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
392. For RCTs, were randomization and allocation concealment adequate?					x	
393. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
394. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
395. Were groups similar at baseline?			x			Some differences in demographics as expected for non-randomized study
396. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				x		Outcome is survival, not important
397. Were outcomes assessed using valid and	x					

reliable measures, implemented consistently across all study participants?						
398. Was overall attrition less than 30%?				x		
399. Was differential attrition less than 15%?				x		
400. Does the analysis control for baseline differences between groups?	x					
401. Does the analysis account for differences in treatment received by the groups?		x				
402. Are the statistical methods used to assess the outcomes appropriate?	x					
403. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3411	Gligorov	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High	Unclear	N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	Unclear risk of bias; abstract only from conference; insufficient information presented to assess risk of selection bias and measurement bias.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
404. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
405. For RCTs, were randomization and allocation concealment adequate?					X	
406. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		No inclusion/exclusion criteria in the abstract
407. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
408. Were groups similar at baseline?					X	It's a pre-post study of changes in decisions with/without Oncotype Dx
409. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
410. Were outcomes assessed using valid and				X		

reliable measures, implemented consistently across all study participants?						
411. Was overall attrition less than 30%?	X					
412. Was differential attrition less than 15%?	X					
413. Does the analysis control for baseline differences between groups?					X	
414. Does the analysis account for differences in treatment received by the groups?					X	
415. Are the statistical methods used to assess the outcomes appropriate?				X		
416. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: Abstract only, from conference, preliminary data on 92 of 100 subjects						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3443	Gonzalez De Castro	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	X	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
417. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
418. For RCTs, were randomization and allocation concealment adequate?						
419. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
420. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
421. Were groups similar at baseline?						
422. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
423. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
424. Was overall attrition less than 30%?						
425. Was differential attrition less than 15%?						
426. Does the analysis control for baseline						

differences between groups?						
427. Does the analysis account for differences in treatment received by the groups?						
428. Are the statistical methods used to assess the outcomes appropriate?						
429. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?	X						
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?			X				
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
579	Gryfe	2000

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
430. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
431. For RCTs, were randomization and allocation concealment adequate?					X	
432. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
433. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
434. Were groups similar at baseline?			X			Differences for synchronous or metachronous, location (more proximal among the MSI group), grade (more poorly

						differentiated among MSI group), and stage (fewer stage 4 in MSI group)
435. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	X					
436. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
437. Was overall attrition less than 30%?	X					
438. Was differential attrition less than 15%?	X					
439. Does the analysis control for baseline differences between groups?	X					
440. Does the analysis account for differences in treatment received by the groups?		X				Treatment differences were not included in the multivariate analysis; and information on chemo initiated within 120 days was only available for 65% of subjects
441. Are the statistical methods used to assess the outcomes appropriate?	X					
442. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					See comments below
Comments: Multivariate model obtained with step-down variable selection, in which all prognostic factors were initially entered and those with P>0.1 were rejected. Model did not include treatment differences and the outcome of the model is OS, not recurrence, so there are still a number of other potential confounders that could alter risk of death that the study does not consider (e.g., heart disease, diabetes).						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							

Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3751	Guan	2013

Indicate the appropriate quality rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable **AND** for an Overall rating. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
443. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
444. For RCTs, were randomization and allocation concealment adequate?					X	
445. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
446. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?			X			Some confusion in the description of the exclusion criteria which eliminated patients with EGFR mutations at exon 18 or 20 and the matched EGFR mutation group.

447. Were groups similar at baseline?	X					
448. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
449. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
450. Was overall attrition less than 30%?	X					
451. Was differential attrition less than 15%?	X					
452. Does the analysis control for baseline differences between groups?	X					
453. Does the analysis account for differences in treatment received by the groups?		X				
454. Are the statistical methods used to assess the outcomes appropriate?	X					
455. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Primary analysis for OS did not control for treatment. Matching description a little confusing						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
581	Guidoboni	2001

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
456. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
457. For RCTs, were randomization and allocation concealment adequate?					X	
458. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
459. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
460. Were groups similar at baseline?		X				Higher proportion 60 and older, with poorly differentiated tumor, and with mucoid histological type in the MSI-H group
461. Were the outcome assessors blinded to the test result/intervention/exposure status of				X		

participants?						
462. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
463. Was overall attrition less than 30%?	X					
464. Was differential attrition less than 15%?	X					
465. Does the analysis control for baseline differences between groups?		X				Other than age, the things listed above were not included in the analysis
466. Does the analysis account for differences in treatment received by the groups?		X				But a very similar proportion of subjects in both groups received adjuvant chemo
467. Are the statistical methods used to assess the outcomes appropriate?	X					
468. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Adjusted for age, sex, and stage (and all tumors were right sided/proximal); did not adjust for grade or cellular subtypes
Comments: Small to moderate risk of measurement bias with unclear masking of outcome assessors; moderate risk of confounding with some baseline differences between groups not considered in the analysis						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have							

introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
196	Habel	2006

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
469. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
470. For RCTs, were randomization and allocation concealment adequate?					x	
471. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
472. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
473. Were groups similar at baseline?			x			Matched on age, race, tamo tx, year, facility
474. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				x		Outcome was death
475. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					Death determined by insurance records

476. Was overall attrition less than 30%?					x	Full f/u was a requirement for inclusion
477. Was differential attrition less than 15%?					x	
478. Does the analysis control for baseline differences between groups?	x					
479. Does the analysis account for differences in treatment received by the groups?	x					
480. Are the statistical methods used to assess the outcomes appropriate?	x					
481. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
603	Hancer	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low	*	Medium	*	High	*	N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	*Overall, unclear risk of bias due to inadequate reporting							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
482. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
483. For RCTs, were randomization and allocation concealment adequate?						
484. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
485. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
486. Were groups similar at baseline?						
487. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
488. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
489. Was overall attrition less than 30%?						
490. Was differential attrition less than 15%?						
491. Does the analysis control for baseline						

differences between groups?							
492. Does the analysis account for differences in treatment received by the groups?							
493. Are the statistical methods used to assess the outcomes appropriate?							
494. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?							X
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			X				
If a threshold was used, was it pre-specified?			X				
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?			X				
Could the reference standard, its conduct, or its interpretation have introduced bias?							X
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: Overall, unclear risk of bias due to inadequate reporting							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
137	Henry	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
495. What is the study design?						x
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
496. For RCTs, were randomization and allocation concealment adequate?					x	
497. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
498. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					pre-post test design: pre-test decisions compared to post-test decisions
499. Were groups similar at baseline?	x					same patients pre/post
500. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?			x			blinded for pre test
501. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					

502. Was overall attrition less than 30%?						x	
503. Was differential attrition less than 15%?						x	
504. Does the analysis control for baseline differences between groups?						x	
505. Does the analysis account for differences in treatment received by the groups?						x	
506. Are the statistical methods used to assess the outcomes appropriate?	x						descriptive statistics only
507. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2051	Hiramatsu	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	High risk of selection bias, ascertainment bias, and confounding.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
508. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
509. For RCTs, were randomization and allocation concealment adequate?					X	
510. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		Methods of selecting included cases NR
511. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					Probably, but methods of selecting the full sample are not described
512. Were groups similar at baseline?				X		Baseline data are not reported by mutation status
513. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		

514. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		No methods reported for how the prognostic outcomes were defined or ascertained
515. Was overall attrition less than 30%?		X				
516. Was differential attrition less than 15%?				X		
517. Does the analysis control for baseline differences between groups?				X		
518. Does the analysis account for differences in treatment received by the groups?		X				
519. Are the statistical methods used to assess the outcomes appropriate?	X					
520. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: High risk of selection bias, ascertainment bias, and confounding. Methods of selecting the 193 lung cancer cases NR; only evaluated EGFR and KRAS mutation status for 93/193 (48%) and reasons for selecting those 93 were NR; no description of how outcomes were defined or ascertained (it is described in the results as "survival", but unclear if it is disease-specific or overall survival; but seems more likely overall survival); analyses don't account for differences in treatment received.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3507	Holt	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
521. What is the study design?		**Uncontrolled trial**				
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
522. For RCTs, were randomization and allocation concealment adequate?					X	
523. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
524. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
525. Were groups similar at baseline?				X		
526. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
527. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
528. Was overall attrition less than 30%?	X					

529. Was differential attrition less than 15%?	X					
530. Does the analysis control for baseline differences between groups?		X				
531. Does the analysis account for differences in treatment received by the groups?	X					
532. Are the statistical methods used to assess the outcomes appropriate?	X					
533. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
630	Hong	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
534. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
535. For RCTs, were randomization and allocation concealment adequate?					X	
536. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
537. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
538. Were groups similar at baseline?		X				Differences for age, family history, stage, tumor type (colon or rectum), site of tumor, grade, CEA level
539. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		

540. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
541. Was overall attrition less than 30%?	X					
542. Was differential attrition less than 15%?	X					
543. Does the analysis control for baseline differences between groups?			X			Controls for age, sex, stage
544. Does the analysis account for differences in treatment received by the groups?		X				Table 5 shows additional analysis stratified by whether they got 5-FU chemo, but these are set up to focus on prediction, not to adjust for differences in treatment within the multivariate analysis of MSI-H vs. MSS/MSI-L (and nothing to account for radiation)
545. Are the statistical methods used to assess the outcomes appropriate?	X					
546. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?			X			It controls for age, sex, stage, but not for grade, cellular type, CEA, location; they also stratify colon and rectal cancers
Comments:						

Key Question 2				If study does NOT apply to this KQ, place an 'X' here (_X_)			
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced							

bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments: Overall, unclear risk of bias due to inadequate reporting							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1621	Imamura	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
547. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
548. For RCTs, were randomization and allocation concealment adequate?					X	
549. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
550. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
551. Were groups similar at baseline?		X				Baseline differences for sex, tumor location, stage, differentiation, MSI status
552. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
553. Were outcomes assessed using valid and	X					

reliable measures, implemented consistently across all study participants?						
554. Was overall attrition less than 30%?				X		
555. Was differential attrition less than 15%?				X		
556. Does the analysis control for baseline differences between groups?	X					
557. Does the analysis account for differences in treatment received by the groups?		X				
558. Are the statistical methods used to assess the outcomes appropriate?	X					
559. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?				X		Unable to determine what was in the final model. They report what went into the initial model, and process for selecting final model (but not what was in it)
Comments: High risk of selection bias and confounding. The variables in the final model were not reported, but the process described for selecting the variables seems reasonable. The article reports that the regression model was stage-stratified, but the results reported in Table 3 and in the text are not stage-stratified (there is just one HR for codon 12 mutants and one for codon 13 mutants for each outcome); so it is unclear that the HRs reported did anything to account for stage. Given these issues, high risk of confounding bias. With the codon 12 mutants found to have HRs just above 1 (1.30 and 1.24 for cancer-specific mortality and overall mortality), the findings might become non-significant with relatively minor changes in what was adjusted for in the model, and how stage was handled.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							

Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3281	Iwamoto	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

RoB Rating: Unclear

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low	*	Medium	*	High	*	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
560. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
561. For RCTs, were randomization and allocation concealment adequate?					X	
562. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		
563. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
564. Were groups similar at baseline?				X		
565. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
566. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
567. Was overall attrition less than 30%?				X		

568. Was differential attrition less than 15%?				X		
569. Does the analysis control for baseline differences between groups?				X		
570. Does the analysis account for differences in treatment received by the groups?					X	From description, all got same treatment
571. Are the statistical methods used to assess the outcomes appropriate?	X					
572. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3456	Jancik	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High	H	N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
573. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
574. For RCTs, were randomization and allocation concealment adequate?						
575. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
576. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
577. Were groups similar at baseline?						
578. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
579. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
580. Was overall attrition less than 30%?						
581. Was differential attrition less than 15%?						
582. Does the analysis control for baseline						

differences between groups?							
583. Does the analysis account for differences in treatment received by the groups?							
584. Are the statistical methods used to assess the outcomes appropriate?							
585. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?	X						
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
673	Jensen	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
586. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
587. For RCTs, were randomization and allocation concealment adequate?					X	
588. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
589. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
590. Were groups similar at baseline?		X				Differences for age, tumor site, grade, and bowel obstruction
591. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
592. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?			X			Yes for death, unclear for recurrence (used

						databases on hospital admission)
593. Was overall attrition less than 30%?	X					
594. Was differential attrition less than 15%?	X					
595. Does the analysis control for baseline differences between groups?			X			Initial model reported to include all candidate prognostic variables; final model did not include age, site, grade (and all were different at baseline)
596. Does the analysis account for differences in treatment received by the groups?					X	All received same adjuvant treatment
597. Are the statistical methods used to assess the outcomes appropriate?	X					
598. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?		X				Final model adjusted for stage, vascular invasion, perineural invasion, and ileus.
Comments: Final model adjusted for stage, vascular invasion, perineural invasion, and ileus. Initial model reported to include all candidate prognostic variables (but they don't report what that full list included), and they used step-down variable selection; final model did not include age, site, grade (and all were different in baseline characteristics) or cellular subtypes (NR at baseline or as something included in models) or sex.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have							

introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
110	Joh	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
599. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
600. For RCTs, were randomization and allocation concealment adequate?					X	
601. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
602. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
603. Were groups similar at baseline?		x				would not be expected to be similar in retrospective design
604. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	x					
605. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						

606. Was overall attrition less than 30%?					x	
607. Was differential attrition less than 15%?					x	
608. Does the analysis control for baseline differences between groups?					x	
609. Does the analysis account for differences in treatment received by the groups?					x	
610. Are the statistical methods used to assess the outcomes appropriate?	x					
611. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					x	
Comments: Study was a survey of providers regarding treatment recommendations with or without information from the oncotype test. Answered these questions with patients as "participants" and providers as "assessors" since no comparative data were given regarding demographics of the small # of providers in the study.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
691	Kakar	2008

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	High risk of selection bias and confounding. Small sample size and it's unclear how subjects were selected from among those undergoing resection from 1996 to 2000 (NR whether consecutive or what the criteria were). Some baseline differences between groups and some known prognostic factors were not considered in the analyses (i.e., multivariate analysis did not adjust for some important factors). Just 10 subjects with BRAF mutation. Unable to assess potential for measurement bias because little information reported. The magnitude of benefit is large (HR above 5), but with such a small sample size, still high risk of confounding.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
612. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
613. For RCTs, were randomization and allocation concealment adequate?					X	
614. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		
615. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?				X		
616. Were groups similar at baseline?			X			Similar for sex, age, all were MSS; higher proportion of

						those with BRAF mutation (than those without BRAF mutation) had right sided cancer and stage III or IV cancer
617. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
618. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
619. Was overall attrition less than 30%?				X		
620. Was differential attrition less than 15%?				X		
621. Does the analysis control for baseline differences between groups?			X			Not for differences in tumor location
622. Does the analysis account for differences in treatment received by the groups?		X				
623. Are the statistical methods used to assess the outcomes appropriate?	X					
624. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?			X			Adjusted for age, sex, stage; did not adjust for grade, cellular subtypes, lymphovascular invastion, location, margin status
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have							

introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
693	Kalady	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
625. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
626. For RCTs, were randomization and allocation concealment adequate?					X	
627. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		Unclear how they got from 475 included subjects down to 322 to include in the analyses (59 were not included because they were stage 4, but another 94 that were not included)
628. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
629. Were groups similar at baseline?		X				Differences for age, sex,

						location, differentiation, MSI status
630. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
631. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		No information reported for how outcomes were ascertained
632. Was overall attrition less than 30%?		X				23% of Stage I to III subjects were not included in the survival analyses
633. Was differential attrition less than 15%?				X		NR
634. Does the analysis control for baseline differences between groups?	X					
635. Does the analysis account for differences in treatment received by the groups?		X				
636. Are the statistical methods used to assess the outcomes appropriate?	X					
637. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: 475 included in Table 1, but just 322 included in the survival analyses (related to comments above). Note: Included for KQ3 only and for BRAF only--MSI used the 10 marker panel						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							

Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
170	Kamal	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
638. What is the study design?						Other: uncontrolled trial
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
639. For RCTs, were randomization and allocation concealment adequate?					X	
640. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?					X	study did not form comparison groups for the subjects (the 6 oncologists are the subjects)
641. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?					X	
642. Were groups similar at baseline?					X	
643. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
644. Were outcomes assessed using valid and				X		Details NR

reliable measures, implemented consistently across all study participants?						about how they really gathered data on the oncologists decisions
645. Was overall attrition less than 30%?	X					
646. Was differential attrition less than 15%?	X					
647. Does the analysis control for baseline differences between groups?					X	
648. Does the analysis account for differences in treatment received by the groups?					X	
649. Are the statistical methods used to assess the outcomes appropriate?	X					
650. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: uncontrolled trial evaluating whether 6 medical oncologists would change treatment recommendations when given Oncotype Dx RS result in addition to information about standard demographic and tumor prognostic criteria (as opposed to just being given standard prognostic criteria); some risk of measurement bias (no detailed description of how they gathered outcomes; unlike some other studies that used a formal questionnaire, this sounds less formal and perhaps more subject to social desirability bias, ascertainment bias); relatively small sample (6 oncologists) and no information provided about those 6 (e.g., whether they were from multiple centers or just from 1 group with like-minded approach)						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3369	Kamat	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
651. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
652. For RCTs, were randomization and allocation concealment adequate?					X	
653. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
654. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
655. Were groups similar at baseline?					X	
656. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
657. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
658. Was overall attrition less than 30%?	X					
659. Was differential attrition less than 15%?	X					
660. Does the analysis control for baseline					X	

differences between groups?						
661. Does the analysis account for differences in treatment received by the groups?					X	
662. Are the statistical methods used to assess the outcomes appropriate?	X					
663. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (X__)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
715	Kim	2007

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	x
Risk of bias for KQ 2:	Low		Medium		High		N/A	x
Risk of bias for KQ 3:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	x
Risk of bias for KQ 4b:	Low		Medium		High		N/A	x
Risk of bias for KQ 5:	Low		Medium		High		N/A	x
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
664. What is the study design?				**x**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
665. For RCTs, were randomization and allocation concealment adequate?					x	
666. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
667. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
668. Were groups similar at baseline?				x		
669. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	x					
670. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
671. Was overall attrition less than 30%?	x					
672. Was differential attrition less than 15%?	x					
673. Does the analysis control for baseline				x		Since

differences between groups?						baseline differences not reported
674. Does the analysis account for differences in treatment received by the groups?	x					
675. Are the statistical methods used to assess the outcomes appropriate?						
676. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2165	Kim	2008

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
677. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
678. For RCTs, were randomization and allocation concealment adequate?					X	
679. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					Criteria were that they underwent surgical resection for primary lung cancer between Dec 2000 and Apr 2004 at their University Hospital, path diagnosis of lung adenocarcinoma, and frozen specimens available

680. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
681. Were groups similar at baseline?		X (for EGFR mutation status)		X (for KRAS)		Table 2 only provided gender, age, smoking, BAC features, and stage for EGFR status (some differences for gender, smoking status, and BAC); information is NR for KRAS
682. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
683. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?			X			Methods of detecting recurrence seem valid and reliable; for deaths, no description of methods to ensure capturing all deaths (and article does not describe completeness of follow up)
684. Was overall attrition less than 30%?				X		Missing data and loss to follow up not described in detail for the 71 patients (mean f/u was 25.3 months, range 2.9 to 47.5)
685. Was differential attrition less than 15%?				X		
686. Does the analysis control for baseline differences between groups?	X					
687. Does the analysis account for differences in treatment received by the groups?		X				
688. Are the statistical methods used to assess the outcomes appropriate?	X					
689. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Most
Comments: no details reported for reasons that most of the 653 patients who underwent surgical resection from primary lung cancer were not included; 71 were included in this study (perhaps availability of frozen specimens was the main reason)						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?	

	Yes	No	Unclear	NA	Low	High	Unclear
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3784	Kim	2013

Indicate the appropriate quality rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable **AND** for an Overall rating. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
690. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
691. For RCTs, were randomization and allocation concealment adequate?					X	
692. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
693. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
694. Were groups similar at baseline?		X				
695. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
696. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
697. Was overall attrition less than 30%?	X					
698. Was differential attrition less than 15%?	X					
699. Does the analysis control for baseline	X					

differences between groups?						
700. Does the analysis account for differences in treatment received by the groups?		X				
701. Are the statistical methods used to assess the outcomes appropriate?	X					
702. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Do not seem to have controlled for size, differentiation but do not think that introduces much bias.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
140	Klang	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
703. What is the study design?						**x**
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
704. For RCTs, were randomization and allocation concealment adequate?						
705. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
706. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					pre-post test design
707. Were groups similar at baseline?					x	case series
708. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	x					pre-post design, did not know results in pretest
709. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
710. Was overall attrition less than 30%?					x	
711. Was differential attrition less than 15%?					x	

712. Does the analysis control for baseline differences between groups?					x	
713. Does the analysis account for differences in treatment received by the groups?	x					
714. Are the statistical methods used to assess the outcomes appropriate?	x					
715. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments: The parent study is a cost effectiveness analysis (none of the listed study designs), but case scenarios from 300+ consecutive patients were used to generate estimates so I call it a case series.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
179	Knauer	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
716. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
717. For RCTs, were randomization and allocation concealment adequate?					X	
718. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
719. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
720. Were groups similar at baseline?				X		
721. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
722. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
723. Was overall attrition less than 30%?				X		
724. Was differential attrition less than 15%?				X		
725. Does the analysis control for baseline				X		

differences between groups?						
726. Does the analysis account for differences in treatment received by the groups?	X					
727. Are the statistical methods used to assess the outcomes appropriate?	X					
728. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					

Comments: For the study design, they defined their cohort as a subset (unilateral stage pT1 to 3, HER-2 positive, etc.) of a database comprised of subjects from previous studies. Medium or greater risk of measurement bias (no information presented about whether assessment of outcomes was equal, valid, and reliable) and whether outcome assessors were aware of test results. Difficult to assess selection bias and confounding since they don't report attrition data (overall or differential) or baseline characteristics for the two groups separately. But, their multivariate analysis does account for most or all of the standard prognostic markers, decreasing the concern for confounding somewhat. Was on the fence between medium and high risk of bias, but went with medium for this.

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2176	Kobayashi	2008

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium	X	High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
729. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
730. For RCTs, were randomization and allocation concealment adequate?					X	
731. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
732. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
733. Were groups similar at baseline?		X				Table 2 doesn't report all the numbers and percents for the wild-type group to easily compare them, but they give enough information to

						do the calculations ourselves; groups were different for sex and smoking status at baseline, similar for age CEA level, tumor size (all were same Stage)
734. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
735. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?			X			If people continued to follow up as planned, then the measures are valid and reliable. But, nothing additional done to ensure capturing all deaths beyond using data from scheduled routine follow up (and from the f/u information reported, unable to determine how many subjects only had 1 to 3 years of follow up time captured).
736. Was overall attrition less than 30%?				X		Median f/u among surviving patients 67 months (range 12-134 months); loss to follow up details otherwise NR.
737. Was differential attrition less than 15%?				X		Data NR to allow determination
738. Does the analysis control for baseline differences between groups?	X					
739. Does the analysis account for differences in treatment received by the groups?	X					Since all were Stage IA and <20mm,

						treatment was surgical without chemo or radiotherapy
740. Are the statistical methods used to assess the outcomes appropriate?	X					
741. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					All were stage IA; analyses adjust for age, sex, smoking status, CEA, tumor size, Non-BAC component, and EGFR
Comments: some concern for selection bias, adequacy of follow up information (missing data), and ascertainment bias						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
741	Kobunai	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	X	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (X__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
742. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
743. For RCTs, were randomization and allocation concealment adequate?						
744. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
745. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
746. Were groups similar at baseline?						
747. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
748. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
749. Was overall attrition less than 30%?						
750. Was differential attrition less than 15%?						
751. Does the analysis control for baseline						

differences between groups?							
752. Does the analysis account for differences in treatment received by the groups?							
753. Are the statistical methods used to assess the outcomes appropriate?							
754. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?	X						
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?			X				
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?							X
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: it is unclear in this paper which is the reference standard – direct sequencing is generally thought of as a standard, but the alternative test (real time PCR using PNA clamping) appears to be more sensitive							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2180	Koh	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High	x	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
755. What is the study design?				**x**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
756. For RCTs, were randomization and allocation concealment adequate?					x	
757. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
758. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
759. Were groups similar at baseline?				x		Demographics not reported by EGFR status
760. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		x				Outcome is death
761. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						Outcome is death
762. Was overall attrition less than 30%?					X	

763. Was differential attrition less than 15%?					X	
764. Does the analysis control for baseline differences between groups?	X					
765. Does the analysis account for differences in treatment received by the groups?		X				Excluded patients who got chemo
766. Are the statistical methods used to assess the outcomes appropriate?	X					
767. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments: methods of measuring EGFR not described. % of sample with EGFR mutations not described, limiting the ability to determine whether sample size is a problem. Also unclear how the inclusion of novel markers with uncertain interactions with EGFR positivity in the model would change the effect size/direction with respect to EGFR.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3600	Li	2012

Indicate the appropriate quality rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable **AND** for an Overall rating. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
768. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
769. For RCTs, were randomization and allocation concealment adequate?					X	
770. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
771. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
772. Were groups similar at baseline?		X				
773. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
774. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Ascertainment methods NR
775. Was overall attrition less than 30%?	X					
776. Was differential attrition less than 15%?	X					
777. Does the analysis control for baseline	X					

differences between groups?						
778. Does the analysis account for differences in treatment received by the groups?					X	All received same treatment
779. Are the statistical methods used to assess the outcomes appropriate?	X					
780. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
843	Lin	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
781. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
782. For RCTs, were randomization and allocation concealment adequate?					X	
783. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
784. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
785. Were groups similar at baseline?		X				Differences for histology, mucin-containing tumors, location (proximal), sex, and stage
786. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		

787. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
788. Was overall attrition less than 30%?	X					Follow up was 98% for at least 3 years (not certain beyond 3 years)
789. Was differential attrition less than 15%?	X					
790. Does the analysis control for baseline differences between groups?			X			Yes, for the variables listed below; no for mucinous component, location (proximal or distal), but those were NS in the univariate
791. Does the analysis account for differences in treatment received by the groups?		X				
792. Are the statistical methods used to assess the outcomes appropriate?	X					
793. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Most, not all
Comments: Some risk of measurement bias and confounding. Not large effect size in multivariate analyses for OS (the only one that remained statistically significant). Possible that it also would have been NS with further adjustment for other potential confounders.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							

Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
851	Liou	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
794. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
795. For RCTs, were randomization and allocation concealment adequate?					X	
796. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
797. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
798. Were groups similar at baseline?		X				For the KRAS groups, differences for smoking, age, BMI, alcohol drinking, DM, and location of cancer (proximal vs. distal). For the BRAF groups,

						differences for location of cancer, differentiation.
799. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
800. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X			X		Death ascertained by National Taiwan Mortality Registry. It was equal, probably valid and reliable for death/overall survival, but uncertain if it is valid and reliable to determine cause of death (for cancer-specific death outcomes)
801. Was overall attrition less than 30%?	X					Exact attrition data NR, but median followup range was from 49 to 82 months (suggesting that they had at least 4 years of follow up for all subjects)
802. Was differential attrition less than 15%?	X					
803. Does the analysis control for baseline differences between groups?			X			
804. Does the analysis account for differences in treatment received by the groups?		X				Yes for KRAS for OS, but no for BRAF analyses. But, more than 85% of patients for whom adjuvant chemo was indicated (stage 3 or 4) got the same regimen—5FU, LV, and oxaliplatin; the other 15% got 5FU and LV.
805. Are the statistical methods used to assess	X					

the outcomes appropriate?						
806. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					For BRAF, adjusted for age, sex, stage, lymphatic invasion, venous invasion, tumor differentiation, CEA level (>5 vs. ≤5 ng/mL); For KRAS overall survival analysis: age, sex, stage, use of adjuvant chemotherapy
Comments: Moderate risk of selection bias, measurement bias, and confounding						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2249	Liu	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
807. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
808. For RCTs, were randomization and allocation concealment adequate?					X	
809. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
810. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
811. Were groups similar at baseline?				x		Detailed demographics by group not reported
812. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				Outcome is survival
813. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					Outcome is survival
814. Was overall attrition less than 30%?					X	

815. Was differential attrition less than 15%?					X	
816. Does the analysis control for baseline differences between groups?	X					
817. Does the analysis account for differences in treatment received by the groups?		x				Some pts received radiation or chemo and these were not controlled for
818. Are the statistical methods used to assess the outcomes appropriate?	X					
819. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (X__)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
175	Lo	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
820. What is the study design?						Other, design is an uncontrolled trial
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
821. For RCTs, were randomization and allocation concealment adequate?					X	
822. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?					X	Just one group
823. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?					X	
824. Were groups similar at baseline?					X	
825. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
826. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
827. Was overall attrition less than 30%?	X					

828. Was differential attrition less than 15%?	X					
829. Does the analysis control for baseline differences between groups?					X	
830. Does the analysis account for differences in treatment received by the groups?					X	
831. Are the statistical methods used to assess the outcomes appropriate?	X					
832. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: uncontrolled trial (with the intervention being giving the oncologists the Oncotype Dx information) evaluating whether Oncotype Dx information changes decisions. Good that they attempted to get oncologists from several centers (4 centers in 3 different states—California, Michigan, Illinois) to avoid just representing the opinion of 1 group. Formal questionnaire to assess treatment recommendations and confidence in those recommendations.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3605	Lochhead	2013

Indicate the appropriate quality rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable **AND** for an Overall rating. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
833. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
834. For RCTs, were randomization and allocation concealment adequate?					X	
835. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
836. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
837. Were groups similar at baseline?		X				
838. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
839. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?			X			National Death Index is valid and reliable for all-cause mortality, but it is not clear

						whether having the study physicians determine the cause of death is valid and reliable for cancer-specific mortality
840. Was overall attrition less than 30%?	X					
841. Was differential attrition less than 15%?	X					
842. Does the analysis control for baseline differences between groups?	X					
843. Does the analysis account for differences in treatment received by the groups?		X				
844. Are the statistical methods used to assess the outcomes appropriate?	X					
845. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3458	Lopez-Rios	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low	X	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
846. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
847. For RCTs, were randomization and allocation concealment adequate?						
848. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
849. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
850. Were groups similar at baseline?						
851. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
852. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
853. Was overall attrition less than 30%?						
854. Was differential attrition less than 15%?						
855. Does the analysis control for baseline						

differences between groups?							
856. Does the analysis account for differences in treatment received by the groups?							
857. Are the statistical methods used to assess the outcomes appropriate?							
858. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?		X					
Was a case-control design avoided?	x						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?	X						
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	x						
Were the reference standard results interpreted without knowledge of the results of the index test?	x						
Could the reference standard, its conduct, or its interpretation have introduced bias?					x		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	x						
Did patients receive the same reference standard?	x						
Were all patients included in the analysis?	x						
Could the patient flow have introduced bias?					x		
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
883	Maestro	2007

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
859. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
860. For RCTs, were randomization and allocation concealment adequate?					X	
861. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
862. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
863. Were groups similar at baseline?			X			Some differences for site of tumor, sex
864. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		Not reported
865. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		No details reported of how they determined deaths, and

						which were a consequence of the tumor (which is what they analyzed)
866. Was overall attrition less than 30%?	X					Just 1 lost to followup
867. Was differential attrition less than 15%?	X					
868. Does the analysis control for baseline differences between groups?	X					
869. Does the analysis account for differences in treatment received by the groups?	X					
870. Are the statistical methods used to assess the outcomes appropriate?	X					
871. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Moderate risk of measurement bias; no reporting of masking of outcome assessors; no reporting of methods of ascertainment.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2267	Mak	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
872. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
873. For RCTs, were randomization and allocation concealment adequate?					X	
874. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
875. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
876. Were groups similar at baseline?		X				Differences for smoking status, Stage, tumor size, radiotherapy treatment mode, and whether they had surgical resection of the primary tumor.

877. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
878. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?			X			The definitions of the outcomes are reasonable, but unclear whether reviewing their medical records provided valid and reliable assessments of recurrence and survival; unclear how complete the data was from their medical record review (e.g., how many patients lacked complete f/u or went to other hospitals). No description of methods to ensure capturing deaths.
879. Was overall attrition less than 30%?				X		
880. Was differential attrition less than 15%?				X		
881. Does the analysis control for baseline differences between groups?	X					
882. Does the analysis account for differences in treatment received by the groups?	X					
883. Are the statistical methods used to assess the outcomes appropriate?	X					
884. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Final model didn't have most of them, but they started with considering most of them, and used appropriate stepwise selection to determine those in the final model.
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)

KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
176	Mamounas	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
885. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
886. For RCTs, were randomization and allocation concealment adequate?					X	
887. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
888. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
889. Were groups similar at baseline?				X		
890. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
891. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		The 2 RCTs that they obtained subjects from might have more description of

						how LRR was determined
892. Was overall attrition less than 30%?				X		
893. Was differential attrition less than 15%?				X		
894. Does the analysis control for baseline differences between groups?				X		
895. Does the analysis account for differences in treatment received by the groups?	X					
896. Are the statistical methods used to assess the outcomes appropriate?	X					
897. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: multivariate adjusted analysis included age, tumor size, tumor grade, and treatment differences (all subjects were node negative and ER+ so those did not need to be considered)						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3415	Mamounas	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	Unclear	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	Unclear risk of bias; abstract only from conference; insufficient information presented to assess risk of selection bias, confounding, and measurement bias.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
898. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
899. For RCTs, were randomization and allocation concealment adequate?					X	
900. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		
901. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
902. Were groups similar at baseline?				X		
903. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
904. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
905. Was overall attrition less than 30%?				X		
906. Was differential attrition less than 15%?				X		

907. Does the analysis control for baseline differences between groups?				X		
908. Does the analysis account for differences in treatment received by the groups?	X					
909. Are the statistical methods used to assess the outcomes appropriate?				X		
910. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1653	Mancini	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium	X	High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
911. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
912. For RCTs, were randomization and allocation concealment adequate?						
913. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
914. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
915. Were groups similar at baseline?						
916. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
917. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
918. Was overall attrition less than 30%?						
919. Was differential attrition less than 15%?						
920. Does the analysis control for baseline						

differences between groups?						
921. Does the analysis account for differences in treatment received by the groups?						
922. Are the statistical methods used to assess the outcomes appropriate?						
923. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?	X						
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?	X						
Could the selection of patients have introduced bias?					X		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			X				
If a threshold was used, was it pre-specified?	X						
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?			X				
Could the reference standard, its conduct, or its interpretation have introduced bias?							X
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: For the aspects that were reported, risk of bias seems low. Although it is not clear whether the various tests were interpreted without knowledge of the reference standard result and vice versa, the article implies that there was no masking of outcome assessors to the results of the other tests.							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2288	Matsumoto	2005

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
924. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
925. For RCTs, were randomization and allocation concealment adequate?					X	
926. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
927. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
928. Were groups similar at baseline?		x				Not expected in nonrandomized study
929. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				x		Outcome is death, not relevant
930. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
931. Was overall attrition less than 30%?				X		

932. Was differential attrition less than 15%?				X		
933. Does the analysis control for baseline differences between groups?	X					
934. Does the analysis account for differences in treatment received by the groups?		x				All pts treated pretty similarly
935. Are the statistical methods used to assess the outcomes appropriate?	X					
936. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: my only concern regarding this paper is the sample size, confidence interval was quite wide for main effect of interest. Also, restricted to stage I cancers which have an overall very good prognosis, should not generalize to the entire stage I-III population						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
133	Mook	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
937. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
938. For RCTs, were randomization and allocation concealment adequate?					X	
939. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
940. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
941. Were groups similar at baseline?		X				not expected in a retrospective observational design
942. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
943. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					

944. Was overall attrition less than 30%?				X		
945. Was differential attrition less than 15%?				X		
946. Does the analysis control for baseline differences between groups?	X					
947. Does the analysis account for differences in treatment received by the groups?	X					
948. Are the statistical methods used to assess the outcomes appropriate?	X					
949. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:							

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
109	Mook	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
950. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
951. For RCTs, were randomization and allocation concealment adequate?					X	
952. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?		X				pooled analysis of 7 cohorts with slightly different criteria
953. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
954. Were groups similar at baseline?		X				in non RCT comparison groups would not be expected to be similar
955. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	

956. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
957. Was overall attrition less than 30%?				X		
958. Was differential attrition less than 15%?				X		
959. Does the analysis control for baseline differences between groups?	X					
960. Does the analysis account for differences in treatment received by the groups?	X					
961. Are the statistical methods used to assess the outcomes appropriate?	X					
962. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
156	Mook	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
963. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
964. For RCTs, were randomization and allocation concealment adequate?					x	
965. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
966. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?			x			
967. Were groups similar at baseline?				x		
968. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					x	
969. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
970. Was overall attrition less than 30%?				x		
971. Was differential attrition less than 15%?				x		
972. Does the analysis control for baseline		x				used a

differences between groups?						composite of common prognostic factors including age and overall health, but do not appear to have adjusted for other baseline differences
973. Does the analysis account for differences in treatment received by the groups?		x				
974. Are the statistical methods used to assess the outcomes appropriate?	x					
975. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						see above, did not control separately for prognostic variables, used a composite score
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (_X_)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2342	Naoki	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	x	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_x_)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
976. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
977. For RCTs, were randomization and allocation concealment adequate?						
978. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
979. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
980. Were groups similar at baseline?						
981. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
982. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
983. Was overall attrition less than 30%?						
984. Was differential attrition less than 15%?						
985. Does the analysis control for baseline						

differences between groups?							
986. Does the analysis account for differences in treatment received by the groups?							
987. Are the statistical methods used to assess the outcomes appropriate?							
988. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?	x						
Was a case-control design avoided?	x						
Did the study avoid inappropriate exclusions?	x						
Could the selection of patients have introduced bias?					x		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			x				
If a threshold was used, was it pre-specified?				x			
Could the conduct or interpretation of the index test have introduced bias?					x		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	x						
Were the reference standard results interpreted without knowledge of the results of the index test?			x				
Could the reference standard, its conduct, or its interpretation have introduced bias?					x		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	x						
Did patients receive the same reference standard?	x						
Were all patients included in the analysis?	x						
Could the patient flow have introduced bias?					x		
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
113	Nuyten	2008

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
989. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
990. For RCTs, were randomization and allocation concealment adequate?					X	
991. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
992. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?			X			odd comparison of the test of interest (MammaPrint) to an alternative gene signature
993. Were groups similar at baseline?						
994. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
995. Were outcomes assessed using valid and reliable measures, implemented consistently						

across all study participants?						
996. Was overall attrition less than 30%?				X		
997. Was differential attrition less than 15%?				X		
998. Does the analysis control for baseline differences between groups?	X					
999. Does the analysis account for differences in treatment received by the groups?	X					
1000. Are the statistical methods used to assess the outcomes appropriate?	X					
1001. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: biggest concern about this study is that another gene signature with prognostic value and likely overlapping genetic info was included as a covariate, and this alternate signature is not a standard prognostic marker, skewing the analysis toward non-significance for the test of interest						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1033	Ogino	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1002. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1003. For RCTs, were randomization and allocation concealment adequate?					X	
1004. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1005. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1006. Were groups similar at baseline?			X			Similar for most characteristics; some small differences for MSI status and treatment arm
1007. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		

1008. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Just report how DFS, RFS, OS were defined, but don't report information on how outcomes were ascertained
1009. Was overall attrition less than 30%?	X					
1010. Was differential attrition less than 15%?	X					
1011. Does the analysis control for baseline differences between groups?	X					
1012. Does the analysis account for differences in treatment received by the groups?	X					
1013. Are the statistical methods used to assess the outcomes appropriate?	X					
1014. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: they created cohort from among subjects enrolled in an RCT comparing adjuvant chemotherapies for stage III CRC. This was 508 of the 1264 (those with tumor tissue available for KRAS sequencing). Article does nice job of exploring and addressing potential confounding through multivariate adjustment, stratification, and other statistical analyses. Group enrolled in this study were similar to the 700+ subjects that were in the RCT but not in this trial. Some risk of measurement bias as details are not reported for ascertainment.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1043	Ogino	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1015. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1016. For RCTs, were randomization and allocation concealment adequate?					X	
1017. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1018. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1019. Were groups similar at baseline?		X				For BRAF mutation vs. wild-type, differences for sex, age, tumor location, MSI status, and KRAS
1020. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		

1021. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
1022. Was overall attrition less than 30%?	X					
1023. Was differential attrition less than 15%?	X					
1024. Does the analysis control for baseline differences between groups?	X					
1025. Does the analysis account for differences in treatment received by the groups?	X					
1026. Are the statistical methods used to assess the outcomes appropriate?	X					
1027. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: they created cohort from among subjects enrolled in an RCT comparing adjuvant chemotherapies for stage III CRC. This was 506 of the 1264 (those with tumor tissue available). Article does nice job of exploring and addressing potential confounding through multivariate adjustment, stratification, and other statistical analyses. Group enrolled in this study were similar to the 700+ subjects that were in the RCT but not in this trial. Some risk of measurement bias as details are not reported for ascertainment. For overall survival, the only outcome that remained statistically significantly different between BRAF mutation vs. wild-type, the risk of bias may be higher than for other outcomes because they did not adjust for many potential confounders that would influence overall survival.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1071	Pai	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	High risk of selection bias, confounding, attrition bias, and measurement bias. See comments box below for details.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1028. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1029. For RCTs, were randomization and allocation concealment adequate?					X	
1030. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1031. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1032. Were groups similar at baseline?		X				
1033. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
1034. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?		X				
1035. Was overall attrition less than 30%?				X		
1036. Was differential attrition less than 15%?				X		

1037. Does the analysis control for baseline differences between groups?			X			
1038. Does the analysis account for differences in treatment received by the groups?		X				
1039. Are the statistical methods used to assess the outcomes appropriate?			X			
1040. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?		X				
Comments: High risk of selection bias and confounding. The multivariate analysis only adjusted for stage. It did not consider sex, age, grade, cellular subtype (e.g. signet ring histology), or lymphovascular invasion. The baseline characteristics show potentially important differences for sex, lymph invasion, lymph node metastases, histology. Very little description of methods of assessing outcomes (DFS and OS) in this retrospective cohort. No reporting of masking of outcome assessors to genotypes. High risk of attrition bias as they do not report numbers lost to follow up clearly for the 3 different groups. For the OS analyses, lost to followup was treated the same as death. Not clear how missing data was handled for the DFS analysis.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3276	Paik	2004

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1041. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1042. For RCTs, were randomization and allocation concealment adequate?					X	
1043. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		
1044. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1045. Were groups similar at baseline?				X		
1046. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1047. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Unclear ascertainment methods
1048. Was overall attrition less than 30%?				X		
1049. Was differential attrition less than 15%?				X		
1050. Does the analysis control for baseline				X		Data NR

differences between groups?						
1051. Does the analysis account for differences in treatment received by the groups?		X				
1052. Are the statistical methods used to assess the outcomes appropriate?	X					
1053. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?			X			
Comments: Concern for selection bias, measurement bias, and confounding, but insufficient reporting of Methods, subject characteristics, and Results to adequately determine risk of bias.						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1081	Pang	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High	X	N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	See comments below. This article isn't really designed to answer our questions of interest. I see why we may have included it (although it would be reasonable to exclude it for wrong comparison because it is mainly about comparing results of testing metastases with results of the primary tumor) because there is a small detail that we could mention, but for our questions, this has a high risk of bias given the design and selection.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1054. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1055. For RCTs, were randomization and allocation concealment adequate?						
1056. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
1057. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
1058. Were groups similar at baseline?						
1059. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
1060. Were outcomes assessed using valid and reliable measures, implemented consistently						

across all study participants?						
1061. Was overall attrition less than 30%?						
1062. Was differential attrition less than 15%?						
1063. Does the analysis control for baseline differences between groups?						
1064. Does the analysis account for differences in treatment received by the groups?						
1065. Are the statistical methods used to assess the outcomes appropriate?						
1066. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?		X					
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?						X	
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?				X			
If a threshold was used, was it pre-specified?				X			
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?			X				
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?		X					
Could the patient flow have introduced bias?						X	
Comments: High risk of bias for our questions, with just 11 cases chosen by an unclear method. It's hard to find any relevant, useful information in this article for the questions that we're asking. If we were interested in how similar the results from testing of metastases are to results of testing primary tumors, this would possibly be more useful. Note: Many of the risk of bias questions are not relevant for this particular article because it is not looking at a test of interest compared with a gold standard. It just contributes a very small piece of potentially relevant data about how many samples returned a valid result (8 of 11) and about tissue acceptance criteria (which was something that was predetermined in their methods, not something determined by the results of the study).							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1683	Phipps	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1067. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1068. For RCTs, were randomization and allocation concealment adequate?					X	
1069. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1070. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1071. Were groups similar at baseline?		X				Differences for age, sex, tumor site, stage, MSI status
1072. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1073. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					SEER and National Death Index

1074. Was overall attrition less than 30%?	X					
1075. Was differential attrition less than 15%?				X		Of the 27% without BRAF status, no information available to determine differential attrition
1076. Does the analysis control for baseline differences between groups?	X					
1077. Does the analysis account for differences in treatment received by the groups?		X				They did not have treatment information and did not adjust for differences in treatment
1078. Are the statistical methods used to assess the outcomes appropriate?	X					
1079. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Moderate potential risk of survivor bias; BRAF mutation status was not determined in 27% of enrolled cases, nor was it determined in cases who were eligible for the study but were not enrolled; analyses were based on the 1980/2708 with BRAF results determined; analyses did not account for differences in treatment						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3417	Poulet	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low	*	Medium	*	High	*	N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	unclear							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_X_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1080. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1081. For RCTs, were randomization and allocation concealment adequate?						
1082. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
1083. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
1084. Were groups similar at baseline?						
1085. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
1086. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
1087. Was overall attrition less than 30%?						
1088. Was differential attrition less than 15%?						
1089. Does the analysis control for baseline						

differences between groups?							
1090. Does the analysis account for differences in treatment received by the groups?							
1091. Are the statistical methods used to assess the outcomes appropriate?							
1092. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?	X						
Did the study avoid inappropriate exclusions?			X				
Could the selection of patients have introduced bias?							X
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?	X						
If a threshold was used, was it pre-specified?			X				
Could the conduct or interpretation of the index test have introduced bias?					X		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?				X			
Were the reference standard results interpreted without knowledge of the results of the index test?				X			
Could the reference standard, its conduct, or its interpretation have introduced bias?					NA	NA	NA
Domain 4: Flow and Timing							
Did all patients receive a reference standard?				X			
Did patients receive the same reference standard?				X			
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: No reference standard in this because it's just reporting how many times a valid result was returned for Mammaprint and for Oncotype Dx, and comparing that, as well as how many times they both got low, intermed, or high risk on the same samples.							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
161	Rayhanabad	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1093. What is the study design?						**X**
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1094. For RCTs, were randomization and allocation concealment adequate?					X	
1095. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1096. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?					X	
1097. Were groups similar at baseline?					X	
1098. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
1099. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1100. Was overall attrition less than 30%?					X	
1101. Was differential attrition less than 15%?					X	
1102. Does the analysis control for baseline					X	

differences between groups?						
1103. Does the analysis account for differences in treatment received by the groups?					X	
1104. Are the statistical methods used to assess the outcomes appropriate?	X					
1105. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments: Altho retrospective decision of change in management is based on comparing the NCCN guidelines so do not think there is a risk of bias						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (_X_)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1194	Roth	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1106. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1107. For RCTs, were randomization and allocation concealment adequate?					X	
1108. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1109. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1110. Were groups similar at baseline?				X		
1111. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1112. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1113. Was overall attrition less than 30%?	X					
1114. Was differential attrition less than 15%?	X					
1115. Does the analysis control for baseline				X		

differences between groups?						
1116. Does the analysis account for differences in treatment received by the groups?	X					
1117. Are the statistical methods used to assess the outcomes appropriate?	X					
1118. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Unable to determine baseline comparison for the comparison we're interested in (BRAF mutation vs. wild-type and KRAS mutation vs. wildtype); Study reports baseline data for 3 groups (all subjects, stage II, and stage III). Can't determine if analysis controls for baseline differences between groups, but it does control for many variables, including most known prognostic factors.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3649	Roth	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1119. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1120. For RCTs, were randomization and allocation concealment adequate?					X	
1121. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1122. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1123. Were groups similar at baseline?				X		
1124. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1125. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
1126. Was overall attrition less than 30%?				X		
1127. Was differential attrition less than 15%?				X		
1128. Does the analysis control for baseline				X		

differences between groups?						
1129. Does the analysis account for differences in treatment received by the groups?	X					
1130. Are the statistical methods used to assess the outcomes appropriate?	X					
1131. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2992	Rouquette	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1132. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1133. For RCTs, were randomization and allocation concealment adequate?					x	
1134. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
1135. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
1136. Were groups similar at baseline?				x		
1137. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					x	death is outcome blinding unlikely to matter
1138. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
1139. Was overall attrition less than 30%?				x		
1140. Was differential attrition less than 15%?				x		

1141. Does the analysis control for baseline differences between groups?				x		
1142. Does the analysis account for differences in treatment received by the groups?	x					
1143. Are the statistical methods used to assess the outcomes appropriate?	x					
1144. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments: survival analysis was not the primary focus of the study and risk ratios were not presented, so very difficult to determine if sample size or other factors were drivers of non-significant findings						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3986	Saghatchian	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High				N/A	X
Risk of bias for KQ 2:	Low		Medium		High				N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High				N/A	
Risk of bias for KQ 4a:	Low		Medium		High				N/A	X
Risk of bias for KQ 4b:	Low		Medium		High				N/A	X
Risk of bias for KQ 5:	Low		Medium		High				N/A	X
Explain any **High** ratings:										

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1145. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1146. For RCTs, were randomization and allocation concealment adequate?					X	
1147. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1148. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1149. Were groups similar at baseline?		X				Many differences (Table 1)
1150. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1151. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Outcomes were related to death, metastases, and recurrence

						measures. No description of ascertainment methods
1152. Was overall attrition less than 30%?				X		
1153. Was differential attrition less than 15%?				X		
1154. Does the analysis control for baseline differences between groups?	X					
1155. Does the analysis account for differences in treatment received by the groups?		X				
1156. Are the statistical methods used to assess the outcomes appropriate?	X					
1157. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3652	Samadder	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1158. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1159. For RCTs, were randomization and allocation concealment adequate?					X	
1160. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1161. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1162. Were groups similar at baseline?				X		
1163. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1164. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1165. Was overall attrition less than 30%?	X					
1166. Was differential attrition less than 15%?				X		
1167. Does the analysis control for baseline				X		

differences between groups?						
1168. Does the analysis account for differences in treatment received by the groups?	X					Adjusted for chemo exposure and radiation exposure
1169. Are the statistical methods used to assess the outcomes appropriate?	X					
1170. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1226	Samowitz	2005

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	High risk of selection bias and confounding. See comments below.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1171. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1172. For RCTs, were randomization and allocation concealment adequate?					X	
1173. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1174. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1175. Were groups similar at baseline?		X				
1176. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1177. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1178. Was overall attrition less than 30%?				X		
1179. Was differential attrition less than 15%?				X		
1180. Does the analysis control for baseline			X			

differences between groups?						
1181. Does the analysis account for differences in treatment received by the groups?		X				
1182. Are the statistical methods used to assess the outcomes appropriate?	X					
1183. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?		X				
Comments: Analysis adjusted for age, stage, tumor site, and CIMP; did not consider other prognostic factors, such as grade, cellular subtypes, LV invasion, location (rectum vs. colon), sex, margin status—and there were significant baseline differences between the groups for some of these factors (tumor site, differentiation, mucinous histology). High risk of selection bias and confounding given the baseline differences and many factors not included in the multivariate analysis.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3514	Schneider	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1184. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1185. For RCTs, were randomization and allocation concealment adequate?					X	
1186. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1187. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1188. Were groups similar at baseline?		X				Differences for grade and AOL
1189. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1190. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
1191. Was overall attrition less than 30%?	X					

1192. Was differential attrition less than 15%?	X					
1193. Does the analysis control for baseline differences between groups?		X				
1194. Does the analysis account for differences in treatment received by the groups?	X					
1195. Are the statistical methods used to assess the outcomes appropriate?	X					
1196. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3012	Scoccianti	

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1197. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1198. For RCTs, were randomization and allocation concealment adequate?					x	
1199. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
1200. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
1201. Were groups similar at baseline?		x				non-randomized study
1202. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					x	outcome is survival
1203. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
1204. Was overall attrition less than 30%?	x					

1205. Was differential attrition less than 15%?					x	
1206. Does the analysis control for baseline differences between groups?			x			
1207. Does the analysis account for differences in treatment received by the groups?		x				
1208. Are the statistical methods used to assess the outcomes appropriate?	x					
1209. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments: most standard prognostic markers were used in multivariable analysis (T and N size) however only covariates with significant univariate associations were included in the multivariable model, iffy way of reducing a model						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1264	Shaukat	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1210. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1211. For RCTs, were randomization and allocation concealment adequate?					X	
1212. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1213. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1214. Were groups similar at baseline?				X		analysis was cohort nested in a case-control, full demographics not presented by cohort groups
1215. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	

1216. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1217. Was overall attrition less than 30%?				X		
1218. Was differential attrition less than 15%?				X		
1219. Does the analysis control for baseline differences between groups?			X			adjusted analysis is presented but since baseline comparison between groups not presented, unable to assess if fully adjusted
1220. Does the analysis account for differences in treatment received by the groups?		X				
1221. Are the statistical methods used to assess the outcomes appropriate?	X					
1222. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?			X			
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1272	Shia	2008

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	High risk of selection bias, attrition bias, measurement bias, and confounding. See comments below for additional details.							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1223. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1224. For RCTs, were randomization and allocation concealment adequate?					X	
1225. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1226. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1227. Were groups similar at baseline?				X		For our comparison of interest (by MSI status), no information reported
1228. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1229. Were outcomes assessed using valid and				X		Used a surgical

reliable measures, implemented consistently across all study participants?						database to gather clinical information and outcomes; no further information reported on ascertainment, definitions of outcomes, masking of outcome assessors
1230. Was overall attrition less than 30%?		X				Of 236 patients included, complete clinical information available for only 130 (55%) and MSI detection completed in just 77 (32.6%)
1231. Was differential attrition less than 15%?				X		
1232. Does the analysis control for baseline differences between groups?				X		
1233. Does the analysis account for differences in treatment received by the groups?		X				
1234. Are the statistical methods used to assess the outcomes appropriate?	X					
1235. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?				X		
Comments: High risk of selection bias, attrition bias, measurement bias, and confounding. Just 32.6% of the sample had MSI results and just 55% of the sample had complete clinical information. Because the article is focused on FR alpha, it reports little information about the MSI results and analyses. The multivariate model is not reported, nor are the outcomes data for MSI from the multivariate model. It just tells us that only TNM staging remained significant predictor for DSS in the multivariate analysis (Cox regression with forward selection, which was limited to 3 covariated due to the limited number of events).						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							

Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3348	Solin	2012

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1236. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1237. For RCTs, were randomization and allocation concealment adequate?					X	
1238. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1239. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1240. Were groups similar at baseline?					X	
1241. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	X					
1242. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1243. Was overall attrition less than 30%?	X					
1244. Was differential attrition less than 15%?	X					
1245. Does the analysis control for baseline					X	

differences between groups?						
1246. Does the analysis account for differences in treatment received by the groups?					X	
1247. Are the statistical methods used to assess the outcomes appropriate?			X – Since there were differences by ER positive and negative. Should have stratified or used an interaction term			
1248. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2	X						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1311	Soreide	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1249. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1250. For RCTs, were randomization and allocation concealment adequate?					X	
1251. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1252. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1253. Were groups similar at baseline?		X				Differences for site, size, LN status, grade, ploidy, stage
1254. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1255. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Unclear how outcomes were assessed. No

						reporting of followup plans/schedule; only reports that recurrence was recorded during follow up and was defined as any locoregional recurrence, distant metastases, or both; no description of how recurrence was determined
1256. Was overall attrition less than 30%?	X					
1257. Was differential attrition less than 15%?	X					
1258. Does the analysis control for baseline differences between groups?	X					
1259. Does the analysis account for differences in treatment received by the groups?		X				But the methods suggests that there was little variation in treatment
1260. Are the statistical methods used to assess the outcomes appropriate?	X					
1261. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Unclear if this was prospective or retrospective; it sounds like some information was collected retrospectively; difficult to assess risk of ascertainment bias as very little information reported; no attempt to consider differences in treatment						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							

Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2558	Sriram	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low	x	Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (_x_)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1262. What is the study design?						
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1263. For RCTs, were randomization and allocation concealment adequate?						
1264. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?						
1265. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?						
1266. Were groups similar at baseline?						
1267. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?						
1268. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?						
1269. Was overall attrition less than 30%?						
1270. Was differential attrition less than 15%?						
1271. Does the analysis control for baseline						

differences between groups?							
1272. Does the analysis account for differences in treatment received by the groups?							
1273. Are the statistical methods used to assess the outcomes appropriate?							
1274. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?							
Comments:							

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			x				
Was a case-control design avoided?	x						
Did the study avoid inappropriate exclusions?	x						
Could the selection of patients have introduced bias?					x		
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?			x				
If a threshold was used, was it pre-specified?	x						
Could the conduct or interpretation of the index test have introduced bias?					x		
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	x						
Were the reference standard results interpreted without knowledge of the results of the index test?			x				
Could the reference standard, its conduct, or its interpretation have introduced bias?					x		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	x						
Did patients receive the same reference standard?	x						
Were all patients included in the analysis?							
Could the patient flow have introduced bias?					x		
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3432	Srivastava	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium		High	Unclear	N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

1275. What is the study design?	**RCT**	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
		Uncontrolled trial				
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1276. For RCTs, were randomization and allocation concealment adequate?					X	
1277. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		
1278. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1279. Were groups similar at baseline?					X	
1280. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1281. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
1282. Was overall attrition less than 30%?				X		

1283. Was differential attrition less than 15%?				X		
1284. Does the analysis control for baseline differences between groups?					X	
1285. Does the analysis account for differences in treatment received by the groups?					X	
1286. Are the statistical methods used to assess the outcomes appropriate?				X		
1287. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: Unclear risk of bias; very limited reporting of methods in this abstract from a conference; all physicians were from 1 site; unclear methods of ascertainment; unclear selection bias and measurement bias						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
124	Tang	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

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What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1288. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1289. For RCTs, were randomization and allocation concealment adequate?					X	
1290. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1291. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1292. Were groups similar at baseline?	X					
1293. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
1294. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1295. Was overall attrition less than 30%?				X		
1296. Was differential attrition less than 15%?				X		
1297. Does the analysis control for baseline	X					

differences between groups?						
1298. Does the analysis account for differences in treatment received by the groups?		X				patients all treated similarly
1299. Are the statistical methods used to assess the outcomes appropriate?	X					
1300. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (X__)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1373	Tie	2010

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

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What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1301. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1302. For RCTs, were randomization and allocation concealment adequate?					X	
1303. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		Unclear how they selected the 600 cases from the 1674 in the database
1304. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1305. Were groups similar at baseline?		X				
1306. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1307. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Details of methods of ascertaining outcomes NR; we know they

						used a database (BioGrid Austrialia), but no information about validity of that database is provided, or about how they determined recurrence, etc. to input into the database
1308. Was overall attrition less than 30%?	X					
1309. Was differential attrition less than 15%?	X					
1310. Does the analysis control for baseline differences between groups?			X			Did for age, site, stage, but did not adjust for gender
1311. Does the analysis account for differences in treatment received by the groups?	X					
1312. Are the statistical methods used to assess the outcomes appropriate?	X					
1313. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Adjusted for age, stage, site, adjuvant treatment; did not adjust for grade, cellular subtypes, sex, lymphovascular invasion
Comments: Follow up was perhaps too short to collect good outcomes data on recurrence and mortality (median 38.5 months for stage I to III patients)						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic							

markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3057	Tsao	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1314. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1315. For RCTs, were randomization and allocation concealment adequate?					x	
1316. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
1317. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
1318. Were groups similar at baseline?		x				Not expected in non-randomized trial
1319. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		x				Outcome is survival
1320. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1321. Was overall attrition less than 30%?				X		

1322. Was differential attrition less than 15%?				X		
1323. Does the analysis control for baseline differences between groups?	X					
1324. Does the analysis account for differences in treatment received by the groups?			x			Controls for type of resection, on a clinical trial so distribution of chemo should be similar between groups
1325. Are the statistical methods used to assess the outcomes appropriate?	x					
1326. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3332	Van de Vijver	2002

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1327. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1328. For RCTs, were randomization and allocation concealment adequate?					X	
1329. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1330. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1331. Were groups similar at baseline?		X				
1332. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
1333. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1334. Was overall attrition less than 30%?				X		
1335. Was differential attrition less than 15%?				X		
1336. Does the analysis control for baseline	X					

differences between groups?						
1337. Does the analysis account for differences in treatment received by the groups?	X					
1338. Are the statistical methods used to assess the outcomes appropriate?	X					
1339. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3333	Van't Veer	2002

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium	X	High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1340. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1341. For RCTs, were randomization and allocation concealment adequate?					X	
1342. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1343. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1344. Were groups similar at baseline?				X		
1345. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?					X	
1346. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
1347. Was overall attrition less than 30%?				X		
1348. Was differential attrition less than 15%?				X		
1349. Does the analysis control for baseline				X		

differences between groups?						
1350. Does the analysis account for differences in treatment received by the groups?				X		
1351. Are the statistical methods used to assess the outcomes appropriate?	X					
1352. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: only one paragraph in short article describes multivariate analysis, many details not shown						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (___)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?			X				
Was a case-control design avoided?		X					
Did the study avoid inappropriate exclusions?		X					
Could the selection of patients have introduced bias?						X	
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?				X			
If a threshold was used, was it pre-specified?		X					
Could the conduct or interpretation of the index test have introduced bias?							X
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?	X						
Were the reference standard results interpreted without knowledge of the results of the index test?	X						
Could the reference standard, its conduct, or its interpretation have introduced bias?					X		
Domain 4: Flow and Timing							
Did all patients receive a reference standard?	X						
Did patients receive the same reference standard?	X						
Were all patients included in the analysis?	X						
Could the patient flow have introduced bias?					X		
Comments: Reference standard was ascertainment of actual outcome from medical records, test was not compared to another prognostic test. Patients were selected based on whether they had metastases or not case-control) and article does not state whether they were randomly, consecutively or otherwise selected. All patients were <55 years old and reason for this exclusion is not stated.							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
3354	Venook	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1353. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1354. For RCTs, were randomization and allocation concealment adequate?					X	
1355. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1356. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1357. Were groups similar at baseline?				X		
1358. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1359. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1360. Was overall attrition less than 30%?	X					
1361. Was differential attrition less than 15%?	X					
1362. Does the analysis control for baseline				X		

differences between groups?						
1363. Does the analysis account for differences in treatment received by the groups?				X		
1364. Are the statistical methods used to assess the outcomes appropriate?	X					
1365. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
43	Whitson	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low	x	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1366. What is the study design?			**x**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1367. For RCTs, were randomization and allocation concealment adequate?					x	
1368. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
1369. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
1370. Were groups similar at baseline?		x				non-randomized
1371. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				x		outcome was survival
1372. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	x					
1373. Was overall attrition less than 30%?				x		
1374. Was differential attrition less than 15%?				x		

1375. Does the analysis control for baseline differences between groups?	x					
1376. Does the analysis account for differences in treatment received by the groups?					x	all treated similarly
1377. Are the statistical methods used to assess the outcomes appropriate?	x					
1378. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	x					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
2684	Woo	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (__)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1379. What is the study design?			**X**			
If "Other" study design, enter a description in this box: Uncontrolled trial	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1380. For RCTs, were randomization and allocation concealment adequate?					X	
1381. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1382. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1383. Were groups similar at baseline?		X				
1384. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1385. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1386. Was overall attrition less than 30%?				X		
1387. Was differential attrition less than 15%?				X		

1388. Does the analysis control for baseline differences between groups?		X				
1389. Does the analysis account for differences in treatment received by the groups?					X	
1390. Are the statistical methods used to assess the outcomes appropriate?	X					
1391. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments:						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
4004	Yamauchi	2013

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High		N/A	X
Risk of bias for KQ 4a:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1392. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1393. For RCTs, were randomization and allocation concealment adequate?					X	
1394. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					Consecutive sample was offered entry, To participate in the study, patients were required to incur the costs of the assay as an out-of-pocket expense
1395. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1396. Were groups similar at baseline?				X		Not reported by test result
1397. Were the outcome assessors blinded to				X		But, not as important as

the test result/intervention/exposure status of participants?						some studies since the outcome is the questionnaire result
1398. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		Modified a published questionnaire; no information provided on the validity and reliability
1399. Was overall attrition less than 30%?	X					
1400. Was differential attrition less than 15%?	X					
1401. Does the analysis control for baseline differences between groups?		X				
1402. Does the analysis account for differences in treatment received by the groups?					X	
1403. Are the statistical methods used to assess the outcomes appropriate?			X			Some concern for stopping enrollment early, and their justification for doing so. See comments below
1404. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?					X	
Comments: The study was designed to enroll 200 patients, with the original intent to estimate a decision change rate of 20% with a precision of `5% to 6%. However, it was decided to halt enrollment after 124 patients were enrolled because the accumulating data indicated that there were statistically significant reductions in treatment recommendations for chemotherapy in N0 and Nþ patient subgroups.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have							

introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1516	Yoon	2011

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low	X	Medium		High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1405. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1406. For RCTs, were randomization and allocation concealment adequate?					X	
1407. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1408. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1409. Were groups similar at baseline?		X				Differences for age, site, size, LN metastases, histology and synchronous adenomas
1410. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?				X		
1411. Were outcomes assessed using valid and	X					

reliable measures, implemented consistently across all study participants?						
1412. Was overall attrition less than 30%?	X					
1413. Was differential attrition less than 15%?	X					
1414. Does the analysis control for baseline differences between groups?	X					
1415. Does the analysis account for differences in treatment received by the groups?			X			Accounts for differences in adjuvant chemo, but not differences in radiation (among the 770 with rectal cancer, 41% got postop radiation); however, not too concerning because 97% of the rectal cancer cases were MSS and analyses found MSI-H to have better outcomes
1416. Are the statistical methods used to assess the outcomes appropriate?	X					
1417. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					Table 3
Comments: age, sex, location, CEA, growth type, LN metastasis, distant metastasis, lymphovascular invasion, histology, and adjuvant chemotherapy included in the multivariate analysis in Table 3 for DFS and OS.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							

Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
162	Yorozuya	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	
Risk of bias for KQ 2:	Low		Medium		High		N/A	
Risk of bias for KQ 3:	Low		Medium	x	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	
Risk of bias for KQ 4b:	Low		Medium		High		N/A	
Risk of bias for KQ 5:	Low		Medium		High		N/A	
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)
KQ 1: Overarching Question Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?	
KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?	
KQ 4a. Clinical Utility. What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?	
KQ 4b. Clinical Utility. What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?	
KQ 5. Harms. What are the harms associated with treatment decisions that are informed by the genetic tests?	

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1418. What is the study design?					**x**	
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1419. For RCTs, were randomization and allocation concealment adequate?					x	
1420. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	x					
1421. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	x					
1422. Were groups similar at baseline?			x			matched on age but some differences bet cases and controls on clinical covariates
1423. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		x				
1424. Were outcomes assessed using valid and				x		assuming outcome of

reliable measures, implemented consistently across all study participants?						metastatic disease was determined by chart review, but not stated
1425. Was overall attrition less than 30%?					x	
1426. Was differential attrition less than 15%?					x	
1427. Does the analysis control for baseline differences between groups?	x					
1428. Does the analysis account for differences in treatment received by the groups?		x				does not control although cases and controls were similar in this respect
1429. Are the statistical methods used to assess the outcomes appropriate?	x					
1430. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?						
Comments:						

Key Question 2					If study does NOT apply to this KQ, place an 'X' here (_X_)		
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
67	Zellweger	2006

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium		High	X	N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:	See comments below in comments box							

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1431. What is the study design?			**X**			
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1432. For RCTs, were randomization and allocation concealment adequate?					X	
1433. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?				X		
1434. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1435. Were groups similar at baseline?				X		
1436. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?		X				
1437. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?				X		
1438. Was overall attrition less than 30%?				X		
1439. Was differential attrition less than 15%?				X		
1440. Does the analysis control for baseline				X		

differences between groups?						
1441. Does the analysis account for differences in treatment received by the groups?		X				
1442. Are the statistical methods used to assess the outcomes appropriate?			X			
1443. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?				X		

Comments: High risk of selection bias as the article does not report inclusion/exclusion criteria, method of selecting the 138 subjects (e.g. was it consecutive? Random? Other?), or comparison of groups at baseline. There is no information provided that allows us to determine how similar/different the FISH + vs. FISH – groups were at baseline. Also, no information about attrition provided: of the 138, median follow up was 19 months (range 4 - 52), but no data reported about how many were lost early in the study and whether it was differential for the FISH + vs. FISH – groups. High risk of measurement bias as they don't give any information about how recurrence was determined and they don't describe any masking of outcome assessors. Although it's hard to assess their multivariate analysis because they don't report what they adjusted for in the Cox model, I would probably consider the analysis to have a high risk of confounding because they don't describe adjustment for known prognostic factors (e.g., stage, presence of CIS). In addition, since we don't have information on how demographics or potential confounders were distributed at baseline, we can't even assess what else they should have adjusted for.

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (_X_)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Worksheet for Risk of Bias of Individual Studies – GTRRC (v. 4-23-13)

Ref ID #	Author	Year
1533	Zlobec	2009

Indicate the appropriate risk of bias rating by placing an 'X' in the shaded space to the right of the rating for each KQ that is applicable. If a study does not apply to a KQ, put an 'X' next to N/A.

Risk of bias for KQ 1:	Low		Medium		High		N/A	X
Risk of bias for KQ 2:	Low		Medium		High		N/A	X
Risk of bias for KQ 3:	Low		Medium	X	High		N/A	
Risk of bias for KQ 4a:	Low		Medium		High		N/A	X
Risk of bias for KQ 4b:	Low		Medium		High		N/A	X
Risk of bias for KQ 5:	Low		Medium		High		N/A	X
Explain any **High** ratings:								

Low: Study has minimal bias. High degree of confidence that the true effect size is similar in size and direction to that reported.
Medium: Study has at least one significant source of bias, but does not have flaws that are likely to cause major bias. Moderate degree of confidence that the actual effect is in the direction reported by the study; moderate degree of confidence that the true effect size is similar to that reported.
High: Study has at least one major sources of bias or multiple significant sources of bias. Low degree of confidence that the true effect size is similar in size or direction to that reported.
NA: Not applicable to that key question.

Key Questions 1, 3, 4a, 4b, and 5	If the study does NOT apply to any of these KQs, place an 'X' here (___)

KQ 1: Overarching Question
Is there direct evidence that the addition of the following genetic tests used alone or in combination with traditional prognostic factors change physician decision making and improve outcomes for adult patients with lung, breast, colorectal, and bladder cancer compared with the use of traditional factors to predict risk of recurrence for adults with these cancers?

KQ 3. Clinical Validity. Does existing evidence establish the prognostic accuracy of the tests for predicting recurrence?

KQ 4a. Clinical Utility.
What is the evidence that the prognostic information provided by the genetic tests modifies physician decisions regarding use of adjuvant anti-neoplastic chemo- and/or radiotherapy; enhanced diagnostic testing for recurrence; and/or preventive surgery among adult patients with malignant tumors?

KQ 4b. Clinical Utility.
What is the evidence that modified decisions lead to improved overall survival, disease free survival, time to recurrence, quality of life, or incidence of serious side effects of adjuvant therapy in adult patients with malignant tumors?

KQ 5. Harms.
What are the harms associated with treatment decisions that are informed by the genetic tests?

	RCT	**non-RCT**	**Prospective cohort study**	**Retrospective cohort study**	**Case-control study**	**Case Series**
1444. What is the study design?				**X**		
If "Other" study design, enter a description in this box:	**Yes**	**No**	**Partially**	**Can't Determine**	**NA**	**Explanation**
1445. For RCTs, were randomization and allocation concealment adequate?					X	
1446. Did the study apply inclusion/exclusion criteria uniformly to all comparison groups of the study?	X					
1447. Is the selection of the comparison group appropriate, after taking into account feasibility and ethical considerations?	X					
1448. Were groups similar at baseline?		X				
1449. Were the outcome assessors blinded to the test result/intervention/exposure status of participants?	X					
1450. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	X					
1451. Was overall attrition less than 30%?	X					
1452. Was differential attrition less than 15%?	X					
1453. Does the analysis control for baseline	X					

differences between groups?						
1454. Does the analysis account for differences in treatment received by the groups?		X				Tissues were from pre-operatively untreated patients. No information about treatment
1455. Are the statistical methods used to assess the outcomes appropriate?	X					
1456. **For KQ3 ONLY** – Did analyses adjust for all or most of the standard prognostic markers?	X					
Comments: Treatment & Distant metastasis information not available. Both factors affect CSS.						

Key Question 2	If study does NOT apply to this KQ, place an 'X' here (___)						
KQ 2. Analytic Validity. Does existing evidence establish the technical accuracy and reliability of the tests for detecting the relevant genetic markers?							
	Yes	**No**	**Unclear**	**NA**	**Low**	**High**	**Unclear**
Domain 1: Patient Selection							
Was a consecutive or random sample of patients enrolled?							
Was a case-control design avoided?							
Did the study avoid inappropriate exclusions?							
Could the selection of patients have introduced bias?							
Domain 2: Index Test(s)							
Were the index test results interpreted without knowledge of the results of the reference standard?							
If a threshold was used, was it pre-specified?							
Could the conduct or interpretation of the index test have introduced bias?							
Domain 3: Reference Standard							
Is the reference standard likely to correctly classify the genetic markers?							
Were the reference standard results interpreted without knowledge of the results of the index test?							
Could the reference standard, its conduct, or its interpretation have introduced bias?							
Domain 4: Flow and Timing							
Did all patients receive a reference standard?							
Did patients receive the same reference standard?							
Were all patients included in the analysis?							
Could the patient flow have introduced bias?							
Comments:							

Appendix D. Strength of Evidence (SOE)

Table D-1. EGFR for lung carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort: 6 (1,870)	Medium	Indirect	Consistent	Imprecise	None	0.87(0.65,1.15) No Association[a]	Low
Cancer-specific mortality	None	NA	NA	NA	NA	NA	NA	NA
Overall survival	Cohort: 6 (1,820)	Medium	Indirect	Consistent	Imprecise	NA	0.77(0.5, 1.19) No Association[b]	Insufficient
Decisions about treatment								
Analytic Validity	5;898	Low	Direct	Consistent	Precise			High
Sensitivity and specificity	5;898	Low	Direct	Consistent	Precise			High
Positive and negative predictive value	5;898	Low	Direct	Consistent	Precise			High
Cross-lab validity	5;636	Low	Direct	Consistent	Precise	CAP Report Data		High

[a] Two studies did not report a hazard ratio for risk of recurrence, therefore 4 studies (N=936) contributed data to the meta-analysis.

[b] Two studies did not report a hazard ratio for overall survival, therefore 4 studies (N=834) contributed data to the meta-analysis.

Abbreviations: CI = confidence interval; N = number.

Table D-2. KRAS for lung carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort: 4 (N=611)	Medium	Indirect	Inconsistent	Imprecise	None	2.84(1.14, 7.1)[a] KRAS mutation associated with greater RR	Insufficient
Cancer-specific mortality	None	NA	NA	NA	NA	NA	NA	NA
Overall survival	Cohort: 2 (N=253)	Medium	Indirect	Unknown (single study)	Unknown (single study)	None	OR, 2.69(1.91,3.80)) ; 3.33 (1.03, 10.82) mutation associated with lower OS	Insufficient
Decisions about treatment	0/0	NA	NA	NA	NA	NA	NA	NA
Analytic Validity	2;188	Low	Direct	Consistent	Precise			Moderate
Sensitivity and specificity	1;114	Low	Direct	Consistency Unknown (Single Study)	Precise			Low
Positive and negative predictive value	1;114	Low	Direct	Consistency Unknown (Single Study)	Precise			Low
Cross-lab validity	4;686	Low	Direct	Consistent	Precise	CAP Report Data		High

[a] Three studies representing 4 cohorts (N=611) contributed to the meta-analysis.

Abbreviations: CI = confidence interval; N = number.

Table D-3. Microsatellite instability for colorectal carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort studies: 10 (7,130)	Medium	Indirect	Inconsistent	Imprecise	NA	MSI-H vs. MSS: HR, 0.60 (0.50 to 0.72) for the association between MSI status and recurrence	Insufficient
Cancer-specific mortality	Cohort studies: 6 (3,439)	Medium	Indirect	Inconsistent	Imprecise	NA	MSI-H vs. MSS: HR, 0.65 (0.51 to 0.82)[a] for the association between MSI status and cancer-specific mortality	Insufficient
Overall survival	Cohort studies: 12 (8,839)	Medium	Indirect	Inconsistent	Imprecise	NA	MSI-H vs. MSS: HR, 0.57 (0.43 to 0.77)[b] for the association between MSI status and OS;	Insufficient
Decisions about treatment	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Analytic Validity	NA	NA	NA	NA	NA		NA	NA
Sensitivity and specificity	NA	NA	NA	NA	NA		NA	NA
Positive and negative predictive value	NA	NA	NA	NA	NA		NA	NA
Cross-lab validity	3;291	NA	Indirect	Consistent	Precise	CAP Report Data	NA	High

[a] Five studies (N = 3252) contributed data to the meta-analysis.

[b] Eleven studies (N = 8626) contributed to the meta-analysis)

Abbreviations: CI = confidence interval; N = number; NA = not applicable.

Table D-4. KRAS for colorectal carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort studies: 5 (4,085)	Medium	Indirect	Consistent	Precise	NA	Wild type vs. mutated: HR, 1.02 (0.91 to 1.14); no association found in adjusted analyses;	Low
Cancer-specific mortality	2 (1,747)	Medium	Indirect	Inconsistent	Imprecise	NA	Wild type vs. mutated: HR, 1.30 (1.02 to 1.66) KRAS mutation for the association between KRAS mutation and CSS	Insufficient
Overall survival	Cohort studies: 10 (5,328)	Medium	Indirect	Inconsistent	Imprecise	NA	Wild type vs. mutated: HR, 1.22 (0.95 to 1.55);	Low
Decisions about treatment	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Analytic Validity	5;576	Low	Direct	Consistent	Precise		NA	High
Sensitivity and specificity	5;576	Low	Direct	Consistent	Precise		NA	High
Positive and negative predictive value	5;576	Low	Direct	Consistent	Precise		NA	High
Cross-lab validity	5;747	Low	Direct	Consistent	Precise	CAP Report Data	NA	High

Abbreviations: CI = confidence interval; N = number; NA = not applicable.

Table D-5. BRAF for colorectal carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort studies: 5 (4,106)	Medium	Indirect	Inconsistent	Imprecise	NA	Wild type vs. mutation: HR, 1.07 (0.76 to 1.52);	Low
Cancer-specific mortality	Cohort studies: 7 (5,409)	Medium	Indirect	Consistent (all trend for association of mutation with worse outcome)	CND	NA	Wild type vs. mutation: HR, 1.5 (1.27 to1.77) for the association between mutation and cancer-specific mortality	Insufficient
Overall survival	Cohort studies: 11 (7,610)	Medium	Indirect	Consistent	CND	NA	Wild type vs. mutation: HR 1.45 (1.29 to 1.62),) for the association between mutation and survival	Insufficient
Decisions about treatment	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Analytic Validity	1;117	Medium	Direct	Consistency Unknown (Single Study)	Imprecise			Low
Sensitivity and specificity	1;117	Medium	Direct	Consistency Unknown (Single Study)	Imprecise			Low
Positive and negative predictive value	1;117	Medium	Direct	Consistency Unknown (Single Study)	Imprecise			Low
Cross-lab validity	3;533	NA	Direct	Consistent	Precise	CAP Report Data	NA	High

Abbreviations: CI = confidence interval; N = number; NA = not applicable.

Table D-6. Oncotype Dx colon multi-gene assay for colorectal carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort: 1 (690)	Medium	Indirect	Unknown	Imprecise	NA	HR, 1.68 (1.18 to 2.38)	Insufficient
Cancer-specific mortality	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Overall survival	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Decisions about treatment	0 (0)[a]	NA	NA	NA	NA	NA	NA	Insufficient
Analytic Validity	1;NR	Medium	Indirect	Consistency Unknown (Single Study)	Precise		NA	Low
Sensitivity and specificity	NA	NA	NA	NA	NA		NA	NA
Positive and negative predictive value	NA	NA	NA	NA	NA		NA	NA
Cross-lab validity	NA	NA	NA	NA	NA		NA	NA

[a] We identified no low or medium risk of bias studies. But we found three studies rated as high[1] or unclear[2] risk of bias that evaluated decisions of a total of 221 physicians (502 patients)

Abbreviations: CI = confidence interval; N = number; NA = not applicable.

Table D-7. Oncotype Dx breast multi-gene assay for breast carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort: 6 (N=3,222) Case-control: 1 (N=40)	Medium	Indirect	Consistent	Precise	None	2.97(2.19,4.02)[a] High Risk vs. Low Risk	Insufficient
Cancer-specific mortality	Cohort: 1 (N=668) Case-control: 1 (N=566)	Low	Indirect	Consistent	Imprecise	None	2.02(1.35, 3.00) High Risk vs. Low Risk	Low
Overall survival	Cohort: 1 (N=668)	Low	Indirect	Unknown	Unknown	None	HR, 1.77 (1.35 , 2.33) High Risk vs. Low Risk	Insufficient
Decisions about treatment	Cohort: 6 (N=990) Uncontrolled trial: 10 (N=1,261)	Low	Indirect	Consistent	Precise	None	~30% of tx decisions changed by test	Moderate
Analytic Validity	NA	NA	NA	NA	NA		NA	NA
Sensitivity and specificity	NA	NA	NA	NA	NA		NA	NA
Positive and negative predictive value	NA	NA	NA	NA	NA		NA	NA
Cross-lab validity	NA	NA	NA	NA	NA		NA	NA

[a] Four studies (N = 2327) contributed data to the meta-analysis.

Abbreviations: CI = confidence interval; N = number; NA = not applicable; tx = treatment.

Table D-8. Mammaprint multi-gene assay for breast carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort: 6 (N=1,913)	Medium	Indirect	Consistent	Imprecise	None	2.84(2.11,3.82) For poor prognosis vs. good prognosis	Insufficient
Cancer-specific mortality	Cohort: 5 (N=1615)	Medium	Indirect	Consistent	Imprecise	None	3.3 (2.22, 4.9) for poor prognosis vs. good prognosis	Insufficient
Overall survival	Cohort: 1 (N = 144)	Medium	Indirect	Unknown (single study)	Unknown (single study)	None	HR, 1.67 (0.73, 3.82) for poor prognosis vs. poor prognosis	Insufficient
Decisions about treatment	Cohort: 1 (N=427)	Low	Indirect	Unknown (single study)	Unknown (single study)	None	NA	Insufficient
Analytic Validity	2;399	Medium	Indirect	Consistent	Imprecise		NA	Moderate
Sensitivity and specificity	2;399	Medium	Indirect	Consistent	Imprecise		NA	Moderate
Positive and negative predictive value	2;399	Medium	Indirect	Consistent	Imprecise		NA	Moderate
Cross-lab validity	1; 100	Low	Indirect	Consistent	Imprecise		NA	Low

Abbreviations: BRCA = breast cancer; CI = confidence interval; N = number; NA = not applicable.

Table D-9. Urovysion multi-gene assay for urinary bladder carcinoma

Outcome	Study Design: No. Studies (N)	Risk of Bias	Directness	Consistency	Precision	Other	Result/ Effect Size (CI)	Strength of Evidence
Risk of Recurrence	Cohort study: 2 (168)	Low	Indirect	Consistent	Imprecise	Actual adjusted data NR in the larger (N=126) study, just qualitative description	Association between mutation and risk of recurrence in 2 small studies;[a] no evidence that test use leads to improved outcomes	Insufficient
Cancer-specific mortality	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Overall survival	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Decisions about treatment	0 (0)	NA	NA	NA	NA	NA	NA	Insufficient
Analytic Validity	NA	NA	NA	NA	NA		NA	NA
Sensitivity and specificity	NA	NA	NA	NA	NA		NA	NA
Positive and negative predictive value	NA	NA	NA	NA	NA		NA	NA
Cross-lab validity	4;555	NA	Indirect	Consistent	Precise	CAP Report Data	NA	High

[a] One study (N=126) only reported unadjusted data, and reported that the association between FISH results and tumor recurrence "persisted after accounting for other disease factors (stage, grade, history of prior tumors, concomitant CIS, use of post-operative mitomycin, etc.) at all time points."

Abbreviations: CI = confidence interval; N = number; NA = not applicable.

References

1. Cartwright T, Chao C, Lee M, et al. Effect of the 12-gene colon cancer assay results on adjuvant treatment recommendations in patients with stage II colon cancer. Curr Med Res Opin. 2013 Nov 7PMID: 24127781.

2. Srivastava G, Renfro LA, Behrens RJ, et al. Prospective evaluation of a 12-gene assay on treatment recommendations in patients with stage II colon cancer. ASCO Gastrointestinal Symposium; 2013 January; San Francisco, CA.

NICE National Institute for Health and Care Excellence

Gene expression profiling and expanded immunohistochemistry tests for guiding adjuvant chemotherapy decisions in early breast cancer management: MammaPrint, Oncotype DX, IHC4 and Mammostrat

Issued: September 2013

NICE diagnostics guidance 10

www.nice.org.uk/dg10

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1 Recommendations

1.1 Oncotype DX is recommended as an option for guiding adjuvant chemotherapy decisions for people with oestrogen receptor positive (ER+), lymph node negative (LN−) and human epidermal growth factor receptor 2 negative (HER2−) early breast cancer if:

- the person is assessed as being at intermediate risk **and**
- information on the biological features of the cancer provided by Oncotype DX is likely to help in predicting the course of the disease and would therefore help when making the decision about prescribing chemotherapy **and**
- the manufacturer provides Oncotype DX to NHS organisations according to the confidential arrangement agreed with NICE.

1.2 NICE encourages further data collection on the use of Oncotype DX in the NHS (see section 7).

1.3 MammaPrint, IHC4 and Mammostrat are only recommended for use in research in people with ER+, LN− and HER2− early breast cancer, to collect evidence about potentially important clinical outcomes and to determine the ability of the tests to predict the benefit of chemotherapy (see section 7). The tests are not recommended for general use in these people because of uncertainty about their overall clinical benefit and consequently their cost effectiveness.

> The analysis leading to recommendation 1.1 was based on intermediate risk of distant recurrence being defined as a Nottingham Prognostic Index (NPI) score above 3.4. It is anticipated that an NPI score can be simply calculated from information that is routinely collected about people with breast cancer. Other decision-making tools or protocols are also currently used in the NHS and these may also be used to identify people at intermediate risk.

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2 The technologies

2.1 Four tests available to the NHS were evaluated. Two are based on gene expression profiling: MammaPrint (Agendia) and Oncotype DX (Genomic Health). Two are based on immunohistochemistry (also known as protein expression profiling): IHC4 (academic sponsor – Royal Marsden Hospital and Queen Mary University, London) and Mammostrat (Clarient). These tests measure multiple markers within the tumour that may indicate how the tumour is likely to develop. Additional details are provided in section 4.

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3 Clinical need and practice

The problem addressed

3.1 Gene expression profiling and immunohistochemistry tests aim to improve the targeting of chemotherapy in breast cancer by more accurately identifying patients who will gain the most benefit. This rationale is based on the knowledge that certain biological features of cancers may indicate an increased likelihood of rapid growth and metastasis (in particular, distant recurrence). Distant recurrence is the return of detectable cancer in another part of the body. The tests may also identify, in some instances, which patients are most likely to benefit from chemotherapy. Some tools or tests provide mainly prognostic information (such as the Nottingham Prognostic Index [NPI] and Adjuvant! Online). Others may or may not be able to predict the extent to which the patient could benefit from chemotherapy (such as Oncotype DX, MammaPrint, Mammostrat and IHC4). Breast cancer patients face significant emotional and psychological strain when considering chemotherapy. It can be particularly distressing for patients in whom the decision to have chemotherapy is unclear using currently available tools (especially for people with an intermediate risk of distant recurrence). Tools or tests that help people decide whether or not to have chemotherapy are likely to be greatly appreciated by patients. The aim of this evaluation is to determine whether using gene expression profiling and expanded immunohistochemistry tests (MammaPrint, Oncotype DX, IHC4 and Mammostrat), in conjunction with current decision-making protocols (including tools such as the NPI and Adjuvant! Online) to guide the use of adjuvant chemotherapy, cost-effectively improves health outcomes and quality of life of people with early stage breast cancer, compared with current decision-making protocols alone.

The condition

Epidemiology and incidence

3.2 Breast cancer is the most commonly diagnosed cancer in women in England and Wales, but it can affect both men and women. In 2010 there were approximately 49,600 new cases in women and 400 in men. For both sexes,

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incidence varies with age. Just over 80% of cases occur in women aged 50 years and over. In England and Wales, 2006–2008 data demonstrate highest incidence rates for women in the 60- to 70-year age range.

3.3 Incidence also varies with family origin. In England, people of Asian, Chinese and black family origin and those with mixed heritage have a lower incidence than those of white family origin. Incidences are 0.65, 0.75, 0.49 and 0.58 that of those of white family origin respectively.

3.4 Breast cancer is the second largest cause of cancer-related death in women after lung cancer, with an age-standardised mortality rate of 24 per 100,000 women. In 2010 this constituted 10,328 deaths for women in England and Wales.

Prognosis

3.5 Overall, 5-year age-standardised survival rates for breast cancer are around 80%. Breast cancer survival rates have improved over the last 2 decades and now almost 2 out of 3 women with breast cancer survive beyond 20 years. Survival varies with age, stage of disease, family origin, socioeconomic status and tumour characteristics.

3.6 Clinicians currently estimate prognosis using tools such as the NPI (see section 4.10) or Adjuvant! Online (see section 4.11). The NPI takes into account grade as well as size and spread of the tumour, whereas Adjuvant! Online uses age of the patient, tumour size, nodal involvement, hormonal receptor status, histological grade and comorbidities to predict disease course and treatment options. Better prognosis is associated with small tumour size, younger age, lymph node negative (LN−), oestrogen receptor positive (ER+) and progesterone receptor positive (PR+) status. Human epidermal growth factor receptor 2 (HER2) over-expression (also known as HER2+) is associated with a poor prognosis. A tool called PREDICT, which is based on cancer registry data for women treated in England (East Anglia) and includes HER2 and Ki-67 status, has recently become available to the NHS.

3.7 Some patients considered to have a 'good' prognosis using current tools may still have recurrence after curative surgery and adjuvant therapy. Some

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patients considered to have a 'poor' prognosis may never develop metastatic disease. It is therefore challenging to decide whether to treat early stage breast cancer with adjuvant chemotherapy.

3.8 The decision whether to offer adjuvant chemotherapy is uncertain in people with ER+, LN− and HER2− early breast cancer. The External Assessment Group and clinical specialists who were consulted advised that the tests being evaluated would most likely be of benefit to the NHS in this patient group. Moreover, the evidence base was most robust for this population. Therefore the economic analysis for this evaluation focused on people with ER+, LN− and HER2− early breast cancer.

The diagnostic and care pathways

3.9 Patients diagnosed with early breast cancer currently follow the diagnosis/ treatment pathway described in figure 1.

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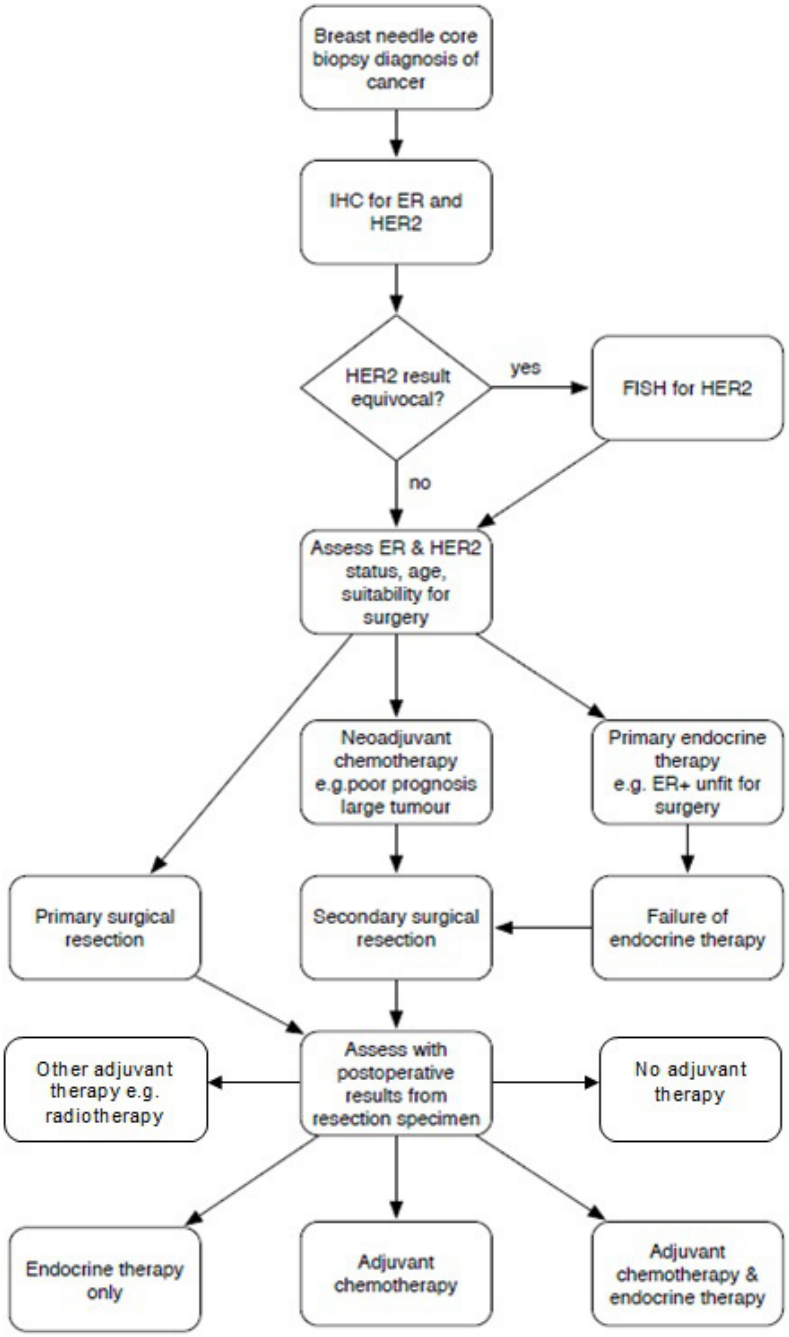
Figure 1 Diagnosis and management pathway in breast cancer



FISH: fluorescence in situ hybridisation

3.10 For the purposes of this assessment, chemotherapy is defined as the use of cytotoxic drugs with the intention of preventing cancer recurrence and does not include other forms of systemic therapy such as endocrine treatments or

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targeted biological therapy. Generally, chemotherapy regimens containing anthracyclines are used after cancer surgery (in the adjuvant setting).

Current guidelines

3.11 NICE cancer service guidance Improving outcomes in breast cancer recommends that women at intermediate or high risk of recurrence who have not had neoadjuvant chemotherapy should normally be offered multi-agent chemotherapy, which includes anthracyclines.

3.12 Early and locally advanced breast cancer: diagnosis and treatment (NICE clinical guideline 80) recommends that adjuvant therapy should be considered for all patients with early invasive breast cancer after surgery, based on assessment of the prognostic and predictive factors, and the potential benefits and side effects of the treatment. These guidelines do not refer to the use of gene expression profiling and expanded immunohistochemistry tests to aid decision making. NICE clinical guideline 80 recommends that decisions should be made following discussion of these predictive and prognostic factors with the patient and that Adjuvant! Online should be considered to support estimations of individual prognosis and the absolute benefit of adjuvant treatment. The NPI is also commonly used locally to aid decisions about chemotherapy for patients with early stage breast cancer and is discussed in NICE clinical guideline 80.

3.13 In the UK, local guidance based on the NPI and Adjuvant! Online has been developed to help clinicians decide about the benefits of adjuvant chemotherapy for a particular patient. However, it has been suggested that these tools may be imperfect and different local approaches to the use and interpretation of these tools leads to a proportion of people with early stage breast cancer being over- or under-treated. This may result in unnecessary use of expensive chemotherapy with its associated adverse effects for people who derive little or no benefit. In addition, there may be avoidable deaths in people who would have benefitted from chemotherapy had it been offered.

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4 The diagnostic tests

The individual tests: MammaPrint, Oncotype DX, IHC4, Mammostrat

4.1 Gene expression profiling and immunohistochemistry tests typically report 1 or 2 types of information – breast cancer subtype and risk of recurrence. Tests developed to provide information on subtypes can be used either before surgery to inform decisions on neoadjuvant therapy or after primary surgery (for removal of the tumour, which may also be used for further assessment of the tumour characteristics) to inform decisions on adjuvant chemotherapy (see figure 1). Tests predicting the risk of recurrence in a specific population are typically used after surgery, in conjunction with other information such as tumour size and grade, to guide the use of adjuvant chemotherapy. Such tests are typically indicated for women with oestrogen receptor positive (ER+) and lymph node negative (LN−) (and sometimes LN+ if the number of nodes is small) breast cancer in whom there is significant uncertainty about the value of chemotherapy. The current evaluation addresses the use of MammaPrint, Oncotype DX, IHC4 and Mammostrat after primary surgery to inform decisions on the use of adjuvant chemotherapy.

4.2 Three tests (MammaPrint, Oncotype DX and Mammostrat) require that samples are sent to a central laboratory for processing following surgery, with an estimated shipping and processing time of up to 7–10 days. IHC4 is processed in a local laboratory with estimated turnaround times of less than 1 week.

Gene expression profiling

4.3 Some gene expression profiling tests work by identifying and quantifying mRNA transcripts in a specific tissue sample. Because only a fraction of the genes encoded in the genome of a cell are transcribed into mRNA, gene expression profiling provides information about the activity of genes that give rise to these mRNA transcripts. Other gene expression profiling tests work by measuring levels of cDNA, which is synthesised from mRNA. There are a

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range of different techniques for measuring mRNA levels in breast cancer tumour samples, including real-time reverse transcription polymerase chain reaction (RT-PCR) and DNA microarrays.

4.4 Different tests use different protocols for preparing the samples (for example, formalin fixation, paraffin embedding, snap freezing and fresh samples) and different methods for preparing the RNA. Furthermore, there are different algorithms for combining the raw data into a summary profile. All of these factors can affect the reproducibility and reliability of gene expression profiling tests.

4.5 The 2 gene expression profiling tests included in this evaluation are described below:

- MammaPrint is based on microarray technology and uses an expression profile of 70 genes. MammaPrint is intended as a prognostic test for women of all ages, with LN− and LN+ (up to 3 nodes positive) breast cancer with a tumour size of 5 cm or less. MammaPrint is used to estimate the risk of distant recurrence of early breast cancer. It stratifies patients into 2 distinct groups – low risk (good prognosis) or high risk (poor prognosis) of distant recurrence. MammaPrint has been cleared by the Food and Drug Administration as an In Vitro Diagnostic Multivariate Index Assay. The test uses fresh or formalin-fixed paraffin-embedded samples that are processed centrally at laboratories run by the manufacturer in the USA or The Netherlands.
- Oncotype DX quantifies the expression of 21 genes in breast cancer tissue by RT-PCR. It predicts the likelihood of recurrence in women of all ages with newly diagnosed stage I or II, ER+, LN− or LN+ (up to 3 nodes positive) breast cancer treated with tamoxifen. The test assigns the breast cancer a continuous recurrence score (RS) and a risk category – low (RS<18), intermediate (18≤RS≤30) or high (RS≥31). The test also reports ER, progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status. The test uses formalin-fixed paraffin-embedded samples that are processed centrally at a laboratory run by the manufacturer in the USA.

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Immunohistochemistry (protein expression profiling)

4.6 Immunohistochemistry tests measure protein levels in the tumour sample rather than RNA or cDNA. Some of these tests offer the advantage of using existing immunohistochemical markers (such as ER and HER2), which are routinely tested in UK pathology departments. The term 'expanded' has been used to describe the immunohistochemistry tests evaluated in this assessment that are used in addition to standard immunohistochemistry testing (such as ER and HER2) for early invasive breast cancer. Immunohistochemistry uses staining to identify protein expression and reports the level of protein expression in tumour tissue. Differences in immunohistochemistry values can be caused by variability in several factors, including fixation of tissue, antigen retrieval (used to enhance staining), reagents, and interpretation.

4.7 The expanded immunohistochemistry tests included in this evaluation are described below:

- IHC4 measures the levels of 4 key proteins (ER, PR, HER2 and Ki-67) in addition to classical clinical and pathological variables (for example, age, nodal status, tumour size and grade) and calculates a risk score for distant recurrence using an algorithm. Quantitative assessments of ER, PR, and Ki-67 are needed for the IHC4 test. An online calculator for IHC4 is in development. The test uses formalin-fixed paraffin-embedded samples that can be processed in local NHS laboratories.
- Mammostrat uses 5 immunohistochemical markers (SLC7A5, HTF9C, P53, NDRG1 and CEACAM5) to stratify patients into risk groups to inform treatment decisions. These markers are independent of one another and do not directly measure either proliferation or hormone receptor status. The test calculates the relative risk of recurrence by using a weighted algorithm that is interpreted in the context of published clinical studies of appropriate patient populations. Patients are classified into 3 risk categories: prognostic index ≤0 defined as the 'low risk' group; prognostic index >0 and ≤0.7 defined as the 'moderate-risk' group; prognostic index >0.7 defined as the 'high risk' group. The test uses formalin-fixed paraffin-embedded samples that are processed centrally at a laboratory run by the manufacturer in the USA.

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The comparator

4.8 The comparator is standard practice in England. Although this varies between hospitals, Adjuvant! Online and/or the Nottingham Prognostic Index (NPI) are often used to guide decisions on which patients with early breast cancer should be offered adjuvant chemotherapy. The economic analysis used cancer registry data on levels of chemotherapy prescribing to reflect standard practice in England and, therefore, is likely to incorporate the impact on the decision to use chemotherapy based on a range of different decision tools currently used in the NHS.

4.9 Further information on the importance of individual molecular markers (for example, ER and HER2, which are routinely assessed for early breast cancer) in the decision to offer adjuvant chemotherapy (and other therapies such as endocrine therapy) has led to varying local practice. Although some hospitals use Adjuvant! Online and the NPI in their original forms, others use adaptations of these tools. Adjuvant! Online is often used in conjunction with the HER2 score. Management algorithms based on the combined use of the NPI and molecular markers such as ER and HER2 are also used.

Nottingham Prognostic Index

4.10 The NPI is a composite prognostic parameter involving both time-dependent factors and aspects of tumour aggressiveness. The NPI score is based on a mix of grade, lymph node involvement and tumour size. The score is calculated by adding numerical grade (1, 2 or 3), lymph node score (negative=1, 1 to 3 nodes=2, >3 nodes=3) and 0.2 times tumour size in centimetres. Patients can be divided into 3 prognostic groups (other subdivisions are also possible, for example 5 prognostic groups) on the basis of the NPI score: a good prognostic group (NPI≤3.4), a moderate prognostic group (3.4<NPI≤5.4) and a poor prognostic group (NPI>5.4).

Adjuvant! Online

4.11 The Adjuvant! Online computer programme is designed to provide estimates of the benefits of adjuvant endocrine therapy and chemotherapy. The current version of Adjuvant! Online does not include HER2 status. Patient and tumour

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characteristics are entered into the programme and provide an estimate of the baseline risk of mortality or relapse for patients without adjuvant therapy. Information about the efficacy of different therapy options was derived from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analyses and provides estimates of reduction in risk of breast cancer-related death or relapse at 10 years for selected treatments. These estimates are then provided on printed sheets in simple graphical and text formats to be used in consultations.

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5 Outcomes

5.1 The Diagnostics Advisory Committee (section 11) considered evidence from a number of sources (section 12).

How outcomes were assessed

5.2 The assessment consisted of a systematic review of the evidence on test performance and clinical-effectiveness data for the 4 tests included in the evaluation. The outcome measures included:

- Analytical validity, defined as the ability of the test to accurately and reliably measure the expression of mRNA or proteins by breast cancer tumour cells (that is, repeatability and reproducibility).
- Clinical validity, defined as prognostic ability or the degree to which the test can accurately predict the risk of an outcome (for example, the risk of distant metastases in 10 years).
- Clinical utility, defined as the ability of the test to improve clinical outcomes such as overall survival. This includes direct harms arising from the test, reclassification of risk compared with existing tools, its impact on clinical decision-making and the ability of the test to predict benefit from chemotherapy. Within the context of clinical utility, the predictive ability of a test refers to the capability of the test to accurately predict patients who will benefit most, in relative terms, from chemotherapy, that is, whether patients classified as high risk benefit more in relative terms than patients classified as low risk.

5.3 In the base-case economic analysis, the External Assessment Group used the available data on the clinical validity and clinical utility of the tests to populate the model. The risk of distant recurrence (prognosis) was computed from data on the clinical validity of the tests. The reclassification of risk by the new tests (presented as 2 Nottingham Prognostic Index [NPI] subgroups), the impact of the test results on clinical decision-making (the proportion of patients receiving adjuvant chemotherapy) and the predicted benefit of chemotherapy by risk group (reduction in the risk of distant recurrence) were based on data on the clinical utility of the tests. In all cases, the systematic review showed that data

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on the clinical validity of the tests were more robust than data on their clinical utility. Therefore the External Assessment Group used sensitivity analysis to explore alternative scenarios with different assumptions of the clinical utility of the tests and, in some cases alternative assumptions of clinical validity.

5.4 For 2 of the 4 tests (Oncotype DX and MammaPrint), the current systematic review updated an existing systematic review of gene expression profiling tests for breast cancer. Two previous systematic reviews (one an update of the other) reviewed the literature relating to both Oncotype DX and MammaPrint. Marchionni et al. (2008) is an exhaustive literature review of various electronic databases covering biomedical literature between 1990 and 2006. In 2010, Smartt updated this systematic review and included all relevant evidence that became available between 2007 and 2009.

5.5 The External Assessment Group undertook a systematic review of the evidence on cost effectiveness for the 4 tests. Genomic Health (Oncotype DX) submitted an economic model and Clarient submitted a report detailing an economic analysis of Mammostrat. The External Assessment Group constructed a de novo economic model. The outcomes of interest for the economic evaluation were the morbidity and mortality associated with invasive breast cancer and its treatment. These included survival and health-related quality of life, including the impact of adverse events associated with chemotherapy. The de novo economic model followed a linked evidence approach in which intermediate outcomes (results of the tests) were linked to treatment outcomes and hence quality-adjusted life year (QALY) gains. Costs and QALYs were assigned to each of the 4 tests and the comparator.

5.6 The population identified in the scope for this evaluation included assessment of the gene expression profiling and expanded immunohistochemistry tests in men with breast cancer if data were available. No such data that would allow the evaluation of these technologies in men were identified in the systematic review.

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Clinical effectiveness

5.7 The terms analytical validity, clinical validity and clinical utility, used in this section, are defined in section 5.2.

5.8 Much of the clinical evidence was related to the Oncotype DX and MammaPrint tests because these tests are much further along the validation pathway than IHC4 and Mammostrat. The highest-quality evidence was reported for Oncotype DX, although limitations or gaps in the clinical data were identified for all tests. Most studies, for all tests, were retrospective in design, analysing archived tumour samples from a cohort of patients with documented information on patient characteristics and outcomes. Retrospective analyses are associated with increased bias compared with prospective randomised controlled trials. Some of the studies involved a prospective analysis of retrospective archived material from a previous randomised controlled trial. Potential issues still remain, including the effects of confounding and the possible incompleteness of some biological specimens.

5.9 Study populations were generally heterogeneous, although most of the evidence on Oncotype DX came from oestrogen receptor positive (ER+), lymph node negative (LN−) populations. Some studies included a small number of participants. Studies including larger sample sizes, in excess of 1000 samples, were available for Oncotype DX, Mammostrat and IHC4. Follow-up was short or not reported in a number of studies. Five studies were specific to a UK population, including 3 for Oncotype DX, 1 for IHC4 and 1 for Mammostrat.

MammaPrint

5.10 A range of studies provided evidence on the prognostic ability of MammaPrint in heterogeneous populations. However, the previous systematic reviews indicated that evidence relating to the clinical validity of MammaPrint was not always conclusive nor supported the prognostic value of the test. Four studies suggested that the test could predict prognosis, 1 study of prognostic utility did not reach statistical significance and in another the methods and results were at variance with other studies. In terms of clinical utility, the previous reviews

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identified 1 prospective observational study (Bueno-de-Mesquita et al. [2007], also known as the RASTER study) demonstrating that MammaPrint had an impact on clinical decision-making when used in addition to current practice in the Netherlands (Dutch Institute for Healthcare Improvement [CBO] guidelines). Adjuvant systemic treatment was advised less often when Dutch CBO guidelines were used compared with use of MammaPrint. Therefore, Bueno-de-Mesquita et al. (2007) reported that the addition of MammaPrint to the standard Dutch clinical assessment of risk (modified by patient preference) in a cohort of 427 patients increased the number of patients receiving adjuvant systemic therapy by 20 (5%). At the time of the previous systematic review, follow-up was not long enough to provide evidence of its effect on clinical end points such as distant metastasis-free survival or its utility in predicting treatment benefit; however, follow-up data at 5 years have recently been published in the study by Drukker et al. (2013). The study reported, among other outcomes, that in the group of patients classified as being at low risk with MammaPrint and at high risk with Adjuvant! Online (of whom 76% had not received adjuvant chemotherapy), the 5-year distant recurrence-free interval was 98.4%. The previous systematic reviews recommended that further evidence from randomised controlled trials was needed in addition to robust evidence on the prediction of benefit from chemotherapy.

5.11 The External Assessment Group identified 7 additional, non-UK-based, studies of MammaPrint. Of these 7 studies, 4 on the clinical validity of MammaPrint demonstrated that the MammaPrint score is a strong independent prognostic factor, and may provide additional value to standard clinicopathological measures. A mix of evidence exists for outcomes at 5 and 10 years. The population in all these studies was relatively small. One of the studies was of a Japanese population, and follow-up was limited to only 5 years in 2 of the studies. For example, Mook et al. (2010) showed that in 148 women the distant metastasis-free survival at 5 years was 93% in the low-risk group and 72% in the high-risk group with an associated hazard ratio (HR) of 4.6 (95% confidence interval [CI] 1.8 to 12.0, p=0.001). The External Assessment Group did not identify any prospective studies of the impact of MammaPrint on long-term outcomes such as overall survival, but the prospective observational RASTER study published 5-year follow-up data after the External Assessment Group completed its assessment, and this was discussed by the Committee

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(see section 6.13). Six studies with data on the clinical utility of MammaPrint were identified by the External Assessment Group. Five studies reported use of MammaPrint to reclassify patients into high- and low-risk groups and compared this with the risk assigned according to current local guidance. They reported a high level of discordance between MammaPrint and current classification, although these studies did not demonstrate how this would impact on treatment decisions. For example, Bueno-de-Mesquita et al. (2009) compared MammaPrint risk categories and risk assessment based on Adjuvant! Online, St Gallen guidelines, NPI and Dutch CBO guidelines (2004). Discordance between MammaPrint and the other risk assessment measures was 38%, 41%, 26%, and 30% respectively. One study reported that the use of MammaPrint would result in altered treatment advice for 40% of patients based on the assumption that all patients classified as high risk would receive chemotherapy and patients classified as low risk would not receive chemotherapy. Because the study was retrospective, altered treatment advice assumed in the analysis represented potential changes and not actual changes from using the test in clinical practice.

5.12 A study of the benefit of chemotherapy according to risk group stratification by MammaPrint was identified (Knauer et al. 2010) but was omitted from the systematic review because it was based on a pooled analysis of 6 primary studies. The External Assessment Group did not include the pooled analysis in the systematic review to avoid double counting of studies already included in the review. In addition, it did not consider the findings of the pooled analysis to be robust because the authors did not reanalyse the tumour samples, it is unclear how individual patient data were combined, and there were potential issues with the statistical analyses performed (for example, although median follow-up was 7.1 years the data were arbitrarily truncated at 5 years).

5.13 Robust evidence of clinical utility is not available for MammaPrint so it is not yet clear whether using the test will improve the use of adjuvant chemotherapy in the management of breast cancer in the UK. In summary, most studies of MammaPrint were retrospective in design, used small sample sizes and had heterogeneous patient populations and some studies included only pre-menopausal women. Moreover, no studies were conducted in the UK. The evidence for MammaPrint is based on the use of the test with fresh samples. It

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is not clear whether this evidence would apply if the test were used on formalin-fixed paraffin-embedded samples. Overall, the External Assessment Group considered that further robust evidence on the clinical validity and clinical utility of the test would be helpful.

Oncotype DX

5.14 Oncotype DX was reported to be furthest along the validation pathway by previous systematic reviews. In terms of clinical validity, these reviews reported evidence that the Oncotype DX recurrence score was significantly correlated with disease-free survival and overall survival. Furthermore, the recurrence score was shown to be a better predictor of distant recurrence at 10 years than traditional clinicopathological predictors. The evidence on clinical utility was limited. One study (Paik et al. 2006) demonstrated a significantly increased benefit from the use of chemotherapy in the Oncotype DX high-risk group compared with the low-risk group, although the review highlighted that the study may have potential flaws. The study indicated that this benefit difference was caused by the better prognosis without chemotherapy (and hence the reduced absolute benefit these patients would receive) and the decreased relative benefit of chemotherapy in the lower-risk groups. The specific cancers in the low-risk groups were less likely to respond to chemotherapy, independent of actual survival probability. Key gaps were identified in the evidence base related to the extent to which the test added to the management of breast cancer and the proportion of patients who would benefit from the test. The previous systematic reviews indicated that prospective confirmation of the clinical utility of Oncotype DX was needed.

5.15 The External Assessment Group identified 12 additional studies of Oncotype DX. Further larger studies now support the prognostic ability of Oncotype DX. One large-scale UK study in post-menopausal women with ER+, LN− early breast cancer found that an increase in risk score was significantly associated with an increased risk of distant recurrence. Furthermore, the evidence base has been extended to include the LN+ population. The External Assessment Group did not identify any prospective studies of the impact of Oncotype DX on long-term outcomes such as overall survival. Four studies were identified that presented further evidence on the impact of Oncotype DX on clinical decision-making. These indicated that the use of Oncotype DX leads to changes in

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treatment decisions for between 32% and 38% of patients. However, only 1 of these studies was performed in the UK and limitations in relation to the generalisability of the study were identified. In addition, the study only included a small sample of patients (interim results on 106 patients were available for the systematic review, the dataset for 142 patients was available for and used in the External Assessment Group's cost-effectiveness analysis). Four recent publications reported evidence that Oncotype DX predicts benefit from chemotherapy. However, only 1 of these studies on an LN+ population (Albain et al. 2010) presented new data. The other 3 publications (Tang et al. 2011, Mamounas et al. 2010 and Tang et al. 2010) reported the same trial data as Paik et al. (2006). The first evidence of improvements in quality of life and reduced patient anxiety as a result of using Oncotype DX have been reported, but the studies had small sample sizes (for example, Lo et al. [2010] included 89 patients). In summary, Oncotype DX is considered to have the most robust evidence base of the tests reviewed in this guidance, with data on the analytical validity, clinical validity and clinical utility of the test. The studies varied considerably in their size, design and patient populations. Many of the Oncotype DX studies were small and retrospective. A small number of studies were conducted in the UK. The External Assessment Group considered that further robust evidence on the clinical utility of the test would be helpful.

IHC4

5.16 No studies on analytical validity of IHC4 (based on ER, progesterone receptor [PR], human epidermal growth factor receptor 2 [HER2] and Ki-67 in addition to classical clinical and pathological variables combined using an algorithm) were identified. Of the 4 individual tests that make up IHC4, 2 (ER and HER2) are commonly measured in the NHS. However, the quantitative assessment of ER needed for IHC4 calculations is not routinely performed. Outstanding issues around the reproducibility of detecting Ki-67 also exist. This is noteworthy because the test is designed for local use and different local processing methods may potentially lead to different results. The External Assessment Group identified 1 study on the clinical validity of IHC4 (Cuzick, 2011), which reports that the IHC4 score is a highly significant predictor of distant recurrence. The authors validated the test in a cohort of 786 patients with ER+ cancer treated in the UK, and demonstrated that the IHC4 score was highly significantly predictive of outcome, with a hazard ratio of 4.8 (95% CI

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2.2 to 10.2) for a change from the 25th to 75th percentile in a univariate analysis. This study also reported evidence comparing IHC4 against Oncotype DX. The study was rated as high quality. The External Assessment Group did not identify any prospective studies of the impact of IHC4 on long-term outcomes such as overall survival. It did not identify any published evidence on the clinical utility of IHC4 in terms of its ability to change treatment decisions or its ability to predict chemotherapy benefit. In summary, the External Assessment Group considered that the evidence base for IHC4 is currently limited to clinical validity (prognostic ability), although this evidence is considered to be relatively robust, and further evidence would be helpful on analytical validity and clinical utility.

Mammostrat

5.17 The External Assessment Group did not identify any specific studies on the analytical validity of Mammostrat, although some limited evidence on analytical validity was reported in studies of clinical validity and clinical utility. Three studies were identified that provided data to support the use of Mammostrat as an independent prognostic tool for women with ER+, tamoxifen-treated breast cancer. Although the evidence base for Mammostrat is at present relatively limited, these studies included a large sample size, appeared to be of reasonable quality, and 1 study provided data from a UK setting. The External Assessment Group did not identify any prospective studies of the impact of Mammostrat on long-term outcomes such as overall survival. In addition, clinical utility data on Mammostrat (from 1 study) suggest that the low- and high-risk groups benefit from chemotherapy, but not the intermediate-risk group. There was no published evidence on reclassification of risk groups compared with conventional means of risk classification, and no evidence on the impact of the test on clinical decision-making. Overall, the External Assessment Group considered that further evidence of analytical validity and clinical utility would be helpful.

Economic analysis

5.18 Four studies were identified as meeting the inclusion criteria of the systematic review of cost-effectiveness evidence (2 for MammaPrint and 2 for Oncotype

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DX). None were conducted in England. Genomic Health and Clarient also submitted economic analyses on the cost effectiveness of Oncotype DX and Mammostrat in England respectively. Several issues were highlighted in the critique of these analyses, which needed further consideration. These included assumptions about: the baseline level of chemotherapy in clinical practice; the risk of distant recurrence; patients who would be offered the test; the proportion of patients who would be offered chemotherapy after reclassification with the new test; the cost of chemotherapy and therapy used to prevent or treat associated adverse events.

5.19 The External Assessment Group constructed a de novo economic model to specifically address the decision problem for this evaluation and to estimate the cost effectiveness of the 4 tests in England.

5.20 The population assessed in the economic model was women with ER+, LN−, HER2− early breast cancer up to 75 years old at diagnosis. One analysis assumed that all women in the group received the new tests. However, the External Assessment Group's clinical specialists suggested that the new tests may be targeted at a subgroup of this population – those at intermediate risk of distant recurrence for whom the decision about whether or not to give chemotherapy is most uncertain. A subgroup analysis was performed that assumed that the new test was given only to women with an NPI score above 3.4 (used as a proxy for those women at intermediate risk of distant recurrence), based on the assumption that most women at low risk (with an NPI score below 3.4) would not be considered for chemotherapy and that there would not be many women at high risk (with an NPI score of above 5.4) within the population considered.

Clinical outcomes

5.21 Modelling was used to estimate clinical outcomes. All women in the model were assumed to be treated with endocrine therapy. A state transition model was used to simulate breast cancer outcomes for patients treated with endocrine therapy alone or with the addition of chemotherapy. Outcomes associated with breast cancer were simulated using multiple health states including recurrence-free survival, recurrence (distant and local), adverse events from chemotherapy, and death.

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Costs

5.22 The costs included in the economic model were the costs of the different tests, treatment costs (endocrine therapy and chemotherapy), costs of short-term and long-term adverse events associated with chemotherapy (including the secondary prevention of short-term adverse events), costs associated with managing distant recurrence, local recurrence and terminal care.

5.23 The cost of the MammaPrint test is £2675 (this cost was used in the economic model). The Oncotype DX test costs £2580 (this cost was used in the original economic analysis, but a revised cost was used for the economic analysis conducted for the confidential revised price). IHC4 was estimated to cost £100–£200 (£150 was used in the economic model) for quantitative analysis of ER (which may need additional time compared with traditional assessment of ER status), and assessment of PR and Ki-67 (which are not routinely collected) and running the algorithm (it was assumed that HER2 would be measured as part of standard practice). The Mammostrat test has an indicative cost between £1120 and £1620 (£1135 was used in the economic model).

Cost effectiveness

5.24 The primary analysis compared current clinical practice with treatment guided by Oncotype DX and IHC4. The systematic review of the evidence indicated most evidence for Oncotype DX compared with the other tests, and that the evidence base for Oncotype DX, in particular in relation to prognostic ability, was reasonably sound. There was less evidence for IHC4, but there was evidence relating to the performance of IHC4 compared with Oncotype DX. This evidence, with some additional assumptions when compared with the analysis of Oncotype DX, was used to model the cost effectiveness of IHC4. Additional assumptions include the reproducibility of the test and the use of risk groups as opposed to a continuous risk score. There was no evidence on the ability of IHC4 to predict benefit from chemotherapy; in the IHC4 analysis, the predicted benefit of chemotherapy was applied according to the Oncotype DX risk classification.

5.25 In addition to the primary economic analysis, further economic analyses were undertaken for Mammostrat and MammaPrint. These additional analyses were

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deemed exploratory by the External Assessment Group because there are significant limitations in the evidence base and the generalisability of the data to practice in England. Only data from studies conducted in the USA (Mammostrat) and The Netherlands (MammaPrint) were available to estimate the reclassification of patients using the new test. There was concern whether these data are generalisable to England and there was also uncertainty in the data. There were no studies showing the impact of these tests on the management of breast cancer in the England. In addition, the External Assessment Group reported considerable uncertainty in the data used to estimate the predicted benefit of chemotherapy by MammaPrint risk groups.

5.26 All analyses assumed that the new tests were used in addition to current practice (for IHC4 it was assumed that quantitative analysis of ER [which may need additional time compared with traditional assessment of ER status], PR and Ki-67 is carried out in addition to current practice and data combined in an algorithm). Full details of the results can be found in section 5.6 of the diagnostics assessment report. A brief summary of the key results (including incremental cost-effectiveness ratios [ICERs]) of the base-case analysis and sensitivity analyses is presented below. Results of the 3 analyses (Oncotype DX and IHC4; Mammostrat; MammaPrint) cannot be directly compared because the data came from different studies with different patient characteristics and methodologies, and the basis for each model therefore varies significantly.

5.27 The base-case analysis modelled a hypothetical cohort of 1000 women over a lifetime horizon (100 years was used as the upper age limit). Two analyses are presented. In the first the tests were used for all women with ER+, LN−, HER2− early breast cancer aged up to and including 75 years. In the second the tests were used only for women with ER+, LN−, HER2− early breast cancer up to and including 75 years with an NPI score above 3.4 (used as a proxy for those women at intermediate risk of distant recurrence). Results are presented on a per patient basis and any differences in expected values are a result of rounding error.

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Oncotype DX and IHC4

5.28 **Tests used for all women with ER+, LN−, HER2− early breast cancer and Oncotype DX is assessed at the list price of £2580.** In the primary economic analysis comparing Oncotype DX and IHC4 with current practice, the proportions of patients receiving chemotherapy were 19.11%, 9.57% and 14.42% respectively. The model predicted that there would be 64, 71 and 76 distant recurrences when using Oncotype DX, IHC4 or current practice respectively. Total costs and QALYs, assuming predictive benefits (that is, benefits from identifying who will benefit most from chemotherapy) based on Paik et al. (2006), are summarised in table 1.

Table 1 Per-patient costs, QALYs and ICERs in the primary economic analysis (Oncotype DX and IHC4 compared with current practice)[a]

	Mean cost (£)	Mean QALYs	ICER – compared with current practice
Oncotype DX	£9094	13.54	£26,940[b]
IHC4	£6340	13.49	Dominant
Current practice	£6519	13.44	

Abbreviations: ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life year.

[a] The analysis assumed the tests are used for all women with ER+, LN−, HER2− early breast cancer in England. Predictive benefit was based on Paik et al. (2006) and is based on the list price of Oncotype DX.

[b] Rounding error contributes to the difference from expected value.

5.29 Compared with current practice, Oncotype DX was associated with an incremental cost of £2575 and incremental QALYs of 0.1, yielding an ICER of £26,940 per QALY gained. IHC4 was £179 cheaper than current practice (cost saving), with incremental QALYs of 0.05 and was predicted to be dominant (that is, provide more QALYs at a lower cost) compared with current clinical practice. Oncotype DX, IHC4 and current practice were also compared using incremental analysis; that is, the least effective strategy was compared with the next least effective strategy that was neither dominated nor extendedly dominated. The cost-effectiveness acceptability curve showed that the

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probability of IHC4 being cost effective (when compared with current practice) was almost 100% if the maximum acceptable ICER was £20,000 per QALY gained. At the same maximum acceptable ICER, the probability of Oncotype DX being cost effective, when compared with current practice only, was 12.4%.

5.30 Tests used for women with ER+, LN−, HER2− early breast cancer and an NPI score above 3.4, and Oncotype DX is assessed at the list price of £2580. In the primary economic analysis the proportion of these patients predicted to receive chemotherapy was 34.72%, 26.31% and 33.60% with Oncotype DX, IHC4 and current practice respectively. The model predicted that there would be 117, 129 and 144 distant recurrences when using Oncotype DX, IHC4 or current practice respectively. Total costs and QALYs, assuming predictive benefits based on Paik et al. (2006), are summarised in table 2.

Table 2 Per patient costs, QALYs and ICERs in the primary economic analysis (Oncotype DX and IHC4 compared with current practice)[a]

	Mean cost (£)	Mean QALYs	ICER – compared with current practice
Oncotype DX	£10,911	13.06	£9007[b]
IHC4	£8318	12.97	Dominant
Current practice	£8816	12.83	

Abbreviations: ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life year.

[a] The analysis assumed the tests are used for women with ER+, LN−, HER2− early breast cancer and an NPI score above 3.4 in England. Predictive benefit was based on Paik et al. (2006) and is based on the list price of Oncotype DX.

[b] Rounding error contributes to the difference from expected value.

5.31 Compared with current practice, Oncotype DX was associated with an incremental cost of £2095 and incremental QALYs of 0.23, which resulted in an ICER of £9007 per QALY gained. IHC4 was £498 cheaper than current practice (cost saving), with incremental QALYs of 0.14 and was predicted to be dominant (that is, provide more QALYs at a lower cost) compared with current clinical practice. Oncotype DX, IHC4 and current practice were also compared using incremental analysis; that is, the least effective strategy was compared

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with the next least effective strategy that was neither dominated nor extendedly dominated. The cost-effectiveness acceptability curve showed that the probability of IHC4 being cost effective (when compared with current practice) was almost 100% if the maximum acceptable ICER was £20,000 per QALY gained. At the same threshold, the probability of Oncotype DX being cost effective, when compared with current practice only, was 91.6%.

5.32 **Sensitivity analyses (univariate)**. A range of univariate sensitivity analyses were undertaken to explore the impact of varying the main model parameters. Analyses of varying the assumptions underlying the structure of the model were also performed. The ICERs for Oncotype DX compared with current clinical practice, for all women with ER+, LN−, HER2− early breast cancer and those with an NPI above 3.4, were sensitive (defined as changes in the ICER by 10% or more) to some of the assumptions made in the model. These included the time horizon modelled, the starting age of the cohort, the risk of recurrence, the proportion of patients receiving chemotherapy after reclassification with the new test, the benefit of chemotherapy in the different risk groups and the distribution of patients by NPI score. For example, the ICERs for Oncotype DX (compared with current practice) when offered to all women with ER+, LN−, HER2− early breast cancer were £91,300 (assuming 30% relative risk reduction from chemotherapy for all patients) per QALY gained and £64,900 (assuming 40% relative risk reduction from chemotherapy for all patients) per QALY gained. The ICERs for IHC4 compared with current clinical practice, for all women with ER+, LN−, HER2− early breast cancer and those with an NPI score above 3.4, were sensitive to more assumptions (such as the time spent in the distant recurrence health state, the proportion of patients receiving chemotherapy under current practice and the cost of chemotherapy), but IHC4 remained dominant compared with current practice (that is, it provided more QALYs at a lower cost) except when the cost of IHC4 was set at £400 (the resulting ICER was £1557 per QALY gained).

5.33 Following the first consultation, the manufacturer of Oncotype DX submitted a proposal to make it easier for the NHS to access the technology. The proposal makes Oncotype DX available at a revised price. The proposed price is commercial in confidence. The proposal is made for patients at an intermediate risk of distant recurrence, defined as an NPI score above 3.4 in this guidance.

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An External Assessment Group analysis of the proposal, using the proposal price and the assumption that Oncotype DX is validated as a prognostic tool but does not predict the benefit patients will get from chemotherapy, yielded an ICER of £22,600 per QALY gained compared with current clinical practice for patients with an NPI score above 3.4.

Mammostrat (exploratory analysis)

5.34 **Test used for all women with ER+, LN−, HER2− early breast cancer.** The proportion of patients receiving chemotherapy increased with the use of Mammostrat when compared with current practice (21.16% and 14.42% respectively). Current practice was associated with a mean cost of £7699 and mean QALYs of 12.86. Mammostrat was associated with a mean cost of £9040 and mean QALYs of 12.91. The ICER for Mammostrat was estimated to be £26,598 per QALY gained. However there were significant uncertainties and limitations associated with this analysis. These included uncertainty about the generalisability of the risk reclassification data to a UK population of patients with ER+, LN−, HER2− breast cancer, and the lack of evidence on the impact of the test on decision-making. In addition, the robustness of evidence on the predictive ability of the test is uncertain – clinical utility data from 1 study suggest that the low- and high-risk groups benefit from chemotherapy, but not the intermediate-risk group. The cost-effectiveness acceptability curve showed a 36.0% probability of Mammostrat being cost effective if the maximum acceptable ICER is £20,000.

5.35 **Test used for women with ER+, LN−, HER2− early breast cancer and an NPI score above 3.4**. The proportion of patients receiving chemotherapy increased slightly with the use of Mammostrat when compared with current practice (34.27% and 33.60% respectively). Current practice was associated with a mean cost of £9717 and mean QALYs of 12.34. Mammostrat was associated with a mean cost of £10,985 and mean QALYs of 12.29. Mammostrat was shown to be dominated by current practice. The cost-effectiveness acceptability curve showed an 18.0% probability of Mammostrat being cost effective if the maximum acceptable ICER is £20,000.

5.36 **Sensitivity analyses (univariate).** A range of univariate sensitivity analyses were undertaken to explore the impact of varying model parameters. When

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offering the test to all women with ER+, LN−, HER2− early breast cancer the ICER was very sensitive to the proportion of patients who would receive chemotherapy based on the test result. The ICER ranged between £18,879 per QALY gained to being dominated, when using the confidence intervals from the Ross et al. (2008) study for the predicted benefit of chemotherapy in terms of the reduction of distant recurrence. The ICER was not sensitive to the assumptions about utility values, management costs and the time spent in the recurrence health state. Mammostrat remained dominated under the assumptions examined in the sensitivity analysis when the test was offered to women with ER+, LN−, HER2− early breast cancer and an NPI score above 3.4.

MammaPrint (exploratory analysis)

5.37 **Test used for all women with ER+, LN−, HER2− early breast cancer.** The proportion of patients receiving chemotherapy increased with the use of MammaPrint when compared with current practice (44.18% and 14.42% respectively). Current practice was associated with mean costs of between £6408 and £6629, and mean QALYs of between 13.39 and 13.49. MammaPrint was associated with mean costs of between £10,017 and £10,748 and mean QALYs of between 13.47 and 13.78. Because of uncertainty around the evidence on the benefit of chemotherapy for the MammaPrint risk groups, the results for MammaPrint were presented as a range (based on the confidence interval for the benefit of chemotherapy). The ICER was estimated to be between £12,240 and £53,058 per QALY gained. Additional uncertainties include the lack of UK data in a relevant population (patients with ER+, LN−, HER2− breast cancer; particularly in relation to risk reclassification compared with UK practice), the impact of the test on clinical decision-making in the UK and reliance on data mainly from pre-menopausal populations.

5.38 **Test used for women with ER+, LN−, HER2− early breast cancer and an NPI score above 3.4**. The proportion of patients receiving chemotherapy increased with the use of MammaPrint when compared with current practice (90.31% and 33.60% respectively). Current practice was associated with mean costs of between £8281 and £8872 and mean QALYs of between 12.81 and 13.07. MammaPrint was associated with mean costs of between £12,278 and

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£14,014 and mean QALYs of between 12.99 and 13.73. Because of uncertainty around the evidence on the benefit of chemotherapy for the MammaPrint risk groups, the results for MammaPrint were presented as a range (based on the confidence interval for the benefit of chemotherapy). The ICER for MammaPrint was estimated to be between £6053 and £29,569 per QALY gained. Additional uncertainties include the lack of UK data and the reliance on data mainly from pre-menopausal populations.

5.39 **Sensitivity analyses (univariate and multivariate)**. Given the uncertainty in the base-case analysis a limited number of sensitivity analyses were undertaken. Univariate analyses included: assuming no additional cost to the NHS for the use of fresh tissue samples and that 5% of patients classified as good prognosis and 95% of patients classified as poor prognosis received chemotherapy. A multivariate sensitivity analysis explored different values for the benefit of chemotherapy in terms of reduction in the risk of distant recurrence, assuming MammaPrint was used in all women with ER+, LN−, HER2− early breast cancer.

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6 Considerations

6.1 The Diagnostics Advisory Committee discussed the focus of the evaluation and the evidence available for the 4 tests. It noted that gene expression profiling and immunohistochemistry tests other than those included in this evaluation are being developed. The Committee also noted that at present, the level and quality of the available evidence varies for the 4 tests. In particular, evidence on the tests' ability to guide clinical decisions on the use of chemotherapy in England and to predict response to chemotherapy in women with early breast cancer was limited. The External Assessment Group's economic model for women with oestrogen receptor positive (ER+), lymph node negative (LN−) or human epidermal growth factor receptor 2 negative (HER2−) early breast cancer was used by the Committee when considering the likely cost effectiveness of the 4 tests. The Committee considered that the most appropriate use of these tests is in women for whom the decision to offer chemotherapy is uncertain, that is, women at intermediate risk of distant recurrence. It therefore considered that the subgroup analyses of women with a Nottingham Prognostic Index (NPI) score above 3.4 were the most relevant, based on the likelihood that there would not be many women with an NPI score of above 5.4 within the target population.

6.2 The Committee acknowledged the emotional and psychological strain for patients with breast cancer when considering therapy, in particular, chemotherapy and its associated adverse events. The Committee noted that this is likely to be significant in patients for whom the decision about whether or not to have chemotherapy is difficult after receiving the results of current tools used in the NHS (especially patients deemed to be at intermediate risk). The Committee also noted that tools used by the NHS to assess the suitability of patients with breast cancer for adjuvant chemotherapy vary across England. The Committee concluded that any tests that can help to alleviate emotional and psychological strain and promote consistency of practice within the NHS are likely to be appreciated by patients and clinicians alike.

6.3 The Committee discussed the generalisability of the data to men. The Committee acknowledged that breast cancer is not only observed in women and that men make up a small proportion of patients with breast cancer. The

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Committee noted that all the clinical and economic evidence had been based on trials with women; however, experts on the Committee stated that even though there are some subtle gender-specific differences in the pathobiology of breast cancer, the general subtypes are identical in men and women. Therefore, in clinical practice men would be treated in the same way as women. The Committee therefore concluded that the recommendations in this guidance should also apply to men.

6.4 The Committee discussed the evidence base for Oncotype DX and concluded that, in general, it was the most developed of the 4 tests in the evaluation. The Committee discussed the analytical validity of Oncotype DX. The Committee noted that no new evidence was identified in the External Assessment Group review, but that evidence was identified in the previous systematic review (Marchionni et al. 2008) that showed reasonable within-laboratory replicability. The Committee also noted that the test is processed centrally by the manufacturer in the USA and the laboratory is CLIA (Clinical Laboratory Improvement Amendments) certified. Given the above, the Committee was satisfied with the analytical validity of the test. The Committee discussed the prognostic ability (clinical validity) of Oncotype DX. Experts on the Committee pointed out and the Committee agreed that the prognostic ability (the ability to predict the risk of distant recurrence) of Oncotype DX had been well validated. The Committee also considered a study by Sgroi et al. reported in abstract form at the San Antonio Breast Cancer Symposium 2012, which assessed the prognostic value of Oncotype DX (and IHC4) over and above standard clinical variables. The Committee noted that the abstract shows that Oncotype DX does not provide prognostic information for late distant metastasis. The Committee considered that these new data raise potential uncertainty around the long-term benefits of Oncotype DX, but judged that the relatively extensive evidence base supporting the prognostic ability of the test to be satisfactory at this time. The Committee therefore concluded that the prognostic ability of Oncotype DX was supported by robust evidence in the early breast cancer population.

6.5 The Committee then discussed the clinical utility of Oncotype DX. It heard from the External Assessment Group that a key aspect of clinical utility is the ability of a test to accurately predict those patients who will benefit most from

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chemotherapy. The Committee therefore considered whether gains from chemotherapy could differ between patients with different prognoses (that is, patients in different risk groups). Experts on the Committee pointed to data from recent meta-analyses that showed proportional gains from chemotherapy were generally constant across clinical parameters such as tumour diameter and ER status (used to help determine a patient's prognosis). However, these constant proportional gains meant that those with a good prognosis would receive less absolute benefit from chemotherapy than those with a poor prognosis. Furthermore, the possibility that chemotherapy might be more effective both proportionally and absolutely in patients identified by Oncotype DX, given that the test provides information about the biological features of the tumour, was discussed. The possibility that tumours with the genomic characteristics identified by Oncotype DX might be more susceptible to chemotherapy was also explored. The evidence on the predicted benefit of chemotherapy (reduction in the risk of distant recurrence) for women receiving chemotherapy in addition to endocrine therapy compared with endocrine therapy alone was discussed. The Committee heard that data were available that suggest that Oncotype DX can predict the relative benefit of chemotherapy and that the effectiveness of chemotherapy varies depending on the classification of patients by the Oncotype DX test in LN− patients (Paik et al. 2006). These data indicated that women in lower risk groups benefit proportionally less from chemotherapy than those in higher risk groups (see section 5.14). The Committee considered that the Paik study was limited by its design, the sample sizes of individual risk groups, the use of some results from the training dataset (tamoxifen-treated patients of the NSABP B-20 trial) in the study dataset, the applicability of the study population (a younger population that includes patients with HER2+ breast cancer) to the population considered in this guidance, and the fact that the treatments (endocrine therapy and chemotherapy) used are different to those currently used in the NHS. In addition, the Committee considered that the relative benefit from chemotherapy by risk group was unclear. The Committee concluded that the evidence implying a predicted differential relative benefit of chemotherapy according to Oncotype DX risk group in LN− patients (Paik et al. 2006) was not robust. The Committee also reviewed evidence implying a predicted differential relative benefit of chemotherapy according to Oncotype DX risk group in LN+ patients (Albain et al. 2010) and data from the neoadjuvant setting. The

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Committee concluded that these data were not robust enough to support the test's ability to predict the benefit of chemotherapy. In the absence of robust data the Committee concluded that equal benefit of chemotherapy should be assumed across all Oncotype DX risk groups. Therefore, although the Committee considered that adequate evidence supported the prognostic ability of Oncotype DX (that is, its ability to predict the risk of distant recurrence, see section 6.4), it concluded that it was not confident in the ability of Oncotype DX to predict benefit from chemotherapy.

6.6 The Committee discussed the cost effectiveness of Oncotype DX based on the original price proposed by the manufacturer (list price). The Committee considered that the incremental cost-effectiveness ratios (ICERs) from the base-case analysis of Oncotype DX were not the most appropriate for decision-making purposes because of the assumption of a predicted differential relative benefit of chemotherapy according to Oncotype DX risk group. The Committee discussed the ICERs presented in the sensitivity analysis that assumed equal benefit of chemotherapy across all Oncotype DX risk groups (at a level of either 30% or 40% relative risk reduction from chemotherapy). The Committee noted the ICERs for Oncotype DX (compared with current practice) when offered to all women with ER+, LN−, HER2− early breast cancer were £91,300 (30% relative risk reduction from chemotherapy) per quality-adjusted life year (QALY) gained and £64,900 (40% relative risk reduction from chemotherapy) per QALY gained. The Committee considered the ICERs to be too high to recommend Oncotype DX for use in the NHS for all women with ER+, LN−, HER2− early breast cancer. The Committee considered that the overall benefit of chemotherapy was likely to be closer to 27% relative risk reduction from chemotherapy across all Oncotype DX risk groups (EBCTCG overviews 2005, 2011). The Committee noted that there are potential differences in the population included in the EBCTCG review compared with the population in the economic analysis and that the outcome measures differed. Without data specific to the population under consideration, the Committee considered the EBCTCG figure to be the most appropriate for use at this time. The Committee concluded that the most plausible ICER, based on the evidence presented, was likely to exceed £91,300 for all women with ER+, LN−, HER2- early breast cancer. Therefore, based on the original

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proposed price (list price), Oncotype DX would not be a cost-effective use of NHS resources in this group.

6.7 The Committee then considered a proposal submitted by the manufacturer of Oncotype DX. The proposal makes Oncotype DX available to the NHS at a revised price for those people assessed as being at intermediate risk. The proposed price is commercial in confidence. NICE advised the Committee, and the Committee agreed, that the access proposal appeared workable and efficient, and did not appear to constitute an excessive administrative burden on the NHS. The Committee went on to discuss the impact of the proposal on the cost effectiveness of Oncotype DX in people assessed as being at intermediate risk when it was assumed that Oncotype DX was able to predict a patient's prognosis but not the benefit of chemotherapy (relative risk reduction of distant recurrence from chemotherapy). The Committee accepted an analysis performed by the External Assessment Group, which showed that the ICER for Oncotype DX (compared with current practice) in this group of patients was £22,600 per QALY gained, assuming prognostic benefits of the test but no predictive effect. The Committee also noted the ICER could be significantly lower if Oncotype DX was shown to predict the benefit of chemotherapy by robust evidence from future research. The Committee noted that an NPI score above 3.4 was used in the analysis as a mechanism for identifying patients at intermediate risk, but also noted that other methods for determining the risk group were available and in use in the NHS. The Committee believed that the subgroup analysis of people with an NPI score above 3.4 was likely to be a reasonable approximation for people at intermediate risk generally. Therefore, given the strength of the evidence on the prognostic ability of the test (and evidence of analytical validity), the Committee concluded that Oncotype DX for use in people at intermediate risk of distant recurrence, when the decision to prescribe chemotherapy remains unclear, would represent a cost-effective use of NHS resources if acquired at the confidential revised price offered by the manufacturer.

6.8 The Committee discussed the need for further robust evidence to demonstrate the ability of Oncotype DX to identify patients who will benefit most from chemotherapy (see section 6.5). The Committee considered that further information on the clinical utility of the test is warranted. This should comprise

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the development of robust evidence on the impact of Oncotype DX on clinical decision-making in England. The Committee noted that the Oncotype DX score may be combined with existing clinicopathological variables used informally by physicians at the local level, or more formally using a pre-specified algorithm. These 2 approaches should be kept in mind for any future research on the impact of Oncotype DX on clinical decision-making in England. The Committee noted that a decision-impact study in Bristol is near completion. Research should also address the ability of the test to predict the benefit of chemotherapy. The Committee noted there is an ongoing prospective trial (TAILORx) that will provide further information on the benefit of chemotherapy in women classified as intermediate risk by Oncotype DX. As the patient population included in TAILORx is from North America, the Committee encouraged the collection of data on Oncotype DX when used in the NHS in England (see section 7). The Committee was mindful that the extra value of the tests when used in addition to current clinical practice has been shown by the model constructed by the External Assessment Group, and that the use of Oncotype DX in the NHS in England represents an opportunity to collect further data on this. The Committee concluded that multicentre audit should be a priority for further investigation.

6.9 The Committee discussed the evidence available on the analytical validity of IHC4. It noted the test was at a comparatively early stage of development. In particular, it was noted that although there are data on the reliability and reproducibility of the measurement of ER, progesterone receptor (PR) and HER2 markers, data were lacking on the reliability and reproducibility of the Ki-67 marker measurement. The Committee heard that ER, PR and HER2 have an established UK National External Quality Assessment Scheme (NEQAS), and that a study was published recently on the reproducibility of Ki-67 and a UK NEQAS was being investigated for the marker. The Committee noted however that quantitative assessments of ER, PR and Ki-67 (not routinely reported in the NHS) should be appropriately considered in the NEQAS if not already done so. The Committee considered that data are needed on the reproducibility and reliability (analytical validity) of the complete IHC4 test (an algorithm combining 4 markers and classical clinical and pathological variables). This is particularly important as the test is designed for local processing in NHS laboratories. An additional study on quality assurance

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was also considered by the Committee. This was a small preliminary study that did not materially change the results of the External Assessment Group analysis. The Committee concluded that the lack of data on analytical reliability meant that it was not possible to make a recommendation for general use of the IHC4 test at this time.

6.10 The Committee then discussed the clinical validity and clinical utility of IHC4. It noted that only 1 study was available on the clinical validity of the test. The Committee discussed the separate cohort of 786 patients used for external validation of the test in this study and concluded that it was not fully representative of the population of interest in this assessment because approximately 50% of patients did not receive 5 years of endocrine therapy. The Committee also noted that the External Assessment Group review did not identify any data on the clinical utility of IHC4. An additional study on how the test classifies patients by risk group compared with the NPI and Adjuvant! Online was also considered by the Committee. Although encouraging, this was a small preliminary study that did not provide an indication of how management decisions would actually change and did not materially change the results of the External Assessment Group analysis. The Committee also noted the recent availability of further data on IHC4 in the large TEAM study. The Committee considered that the general uncertainty in the clinical effectiveness evidence for IHC4 limited the validity of the economic analysis. It concluded that robust data on how the test might be used in the NHS in England (a continuous risk score, defined risk groups or both) and the impact of the test on clinical decision-making are needed. The Committee also indicated that data on the benefit of chemotherapy according to IHC4 score (or defined risk groups) would be useful. Although IHC4 was found to dominate current practice in the base-case economic analysis when offered to all women with ER+, LN−, HER2−early breast cancer and in a subgroup of women with an NPI score above 3.4, in addition to most of the sensitivity analyses, the Committee concluded that the uncertainty in the estimates of the clinical effectiveness of the test was too great to recommend adoption at this time. The Committee considered that further evidence was needed before the test could be adopted for general use by the NHS. Given the estimated low cost of the test and the modelling results that showed it has the potential to dominate current practice, the Committee considered it prudent to recommend the use of

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IHC4 for research in the NHS to collect information on the analytical validity, and hence, clinical validity and clinical utility of the test (see section 7).

6.11 The Committee discussed the clinical evidence and the uncertainty in the estimates of the cost effectiveness of Mammostrat (because of uncertainty in the clinical evidence underpinning the economic analysis). The Committee noted there were limited published data on the analytical validity of the test. It went on to discuss the clinical validity of the test, and considered the results of the economic analysis to be limited because the risk reclassification data (provided in confidence) derived from a small subset of women included in the study by Ring et al. (2006; US cohort) demonstrated some inconsistencies and were not sufficiently robust in demonstrating the ability of the test to predict which women were at low, intermediate or high risk in the subgroup of women with an NPI score above 3.4. The Committee noted the recent availability of data on the clinical validity of Mammostrat in the TEAM study. The Committee considered that this study provides additional supportive data for a large UK population on the prognostic ability of the test and expands the evidence base to patients treated with aromatase inhibitors, rather than tamoxifen. Although further supportive data are available on the clinical validity of the test, the Committee considered that the economic analysis was also limited because there was uncertainty about the clinical utility of the test; in particular no evidence exists on how the test would affect clinical decision-making in England, and because of the discordant results (Ross et al. 2008) on the benefit of chemotherapy (only the low- and high-risk groups benefitted from chemotherapy but not the intermediate-risk group). Overall, although a limited number of studies have been conducted, the Committee was encouraged by the large sample sizes of the studies showing that Mammostrat can act as an independent prognostic tool. In particular, the Committee noted that a significant portion of the prognostic evidence was generated using UK-based patients. However, the Committee felt that to fully understand the benefits of the test, further data are needed to demonstrate how the test reclassifies people's risk when compared with current practice in England, and to demonstrate the impact of Mammostrat on clinical decision-making in England. The Committee considered that the uncertainty in the clinical-effectiveness evidence for Mammostrat limited the validity of the economic analysis. Therefore, given the uncertainty in the clinical effectiveness of the test (in

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particular, the analytical validity and clinical utility), the Committee was unable to recommend the adoption of Mammostrat for general use in the NHS at this time and recommended the test for research use only. The Committee heard that there is an extensive ongoing research programme for this relatively new test.

6.12 The Committee discussed the clinical evidence and the uncertainty in the estimates of cost effectiveness of MammaPrint. The Committee noted that although the MammaPrint test was created using samples from an untreated breast cancer population, in particular with samples from patients who had not received endocrine therapy, data were available on the use of the test in patients treated with adjuvant endocrine therapy (for example, Kok et al. 2010). The Committee was not aware of evidence on the use of MammaPrint in UK clinical practice. The Committee noted that no new evidence was identified in the External Assessment Group's review on the analytical validity of the test, but that evidence had been identified in the previous systematic review by Marchionni et al. (2008). The Committee went on to consider the different sample types used by the test. The Committee was aware that the use of MammaPrint on formalin-fixed paraffin-embedded samples has been CE marked and that the manufacturer had submitted data to the Food and Drug Administration to demonstrate that the performance of MammaPrint in formalin-fixed paraffin-embedded samples is equivalent to that of fresh samples. The Committee discussed the clinical validity of the test and agreed with the External Assessment group that such evidence, although developing, is based on cohort studies of small sample sizes that have been conducted outside of England in a heterogeneous population of predominantly younger pre-menopausal women (younger women are more likely to be classified as having a poor prognosis using MammaPrint, which may overestimate the benefit of the test in the early breast cancer population as a whole). The Committee discussed the clinical utility of the test and noted that the risk reclassification data and the proportion of patients receiving chemotherapy were taken from studies of Dutch patients that included predominantly pre-menopausal women. Furthermore, the Committee agreed with the External Assessment Group that the impact of the test on decision-making in England had not been demonstrated and that the Knauer et al. study (2010) had considerable methodological limitations (see section 5.12), and therefore the

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Committee considered that the clinical utility of MammaPrint had not been robustly demonstrated. The Committee considered that the uncertainty in the clinical-effectiveness evidence for MammaPrint limited the validity of the economic analysis.

6.13 The Committee also considered additional evidence on MammaPrint forwarded by the manufacturer, including the RASTER study (an updated analysis by Drukker et al. 2013), the IMPAKT 2012 working group statement and a summary of cost-effectiveness results based on NPI scores from 2 patient series data. However, the Committee agreed with the External Assessment Group, which concluded that this evidence did not materially change the results of the analysis. The Committee noted that the RASTER study provided prospective data on the additional prognostic value of MammaPrint when compared with Adjuvant! Online, in the form of an observational study of over 400 patients. However, the Committee considered that this study did not substantially reduce the uncertainty in clinical effectiveness because RASTER, similarly to other studies of MammaPrint, was conducted in the Netherlands in a younger population than that seen in England, with most patients younger than 55 years. The Committee also noted that treatment decisions were based on a range of factors, including the Dutch Institute for Healthcare Improvement (CBO) guidelines that are not used in England. In addition, the RASTER study included some patients with ER− (20%) or HER2+ (11% positive and 5% unknown) breast cancer. Using MammaPrint, these patients would be very likely to be categorised as having a poor prognosis and receive chemotherapy, which may lead to an overestimation of the benefit of the test in the population considered in this evaluation (ER+, LN− and HER2−). The Committee also considered the consensus statement by the IMPAKT 2012 working group and noted that it found the available evidence on the analytical validity and clinical validity of MammaPrint to be convincing. The Committee noted that the consensus statement did not summarise any new evidence not already included in the External Assessment Group's report. The Committee considered that the data on the clinical validity of the test were not generalisable to the population considered in this evaluation for the reasons already stated (that is, the data were from cohort studies of small sample sizes conducted outside England in a heterogeneous population of predominantly younger pre-menopausal women).

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The Committee agreed with the External Assessment Group that the cost-effectiveness results based on NPI scores from 2 patient series lacked detailed descriptions of methodology, and so did not allow a clear assessment of the quality of the evaluation. Therefore, the Committee considered that the uncertainty in the clinical effectiveness data remained, and limited the validity of any economic analysis despite the additional evidence.

6.14 The Committee concluded that the uncertainty in the clinical effectiveness, in particular the clinical validity and clinical utility of the test, was too high to recommend the adoption of MammaPrint for general use in the NHS at this time and recommended the test for research only. The Committee noted that there is an ongoing prospective clinical trial (MINDACT) on the value of MammaPrint in predicting which patients would benefit from chemotherapy and that results from this trial may help to reduce the uncertainty about effectiveness.

6.15 The Committee expressed general concern over the lack of information on the impact of the use of gene expression profiling and expanded immunohistochemistry tests on clinical decision-making in England. It noted that some limited data on clinical decision-making in England were available for Oncotype DX, which were helpful in informing the assessment. The Committee requested further data on the ability of the tests to impact clinical decision-making. The applicable data forwarded by the manufacturers came from studies that were conducted outside England. The Committee agreed with the External Assessment Group that a lower baseline level of chemotherapy prescribing in England than in the USA or many other European countries increases the uncertainty in the generalisability of studies conducted outside England.

6.16 A potential equality issue was raised by the Committee, which was concerned about the lack of evidence on the use of the tests in women older than 75 years. The Committee accepted the evidence had been limited to women younger than 75 years; however, the recommendations in section 1 do not restrict access to the tests based on age of the patient. The Committee also discussed potential equality issues concerning the use of the new tests in men. The Committee heard that given the relatively low number of men with breast

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cancer when compared with women, evidence on the performance of these tests in men was less likely to be generated. Experts on the Committee pointed out that breast cancer in men shared many characteristics with that seen in women and that both groups were treated similarly in clinical practice (see section 6.3). Therefore, the Committee felt it appropriate that the recommendations apply to both men and women.

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7 Recommendations for further research

7.1 The following research is recommended in the context of people with oestrogen receptor positive (ER+), lymph node negative (LN−) or human epidermal growth factor receptor 2 negative (HER2−) early breast cancer.

7.2 **MammaPrint:** research is recommended on the clinical validity of the test in people that are representative of the population in England. In particular, information on how the test reclassifies people when compared with current practice in England and their risk of distant recurrence would be useful. Research into the clinical utility of the test is also recommended; in particular, evidence of the impact of the test on clinical decision-making in England and robust data on its ability to predict the benefit of chemotherapy.

7.3 **Oncotype DX:** research is recommended on the clinical utility of the test, including robust evidence on the impact of Oncotype DX on clinical decision-making in England (containing consideration of informal approaches compared with a formal algorithm for combining the Oncotype DX score with clinicopathological variables) and its ability to predict the benefit of chemotherapy. As part of the adoption of Oncotype DX by the NHS, the Committee encourages the collection of clinical utility and any other useful data by the health system, potentially by a multicentre audit.

7.4 The Committee noted that the MINDACT study is being conducted on MammaPrint and the TAILORx study is being conducted on Oncotype DX. The Committee encourages the availability of data showing the risk of distant recurrence of patients in these trials using tools representative of current practice adopted by the NHS (for example, Nottingham Prognostic Index [NPI] and Adjuvant! Online). Researchers should be mindful of the evolving breast cancer practice in England. For example, the emergence of the PREDICT tool; although a new tool at present, this may be more widely used in the future.

7.5 **IHC4:** research into the analytical validity (reliability and reproducibility) of the complete IHC4 test is recommended (an algorithm combining 4 markers and classical clinical and pathological variables), particularly within the NHS and when performed in local laboratories. Studies to confirm the prognostic ability

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and to determine the impact of IHC4 on clinical decision-making in England and, ideally, to predict the benefit of chemotherapy are recommended.

7.6 **Mammostrat:** research on the analytical validity (reliability and reproducibility) of the test is recommended. Research on the clinical utility of the test is also recommended. In particular, evidence of how the test reclassifies people's risk when compared with current practice in England, evidence on the impact of Mammostrat on clinical decision-making in England, and its ability to predict the benefit of chemotherapy.

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8 Implementation

8.1 NICE will support this guidance with a range of activities to promote the recommendations for further research. This will include incorporating the research recommendations in section 7 into the NICE guidance research recommendations database (available on the NICE website at www.nice.org.uk) and highlighting these recommendations to public research bodies. The research proposed will also be put forward to NICE's Medical Technologies Evaluation Programme research facilitation team for consideration of the development of specific research protocols.

8.2 The manufacturer has offered Oncotype DX to the NHS under a proposal (December 2012) that makes Oncotype DX available to the NHS at a revised price. The proposal price is commercial-in-confidence. It is the responsibility of the manufacturer to communicate details of the proposal to the relevant NHS organisations. Any enquiries from NHS organisations about the access proposal should be directed to Genomic Health UK at NHSOncotype@genomichealth.com or 020 3031 8087.

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9 Related NICE guidance

See www.nice.org.uk for related guidance.

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10 Review

NICE updates the literature search at least every 3 years to ensure that relevant new evidence is identified. NICE will contact product sponsors and other stakeholders about issues that may affect the value of the diagnostic technologies. NICE may review and update diagnostics guidance at any time if significant new evidence becomes available.

Andrew Dillon
Chief Executive
September 2013

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11 Diagnostics Advisory Committee members and NICE project team

Diagnostics Advisory Committee

The Diagnostics Advisory Committee is an independent committee consisting of 22 standing members and additional specialist members. During this assessment the membership of the Diagnostics Advisory Committee changed as some members reached the end of their terms and others were appointed in their place. A full list of the Committee members who participated in this assessment appears below.

Standing Committee members

Dr Trevor Cole
Consultant Clinical and Cancer Geneticist, Birmingham Women's Hospital

Professor Paul Collinson
Consultant Chemical Pathologist and Professor of Cardiovascular Biomarkers, St George's Hospital

Dr Sue Crawford
General Practitioner (GP) Principal, Chillington Health Centre

Professor Ian Cree
Senior Clinical Advisor, National Institute for Health Research (NIHR) Evaluation Trials and Studies Coordinating Centre, University of Southampton

Professor Erika Denton
National Clinical Director for Imaging, Department of Health, Honorary Professor of Radiology, University of East Anglia and Norfolk and Norwich University Hospital

Dr Steve Edwards
Head of Health Technology Assessment, British Medical Journal (BMJ) Evidence Centre

Mr David Evans
Lay member

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Dr Simon Fleming
Consultant in Clinical Biochemistry and Metabolic Medicine, Royal Cornwall Hospital

Professor Lisa Hall
Professor of Analytical Biotechnology, University of Cambridge

Professor Chris Hyde
Professor of Public Health and Clinical Epidemiology, Peninsula Technology Assessment Group (PenTAG)

Professor Noor Kalsheker
Professor of Clinical Chemistry, University of Nottingham

Dr Gail Norbury
Consultant Clinical Scientist, Guy's and St Thomas' NHS Foundation Trust

Dr Mark Kroese (Vice Chair)
Diagnostics Advisory Committee and Consultant in Public Health Medicine, PHG Foundation, Cambridge and UK Genetic Testing Network

Dr Peter Naylor
General Practitioner (GP), Chair Wirral Health Commissioning Consortia

Professor Adrian Newland (Chair)
Diagnostics Advisory Committee

Dr Richard Nicholas
Consultant Neurologist, Honorary Senior Lecturer, Heatherwood and Wexham Park Hospitals

Ms Margaret Ogden
Lay member

Mr Stuart Saw
Director of Finance, North East London and the City primary care trusts

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Professor Mark Sculpher
Professor of Health Economics at the Centre for Health Economics, University of York

Dr Steve Thomas
Consultant Vascular and Cardiac Radiologist at Sheffield Teaching Hospitals Foundation Trust

Mr Paul Weinberger
Chief Executive Officer (CEO), Diasolve Ltd, London

Mr Christopher Wiltsher
Lay representative

Specialist Committee members

Professor Anthony Howell
Director of the Breakthrough Breast Cancer Research

Mr Simon Pain
Consultant Breast and Endocrine Surgeon, Department of General Surgery, Norfolk and Norwich University Hospital

Mrs Ursula Van Mann
Lay member

Mrs Carole Farrell
Nurse Clinician, The Christie NHS Foundation Trust

Professor Louise Jones
Consultant Clinical Scientist, Barts and the London NHS Trust

NICE project team

Each diagnostics assessment is assigned to a team consisting of a Technical Analyst (who acts as the topic lead), Technical Advisers and Project Managers.

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Gurleen Jhuti
Topic Lead

Hanan Bell and Pall Jonsson
Technical Advisers

Jackson Lynn and Robert Fernley
Project Managers

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12 Sources of evidence considered by the Committee

The diagnostics assessment report was prepared by the School of Health and Related Research (ScHARR).

- Ward S, Scope A, Rachid R et al. Gene expression profiling and expanded immunohistochemistry tests to guide the use of adjuvant chemotherapy in breast cancer management, October 2011.

Registered stakeholders

The following organisations accepted the invitation to participate in this assessment as stakeholders. They were invited to attend the scoping workshop and to comment on the diagnostics assessment report and the diagnostics consultation document.

Manufacturers/sponsors:

The technologies under consideration

- Agendia Bvd (MammaPrint)
- Clarient (Mammostrat)
- Genomic Health (Oncotype DX)
- Royal Marsden Hospital and Queen Mary University London – academic sponsor (IHC4)

Comparator technologies

- None

Other

- ARUP Laboratories
- Ipsogen
- Nottingham University
- Randox Laboratories

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- Roche Diagnostics

Professional/specialist and patient/carer groups:

- BHR University NHS Trust
- Breakthrough Breast Cancer
- Bupa
- Cambridge University Hospital
- Cherry Lodge Cancer Care
- Mersey and Cheshire Cancer Network
- Royal College of Physicians
- Southport and Ormskirk Hospital NHS Trust
- The Royal College of Pathologists
- University College London Hospitals NHS Foundation Trust
- University Hospital of South Manchester

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About this guidance

NICE diagnostics technologies guidance is designed to help the NHS adopt efficient and cost-effective medical diagnostic technologies more rapidly and consistently.

The programme concentrates on pathological tests, imaging, endoscopy and physiological measurement, since these represent most of the investigations performed on patients. The types of products that might be included are medical diagnostic technologies that give greater independence to patients, and diagnostic devices or tests used to detect or monitor medical conditions. Diagnostic technologies may be used for various purposes: diagnosis, clinical monitoring, screening, treatment triage, assessing stages of disease progression, and risk stratification.

This guidance was developed using the NICE diagnostic technologies guidance process.

We have produced a summary for patients and carers. Tools to help you put the guidance into practice and information about the evidence it is based on are also available.

Your responsibility
This guidance represents the view of NICE, which was arrived at after careful consideration of the evidence available. Healthcare professionals are expected to take it fully into account when exercising their clinical judgement. However, the guidance does not override the individual responsibility of healthcare professionals to make decisions appropriate to the circumstances of the individual patient, in consultation with the patient and/or guardian or carer.

Implementation of this guidance is the responsibility of local commissioners and/or providers. Commissioners and providers are reminded that it is their responsibility to implement the guidance, in their local context, in light of their duties to have due regard to the need to eliminate unlawful discrimination, advance equality of opportunity, and foster good relations. Nothing in this guidance should be interpreted in a way which would be inconsistent with compliance with those duties.

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2015 Genetic Testing CPT Codes Recommendations

Date: 10/16/14

		Recommendation		Impact of detecting a mutation				Limitations & Comments
CPT Code	Descriptor	Cover	Don't Cover	Change treatment	Change health monitoring	Provide Prognosis	Provide genetic counseling	
81410	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis panel, must include sequencing of at least 9 genes, including FBN1, TGFBR1, TGFBR2, COL3A1,	X		X	X	X	X	
81411	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); duplication/deletion analysis panel, must include analyses for TGFBR1, TGFBR2, MYH11, and COL3A1	X		X	X	X	X	
81415	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis	X		X		X	X	
81416	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)	X		X		X	X	
81417	Exome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)		X					
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis		X					

		Recommendation		Impact of detecting a mutation				Limitations & Comments
CPT Code	**Descriptor**	**Cover**	**Don't Cover**	**Change treatment**	**Change health monitoring**	**Provide Prognosis**	**Provide genetic counseling**	
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)		X					
81427	Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)		X					
81430	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3	X		X	X	X	X	
81431	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes	X		X	X	X	X	
81435	Hereditary colon cancer syndromes (eg, Lynch syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include analysis of at least 7 genes, including APC, CHEK2, MLH1, MSH2, MSH6, MUTYH, and PMS2	X			X			
81436	Hereditary colon cancer syndromes (eg, Lynch syndrome, familial adenomatosis polyposis); duplication/deletion gene analysis panel, must include analysis of at least 8 genes, including APC, MLH1, MSH2, MSH6, PMS2, EPCAM, CHEK2, and MUTYH	X			X			

2015 Genetic Testing Codes
Non-Prenatal

		Recommendation		Impact of detecting a mutation				Limitations & Comments
CPT Code	**Descriptor**	**Cover**	**Don't Cover**	**Change treatment**	**Change health monitoring**	**Provide Prognosis**	**Provide genetic counseling**	
81440	Nuclear encoded mitochondrial genes (eg, neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including BCS1L, C10orf2, COQ2, COX10, DGUOK, MPV17, OPA1, PDSS2, POLG, POLG2, RRM2B, SCO1, SCO2, SLC25A4, S	X		X	X	X	X	
81460	Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary op	X		X	X	X	X	
81465	Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed	X		X	X	X	X	
81470	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL,		X					
81471	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL,		X					

2015 Genetic Testing CPT code review

81410-81411

1) Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis pane
2) Used for diagnosing both symptomatic patients and asymptomatic family members
3) **American College of Medical Genetics (2012)–**evaluation of a patient with possible Marfan's Syndrome
    a. Consider *FBN1* gene sequencing
    b. Searching for mutations in *FBN1*continues to have a circumscribed role in the diagnosis of equivocal cases. Said differently, MFS remains, by and large, a clinical diagnosis.
    c. Does not address screening asymptomatic family members
4) **American College of Cardiology/American Heart Association (2010)** guidelines for diagnosis and management of patients with thoracic aortic disease
    a. Test for genes associated with aortic aneurysm and/or dissection in patients and family members (*FBN1, TGFBR1, TGFBR2,COL3A1, ACTA2, MYH11*)
    b. Use test results to limit aortic imaging to relatives with the genetic mutation. *(LOE: C)*

1) **Canadian Cardiovascular Society (2014)** opinion
    a. We recommend clinical and genetic screening for suspected Marfan syndrome to clarify the nature of the disease and provide a basis for individual counselling (Strong Recommendation, High-Quality Evidence).
    b. Affects monitoring—recommend echocardiographic screening at diagnosis to measure aortic root and ascending aorta diameters, and repeated 6 months thereafter to determine rate of progression. If aortic diameters remain stable, annual imaging is recommended. (Strong Recommendation, High-Quality Evidence).
2) Recommendation:
    a. **Diagnostic** List

81415-81417

1) Exome testing
2) The human exome comprises only 1–2% of the whole genome, it is this portion that codes for proteins, and was thought to contain 85% of all pathogenic mutations causing Mendelian disorders. The exome can be sequenced in only 48 h at roughly a third of the cost, with far fewer data to store and analyse compared to whole genome testing
3) GAP comments:

   a. May find other genetic mutations that do not address the specific reason for the tests (i.e. BRCA1 mutation, etc.); therefore, need careful genetic counseling prior to testing
   b. Used when there are multiple anomalies in a child, or when other specific testing has not found a diagnosis. 20-30% chance of finding a genetic cause for a syndrome or developmental delay in a population of children who already had non-revealing testing
   c. Cost is about $5000 for a test, may be cost effective rather than doing a series of single gene tests
   d. Recommended change to non-prenatal genetic testing guideline: "A genetic counseling/geneticist visit is required prior to ordering test."
   e. From Cary Harding: Regarding 81417: If any validation of an exome result is necessary, that should be done by another method rather than repeating an exome analysis

4) OHP medical director feedback:
   a. My main concerns are these: it appears that there is a great likelihood of identifying lots of "noise" mutations—things that may or may not have anything to do with the actual problem at hand…many [of the] genetic syndromes don't have any specific treatments that improve their outcome, so it might not alter treatment. It is possible that health monitoring might be changed, because we are basically screening for any genetic mutation. Depending on whether a specific diagnosis is reached, prognosis may change; further testing of parents would probably be needed to answer questions regarding family risk. So an argument could be made that it meets guideline D1, although it really is not clear.
   b. I would need CLEAR documentation how the genetic consultant believes the testing will treat the member. I do get provider feedback with a confirmed diagnosis would allow health plan benefits for the member otherwise not received and this is a majority of our current requests. Our CCO rarely approves this request on the basis it appears to be an investigation and is not assisting on a treatment plan.
   c. Until mutations are better known and we know which ones truly would materially benefit treatment/outcome, I concur testing would be investigational and hence not be covered by OHP plan. There also is the ethics concern raised…of uncovering incidental mutations that may be alarming to members and/or their blood relatives with uncertainty in what it means and whether any prophylactic treatment is indicated. I recommend testing be focused based on strong scientific findings for which recommendations include well-reasoned consideration risks vs. benefits.
5) Other expert input (Alison Adams Martinez, Ph.D**.,** Geneticist Oklahoma Health Care Authority)
   a. The published policies of most private payers indicate that they do not cover WES. However, once you get past testing more than 2-3 whole genes, it actually becomes more cost effective to do the WES up front and prevent the parade of single-gene test requests. So, I felt that the literature/other policies would support

us if we wanted to take a hardline stance against covering WES, but at the same time we could justify covering it in cases where the member's clinical features would make them eligible for genetic testing of multiple genes…we agreed that we would reimburse for WES in select cases…[but] if we got too many claims that we would have to revisit the decision and not cover it at all. So far, we have paid for maybe 10 WES claims over the past year. In all cases, they met the criteria outlined by the ACMG. We've paid between $5-7K per WES claim

6) Evidence
    - **a. TEC 2013**
        - i. Has an algorithm indicating when testing may be indicated
        - ii. Diagnostic yield 10-50%
        - iii. Concern for many mutations found that have no or unknown clinical significance or are unrelated to the reason for testing
        - iv. Medical management decisions, including initiation of new treatment or discontinuing inappropriate treatment, may result for only a subset of those diagnosed.
        - v. Reproductive decisions for parents considering an additional pregnancy may be informed by determining the mode of inheritance.
        - vi. Appropriate use of exome sequencing requires considerable genetic, clinical, and genetic counseling expertise.
7) Other coverage policies
    - **a. Aetna 2014**
        - i. Exome testing is experimental
    - **b. Anthem BCBS 2014**
        - i. Diagnostic genetic testing using panels of genes (with or without next generation sequencing), including but not limited to whole genome and whole exome sequencing, is considered **investigational and not medically necessary** unless all components of the panel have been determined to be medically necessary based on the criteria above. .
8) Recommendations:
    - a. 81415, 81416 (whole exome testing, sequence analysis and comparator genome sequencing): **Diagnostic List**
    - b. 81417 (re-evaluation): **Non-covered List**
        - i. Note: exclusion of 81417 was based on expert testimony that was not reviewed by the entire GAP.
    - c. See changes to the non-prenatal genetic testing guideline
        - i. Requirement for genetic consultation prior to covering test (81415, 81416)
        - ii. Statement that 81417 is non-covered

81425-81427

1) Genome testing
2) Sequencing of an entire human genome to test for genetic mutations. The human genome can now be sequenced in 2 weeks for approximately £4000 (€4700, US$6200) in many laboratories.

3) GAP comments: "not ready for clinical prime time"; "premature for use in clinical practice"; "need for data/experience." Decision was to delay coverage until more research has been done to clarify the role of these tests in clinical practice
4) Recommendation: **Non-covered List**
    a. Notation made in the non-prenatal genetic testing guideline

81430-81431

1) 81430: Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3
2) 81431: Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes
3) Similar code placement:
    a. GJB2 and GJB6 gene analysis assays for nonsyndromic hearing loss are on the diagnostic list (81252, 81253)
4) **American College of Medical Genetics 2014,** guideline for evaluation of hearing loss
    a. If nonsyndromic hearing loss is suspected, consider single-gene tests such as *GJB2* and *GJB6*, gene panel tests, or NGS testing based on history and findings
5) GAP comments: Start with the two most likely tests, if normal, use panels. GJB2 and 6 tests find about 50% of genetic problems. GJB2/6 testing costs $500, panels $3-5,000
    a. Recommend adding clause to guideline: GJB2 and GJB6 need to be tested first and be negative in non-syndromic patients prior to panel being covered
6) Recommendation—**Diagnostic List**
    a. See recommended changes to the non-prenatal genetic testing guideline

81435, 81436

1) 81435-6: Hereditary colon cancer syndromes (eg, Lynch syndrome, familial adenomatosis polyposis); duplication/deletion gene analysis panel, must include analysis of at least 7 genes, including APC, CHEK2, MLH1, MSH2, MSH6, MUTYH, and PMS2
2) Similar Prioritized List placements:
    a. Testing for Lynch syndrome (hereditary nonpolyposis colon cancer) (CPT 81292-81300) with a guideline
        i. MLH1, MSH2, MSH6 individual gene tests (81292-81300)
            1. Current MLH1 tests include full sequence analysis, known familial variants, and duplication/deletion variants (81292-81294)
        ii. PMS2 individual gene tests (81317-81319)

3) **National Comprehensive Cancer Network (NCCN) 2014** screening guideline for colon cancer
    a. Recommends screening for MLH1, MSH2, MSH6, FMS2 and EPCAM for high risk family members
    b. Test results change screening frequency and age to begin screening for affected persons
4) Recommendation: **Diagnostic** List
    a. See recommended changes to the Non-Prenatal Genetic Testing Guideline

81440, 81460, 81465

1) Mitochondrial genome testing
2) Mutations of mitochondrial DNA can lead to a number of illnesses including exercise intolerance and Kearns-Sayre syndrome (KSS). Some evidence suggests that they might be major contributors to the aging process and age-associated pathologies. Recently a mutation in mtDNA has been used to help diagnose prostate cancer in patients with negative prostate biopsy.
3) Evidence
    a. **Falk 2010**, review of mitochondrial disease
        i. Recommends whole mitochondrial DNA genome sequencing when a strong suspicion exists based on the symptom constellation and family history.
        ii. Panel testing of approximately a dozen "common" mtDNA point mutations that cause well-characterized disorders…has historical significance but current utility primarily in adults. Indeed, the high cost of such limited point mutation panel testing currently approximates that of sequencing the entire 16,569 basepair mitochondrial DNA genome…it is typically more cost-efficient and ultimately informative to complete whole mitochondrial DNA genome sequence analysis to evaluate individuals (particularly children) with strong maternal pedigrees
        iii. It is important to realize that mtDNA may harbor other genetic abnormalities that are not detectable by whole genome sequence analysis.
4) GAP comments: "May avoid muscle biopsy or other diagnostic studies." Dr. Cary Harding, a metabolic specialist, thought this test was appropriate in certain clinical situations. He recommended putting in a guideline requiring a genetics or metabolic consult prior to ordering the test.
5) Recommendation: **Diagnostic List**
    a. See recommended changes to the Non-Prenatal Genetic Testing Guideline
        i. Requires genetic or metabolic consult prior to ordering test

81470, 81471

1) X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel

2) Refers to forms of intellectual disability which are specifically associated with X-linked recessive inheritance. As with most X-linked disorders, males are more heavily affected than females. Females with one affected X chromosome and one normal X chromosome tend to have milder symptoms. Unlike many other types of intellectual disability, the genetics of these conditions are relatively well understood. It has been estimated there are ~200 genes involved in this syndrome; of these ~100 have been identified. X-linked intellectual disability accounts for ~16% of all cases of intellectual disability in males.
3) The panel testing would include multiple of these known genes
4) Similar code coverage:
    a. CPT 81243, 81244 (Fragile X genetic testing) is covered with the following notation in the genetic testing guideline:
        i. "for individuals with intellectual disability/developmental delay. Although the yield of Fragile X is 3.5-10%, this is included because of additional reproductive implications."
5) GAC input: do not cover. The labs consulted on this question report a pick up rate for significant mutations is quite low, <5%
6) Recommendation: **Non-covered List**
    a. Notation made in the non-prenatal genetic testing guideline

GAC Recommended Changes to the Diagnostic Guideline D1, Non-Prenatal Genetic Testing Guideline
October 16, 2014

Issues:

1) The Non-Prenatal Genetic Testing Algorithm has modifications suggested by the Genetics Advisory Committee. These suggestions have arisen out of the review of the 2015 CPT codes regarding specific genetic tests.

Recommendations:

1) Adopt the amended Diagnostic Guideline 1 as below

DIAGNOSTIC GUIDELINE D1, NON-PRENATAL GENETIC TESTING GUIDELINE

Coverage of genetic testing in a non-prenatal setting shall be determined by the algorithm shown in Figure C.1 unless otherwise specified below.

A) Related to genetic testing for patients with breast/ovarian and colon/endometrial cancer or other related cancers suspected to be hereditary, or patients at increased risk to due to family history.
   1) Services are provided according to the Comprehensive Cancer Network Guidelines.
      a) Lynch syndrome (hereditary colorectal and endometrial cancer, and other
      a) cancers associated with Lynch syndrome) services (CPT 81288, 81292-81300, 81317-81319, 81435, 81436) and familial adenomatous polyposis (FAP) services (CPT 81201-81203) should be provided as defined by the NCCN Clinical Practice Guidelines in Oncology. ~~Colorectal Cancer Screening~~ Genetic/Familial High-Risk Assessment: Colorectal. V.2.2014 ~~V.1.2013~~ (~~5/13/13~~ 5/19/14). ***www.nccn.org***
      b) BRCA1/BRCA2 testing services (CPT 81211-81217) for women without a personal history of breast, ovarian and other associated cancers should be provided to high risk women as defined ~~in Guideline Note 3 or as otherwise defined~~ by the US Preventive Services Task Force or according to the NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast and Ovarian. V.2.2014 (9/23/14). ***www.nccn.org***..
      c) BRCA1/BRCA2 testing services (CPT 81211-81217) for women with a personal history of breast, ovarian and other associated cancers and for men with breast cancer should be provided according to the NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast and Ovarian. V.2.2014 (9/23/14) ~~V.1.2011 (4/7/11).~~ ***www.nccn.org***
      d) PTEN (Cowden syndrome) services (CPT 81321-81323) should be provided as defined by the NCCN Clinical Practice Guidelines in Oncology. Colorectal Screening. V.1.2013 (5/13/13). ***www.nccn.org.***
   2) Genetic counseling should precede genetic testing for hereditary cancer whenever possible.
      a) Pre and post-test genetic counseling should be covered when provided by a suitably trained health professional with expertise and experience in cancer genetics
         i) "Suitably trained" is defined as board certified or active candidate status from the American Board of Medical Genetics, American Board of Genetic Counseling, or Genetic Nursing Credentialing Commission.
      b) If timely pre-test genetic counseling is not possible for time-sensitive cases, appropriate genetic testing accompanied by pre- and post- test informed consent and post-test disclosure performed by a board-certified physician with experience in cancer genetics should be covered.
         i) Post-test genetic counseling should be performed as soon as is practical.
   3) If the mutation in the family is known, only the test for that mutation is covered. For example, if a mutation for BRCA 1 has been identified in a family, a single site mutation analysis for that mutation is covered (CPT 81215), while a full sequence BRCA 1 and 2 (CPT 81211) analyses is not. There is one exception, for individuals of Ashkenazi Jewish ancestry with a known mutation in the family, the panel for Ashkenazi Jewish BRCA mutations is covered (CPT 81212).
   4) Costs for rush genetic testing for hereditary breast/ovarian and colon/endometrial cancer is not covered.

B) Related to diagnostic evaluation of individuals with intellectual disability (defined as a full scale or verbal IQ < 70 in an individual > age 5), developmental delay (defined as a cognitive index <70

on a standardized test appropriate for children < 5 years of age), Autism Spectrum Disorder, or multiple congenital anomalies:

1) CPT 81228, Cytogenomic constitutional microarray analysis for copy number variants for chromosomal abnormalities: Cover for diagnostic evaluation of individuals with intellectual disability/developmental delay; multiple congenital anomalies; or, Autism Spectrum Disorder accompanied by at least one of the following: dysmorphic features including macro or microcephaly, congenital anomalies, or intellectual disability/developmental delay in addition to those required to diagnose Autism Spectrum Disorder. In 2012, this test may also be billed using one of CPT 88384-88386, or stacking CPTs 83890-83915.
2) CPT 81229, Cytogenomic constitutional microarray analysis for copy number variants for chromosomal abnormalities; plus cytogenetic constitutional microarray analysis for single nucleotide polymorphism (SNP) variants for chromosomal abnormalities: Cover for diagnostic evaluation of individuals with intellectual disability/developmental delay; multiple congenital anomalies; or, Autism Spectrum Disorder accompanied by at least one of the following: dysmorphic features including macro or microcephaly, congenital anomalies, or intellectual disability/developmental delay in addition to those required to diagnose Autism Spectrum Disorder; only if (a) consanguinity and recessive disease is suspected, or (b) uniparental disomy is suspected, or (c) another mechanism is suspected that is not detected by the copy number variant test alone. In 2012, this test may also be billed using one of CPT 88384-88386, or stacking CPTs 83890-83915.
3) CPT 81243, 81244, Fragile X genetic testing is covered for individuals with intellectual disability/developmental delay. Although the yield of Fragile X is 3.5-10%, this is included because of additional reproductive implications.
4) A visit with the appropriate specialist (often genetics, developmental pediatrics, or child neurology), including physical exam, medical history, and family history is covered. Physical exam, medical history, and family history by the appropriate specialist, prior to any genetic testing is often the most cost-effective strategy and is encouraged.

C) Related to other tests with specific CPT codes:
1) The following tests are not covered:
   a) CPT 81225, CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *5, *6)
   b) CPT 81226, CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *5, *6, *9, *10, *17, *19, *29, *35, *41, *1XN, *2XN, *4XN).
   c) CPT 81227, CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *5, *6)
   d) CPT 81287, MGMT (O-6-methylguanine-DNA methyltransferase) (eg, glioblastoma multiforme), methylation analysis
   e) CPT 81291, MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
   f) CPT 81330, SMPD1(sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)
   g) CPT 81350, UGT1A1 (UDP glucuronosyltransferase 1 family, polypeptide A1) (eg, irinotecan metabolism), gene analysis, common variants (eg, *28, *36, *37)
   h) CPT 81355, VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variants (eg, -1639/3673)
   i) CPT 81417, re-evaluation of whole exome sequencing
   j) CPT 81425-81427, Genome sequence analysis
   k) CPT 81470, 81471, X-linked intellectual disability (XLID) genomic sequence panels
   l) CPT 81504, Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
2) The following tests are covered only if they meet the criteria for the Non-Prenatal Genetic Testing Algorithm AND the specified situations:
   a) CPT 81205, BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (eg, Maple syrup urine disease) gene analysis, common variants (eg, R183P, G278S,

E422X): Cover only when the newborn screening test is abnormal and serum amino acids are normal

b) Diagnostic testing for cystic fibrosis (CF)
   i) CFTR, cystic fibrosis transmembrane conductance regulator tests. CPT 81220, 81223, 81222: For infants with a positive newborn screen for cystic fibrosis or who are symptomatic for cystic fibrosis, or for clients that have previously been diagnosed with cystic fibrosis but have not had genetic testing, CFTR gene analysis of a panel containing at least the mutations recommended by the American College of Medical Genetics* (CPT 81220) is covered. If two mutations are not identified, CFTR full gene sequencing (CPT 81223) is covered. If two mutations are still not identified, duplication/deletion testing (CPT 81222) is covered. These tests may be ordered as reflex testing on the same specimen.
c) Carrier testing for cystic fibrosis
   i) CFTR gene analysis of a panel containing at least the mutations recommended by the American College of Medical Genetics* (CPT 81220) is covered.
d) CPT 81240. F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant: Factor 2 20210G>A testing should not be covered for adults with idiopathic venous thromoboembolism; for asymptomatic family members of patients with venous thromboembolism and a Factor V Leiden or Prothrombin 20210G>A mutation; or for determining the etiology of recurrent fetal loss or placental abruption.
e) CPT 81241. F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant: Factor V Leiden testing should not be covered for: adults with idiopathic venous thromoboembolism; for asymptomatic family members of patients with venous thromboembolism and a Factor V Leiden or Prothrombin 20210G>A mutation; or for determining the etiology of recurrent fetal loss or placental abruption.
f) CPT 81256, HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D): Covered for diagnostic testing of patients with elevated transferrin saturation or ferritin levels. Covered for predictive testing ONLY when a first degree family member has treatable iron overload from HFE.
g) CPT 81332 SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antiproteinase, antitrypsin, member 1) (eg, alpha-1-antitrypsin deficiency), gene analysis, common variants (eg, *S and *Z): The alpha-1-antitrypsin protein level should be the first line test for a suspected diagnosis of AAT deficiency in symptomatic individuals with unexplained liver disease or obstructive lung disease that is not asthma or in a middle age individual with unexplained dyspnea. Generic testing or the anpha-1 phenotype test is appropriate is the protein test is abnormal or borderline. The genetic test is appropriate for siblings of people with AAT deficiency regardless of the AAT protein test results.
h) CPT 81415-81417, exome testing: A genetic counseling/geneticist consultation is required prior to ordering test
i) CPT 81430-81431, Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel: Testing for mutations in GJB2 and GJB6 need to be done first and be negative in non-syndromic patients prior to panel testing.
j) CPT 81440, 81460, 81465, mitochondrial genome testing: A genetic counseling/geneticist or metabolic consultation is required prior to ordering test.

3) Do not cover a more expensive genetic test (generally one with a wider scope or more detailed testing) if a cheaper (smaller scope) test is available and has, in this clinical context, a substantially similar sensitivity. For example, do not cover CFTR gene sequencing as the first test in a person of Northern European Caucasian ancestry because the gene panels are less expensive and provide substantially similar sensitivity in that context.

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ACMG PRACTICE GUIDELINES | Genetics inMedicine

Evaluation of the adolescent or adult with some features of Marfan syndrome

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Disclaimer: ACMG standards and guidelines are designed primarily as an educational resource for medical geneticists and other health care providers to help them provide quality medical genetic services. Adherence to these standards and guidelines does not necessarily ensure a successful medical outcome. These standards and guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the geneticists should apply their own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from these standards and guidelines.

Individuals who are suspected of having Marfan syndrome are often referred to a medical geneticist for further evaluation and diagnosis. However, there are a number of conditions that share physical manifestations with those of Marfan syndrome; therefore, an approach to diagnosis and evaluation is crucial to the proper long-term follow-up of these individuals. This practice guideline provides guidance for the approach to this cadre of individuals.

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Key Words: aortic aneurysm; aortic dissection; fibrillin; Loeys–Dietz syndrome; marfanoid

INTRODUCTION

A common reason for referral to cardiologists and medical geneticists is the tall, lanky individual who wants clearance for physical activities or who has vague complaints of discomfort in the back, chest, or joints. Obviously, concern for Marfan syndrome (MFS) is appropriate, but frequently the diagnosis cannot be established by the original "Ghent Criteria."[1] The specialist often wonders whether or how to label such an individual, whether further testing (such as computed tomography (CT) of the spine) or molecular genetic testing is necessary, and whether to recommend follow-up evaluation. New diagnostic criteria for MFS have been published recently.[2] A practice guideline that addresses these issues in the context of the new criteria will assist a variety of clinicians in dealing more appropriately with such patients and reduce instances of overly restricting people who do not have MFS or inappropriately reassuring those who do.

The following list of disorders (with OMIM numbers in parentheses) presents considerable diagnostic challenges because of shared features, overlapping phenotypes, similar inheritance patterns, and, at least for some, causation by mutations in the same gene, *FBN1*.

- MFS (154700)
- Ehlers–Danlos syndrome, hypermobile type (EDS) (130020)
- Familial thoracic aortic aneurysm and dissection (reviewed in 607086)
- Loeys–Dietz syndrome (LDS) and other disorders of TGFβ receptors (reviewed in 609192)
- Congenital contractural arachnodactyly (CCA) (121050)
- MASS phenotype (604308)
- Familial arterial tortuosity syndrome (208050)
- Familial mitral valve prolapse (MVP) (157700)
- Familial ectopia lentis (129600)
- Bicuspid aortic valve sequence (109730)
- Familial tall stature
- Familial pectus excavatum (169300)
- Familial scoliosis (181800)
- Stickler syndrome (108300)

Diagnostic criteria for these related disorders are based entirely on expert opinion, but rarely have groups of experts collaborated on refining their ideas.[1,3] The entire field suffers from the lack of any systematic attempt to apply rigorous methodology to categorization. The focus of what follows is the evaluation for the MFS. Comments about the other disorders are included as an appendix.

Medical geneticists, cardiologists, and sports medicine physicians, among others, are frequently consulted about tall, thin children and adolescents out of concern for MFS. The main reason that primary-care providers are interested in ruling MFS in or out is the risk of progressive aortic dilatation and aortic dissection, particularly related to physical activity. In this regard, if the echocardiogram shows an aortic root dimension within the normal range for body surface area,[4] in almost all of the disorders to be considered, the risk of aortic dissection is very low. The one exception is LDS[5] and other phenotypes

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associated with mutations in *TGFBR1* and *TGFBR2*,[6] in some of which both the aorta and its major branches dissect in the absence of much, if any, dilatation. Thus, the echocardiogram is an essential test whenever any of these conditions is considered seriously and whenever concerns about modulating physical activity are raised. Likewise, a slit-lamp examination, with the pupils fully dilated, is essential to exclude ectopia lentis; when MFS is seriously suspected, this test should be performed by an ophthalmologist comfortable with the ocular features of MFS.

Because all of the conditions listed above (except arterial tortuosity syndrome, which is recessive) can be inherited as autosomal dominant traits, the family history is a crucial component of any diagnostic matrix. Historical information may suffice, but usually more detail is needed in the form of medical and autopsy records. Frequently, a grandparent dies of an "aneurysm" and the family does not know what vessel or what region of the aorta was involved. More so in the past than now, a relative who died of an ascending aortic dissection and had a postmortem examination that showed "cystic medical necrosis" was automatically said to have had MFS, even in the absence of any other physical features of that condition. Personally examining the sibs and parents of a child in whom MFS is suspected is at least as important as obtaining a complete family history. Many of the helpful diagnostic features, such as scoliosis, striae atrophicae, disproportionate stature, MVP, and aortic root dilatation are clinically silent and may not be apparent to the relative and those close to him or her.

Almost all of the conditions listed above are "syndromes," in that multiple features are required to establish the diagnosis. For those conditions caused by a mutation in a single gene, the syndromic features are pleiotropic manifestations of the mutation and the pathogenetic mechanisms that derive from it. However, a number of important caveats affect this interpretation. First, virtually all of the features are age dependent; when examining a young relative, usually fewer features will be evident than in older relatives. Second, all genetic syndromes show variable expression beyond age dependency, for reasons that are poorly understood. Third, many of the features of these conditions also occur "sporadically" in the general population for both genetic and nongenetic reasons. Examples are nearsightedness, tall stature, pectus excavatum, scoliosis, joint hypermobility, and MVP. Fourth, many of these same features are more likely to occur among relatives because of polygenic contributions to pathogenesis. Occasional families will show such frequent occurrence of one of these features that Mendelian inheritance is inferred; this led to some of the diagnoses listed above, although with rare exceptions the causative locus has not been identified. Examples are familial forms of pectus excavatum, scoliosis, tall stature, and what the rheumatologists term "familial benign joint hypermobility," which is distinguished from forms of EDS by an absence of involvement of the skin.

The frequency of specific features in the general population contributes to their importance as diagnostic criteria in a Bayesian sense. For example, lumbosacral dural ectasia occurs in very few conditions besides MFS and LDS and almost never in the absence of some systemic disorder. This prompted the inclusion of dural ectasia as a "major criterion" for the diagnosis of MFS in the Ghent scheme.[1] Similar reasoning was used to so distinguish ectopia lentis, aortic dissection, and aortic dilatation. But it is important to emphasize again that these decisions were based on expert opinion and are just beginning to be subjected to formal scrutiny. If the diagnosis of MFS is clear in a patient who has no back or radicular pain, then there is almost no need to image the lower spine. On the other hand, if the diagnosis of MFS hangs on whether dural ectasia is present, then the effort, expense, and radiation (if a CT scan is done) of imaging are warranted. The severity of dural ectasia is clearly age dependent, but no cross-sectional survey of a pediatric population has been performed to determine when radiographic features appear. In the new diagnostic criteria for MFS, the presence or absence of dural ectasia has been reduced in importance.

Some of the diagnoses are ones of exclusion. For example, a family history of aortic aneurysms and mild thoracic-cage abnormalities, with no one meeting criteria for MFS, warrants a label of familial aortic aneurysm (although that implies little about etiology). Likewise, a family history of MVP and mild joint hypermobility, in the absence of features of MASS such as striae, thoracic-cage abnormalities, and myopia, warrants a label of familial MVP. Familial pectus, familial tall stature, and familial ectopia lentis fall into this group. Occasionally, long-term follow-up of patients or introduction of relatives with a wider spectrum of features may prompt reevaluation of the initial diagnosis. This has happened rather often with familial ectopia lentis due to mutations in *FBN1*.

Because echocardiography is an expensive, if benign, test, physicians often wonder whether to rescreen and at what interval. Certainly in MFS, a maximum interval is annually; the larger the aortic root diameter, the more frequently the test should be performed. This holds for familial aortic aneurysm, bicuspid aortic valve with aneurysm, and LDS. For the tall, lanky adolescent with flat feet and myopia, if the family history is unremarkable and the initial echocardiogram is completely normal, the study might be repeated only if cardiovascular symptoms arise. A great many patients fall somewhere in between, and repeating an echocardiogram every 2 to 3 years, or whenever a major increase in physical exertion is planned, seems reasonable. Interestingly, patients followed by cardiologists tend to have echocardiograms more frequently.

Anyone with moderate or severe aortic root dilation and/or a *TGFBR1* or *-2* mutation should be taught the signs and symptoms of aortic dissection and should consider wearing a medical alert bracelet.

EVALUATION FOR MFS

MFS is characterized by autosomal dominant inheritance of features in the skeletal, ocular, cardiovascular, and pulmonary

systems, along with muscular and adipose hypoplasia, dural ectasia, and hernias.[7] As individuals with MFS live longer with aggressive and prophylactic management of their cardiovascular disease, additional features are emerging, such as renal and hepatic cysts.[8] Because about one-third of patients have parents unaffected by MFS, more features are required in them to be certain of the diagnosis. Mutations in *FBN1*, the gene that encodes the large glycoprotein, fibrillin-1, cause MFS, but also cause many of the related conditions. Conversely, conditions that have been confused with MFS, such as CCA and LDS, are due to mutations in genes that encode either related proteins (e.g., fibrillin-2) or proteins involved in pathogenesis of common features (e.g., TGFβ receptors). There is no case of classic, bona fide MFS due to mutations in a gene other than *FBN1*. However, current clinical molecular testing of *FBN1* successfully detects mutations in such unequivocal patients in only about 90–95% of cases.[9] For all of these reasons, searching for mutations in *FBN1* continues to have a circumscribed role in the diagnosis of equivocal cases. Said differently, MFS remains, by and large, a clinical diagnosis.

Diagnostic criteria

If there is no family history of MFS, then the subject has the condition under any of the following four situations:

A dilated aortic root (defined as greater than or equal to two standard deviations above the mean for age, sex, and body surface area, i.e., a *Z*-score of > +2) and ectopia lentis
A dilated aortic root and a mutation in *FBN1* that is clearly pathologic
A dilated aortic root and multiple systemic features (see below) or
Ectopia lentis and a mutation in *FBN1* that has previously been associated with aortic disease

If there is a positive family history of MFS (independently ascertained with these criteria), then the subject has the condition under any of the following three situations:

Ectopia lentis
Multiple systemic features (see below) or
A dilated aortic root (if over 20 years, greater than two standard deviations; if younger than 20, greater than three standard deviations)

The scoring system for systemic features involves the following:

Wrist AND thumb sign = 3 (wrist OR thumb sign = 1)
Pectus carinatum deformity = 2 (pectus excavatum or chest asymmetry = 1)
Hindfoot deformity = 2 (plain pes planus = 1)
Pneumothorax = 2
Dural ectasia = 2
Protrusio acetabuli = 2
Reduced upper-to-lower segment ratio AND increased arm/height AND no severe scoliosis = 1
Scoliosis or thoracolumbar kyphosis = 1
Reduced elbow extension = 1
Facial features (three of five including dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, and retrognathia) = 1
Skin striae = 1
Myopia >3 diopters = 1
Mitral valve prolapse (all types) = 1
Maximum total: 20 points; score of 7 or more indicates systemic involvement

Diagnostic evaluation

1. Physical exam
2. Family history
3. Echocardiogram
4. Dilated eye exam
5. Consider CT or magnetic resonance imaging for evidence of lumbosacral dural ectasia and protrusion acetabulae
6. Consider *FBN1* gene sequencing

Management

Cardiovascular: (recommend management by a skilled cardiologist)

A. Aortic root dilation and/or diagnostic criteria met for MFS:

Annual echocardiogram for root diameter <4.5 cm in an adult and rate of increase <0.5 cm/year

β-Blocker therapy.[10] A randomized controlled trial of losartan versus atenolol is under way, but the results are potentially not available until 2013–14.[11] The final *N* of 604 subjects was reached at the end of January 2011.

Echocardiogram every 6 months if diameter is >2 SD in an adult or rate of increase in size is >0.5 cm/year

Surgical repair for measurements >4.5 cm, rate of increase in size >1 cm/year, or progressive aortic regurgitation

Magnetic resonance angiography or CT of the entire aorta starting in young adulthood. Repeat annually if there is a history of aortic root replacement or dissection, less frequently if not

B. Normal aortic root size with systemic involvement of another system with a positive family/genetic history:

Annual echocardiogram

C. Normal aortic root size with systemic involvement with a negative family/genetic history:

Repeat echocardiogram every 2 to 3 years until adult height is reached. Then repeat if symptomatic or when a major increase in physical activity is planned. The diameter of the aortic root is slightly larger in men than women of the same body size and, in both sexes, increases very slightly and gradually in normal individuals with age, but should not exceed the general upper limit of normal of 40–42 mm, even in tall individuals.

APPENDIX

EDS HYPERMOBILE TYPE

Joint hypermobility in this condition may mimic that in MFS. The skin may be mildly hyperextensible whereas it rarely is in MFS. Aortic root dilation, usually mild, occurs in one-quarter to one-third of individuals with EDS classic and hypermobility types.[12] There is not thought to be a risk of dissection without significant aortic root dilatation. Long-term prognosis is not known. Other features include joint laxity, easy bruising, functional bowel disorders, osteoporosis, and chronic pain.

Diagnostic criteria

Diagnosis is based on clinical evaluation and family history. A small subset of individuals with the hypermobile form of EDS have an insertion or deletion in the *TNXB* gene.

Major diagnostic criteria[13]

All of the following criteria should be met to establish a diagnosis of EDS, hypermobility type:

1. Joint hypermobility confirmed by a score of 5 or more on the 9-point Beighton scale:[14]
   - Passive dorsiflexion of each fifth finger >90 degrees (1 point each side)
   - Passive apposition of each thumb to the flexor surface of the forearm (1 point each side)
   - Hyperextension of each elbow >10 degrees (1 point each side)
   - Hyperextension of each knee >10 degrees (1 point each side)
   - Place palms flat on the floor when bending over with knees fully extended (1 point)
2. Soft or velvety skin with normal or slightly increased extensibility
3. Absence of skin or soft tissue fragility (suggestive of other EDS subtypes)

Minor diagnostic criteria

These criteria are supportive but not sufficient to establish the diagnosis.

1. Autosomal dominant family history of similar features without skin abnormalities
2. Recurrent joint dislocation or subluxation
3. Chronic joint or limb pain
4. Easy bruising
5. Functional bowel disorders (functional gastritis, irritable bowel syndrome)
6. Neurally mediated hypotension or postural orthostatic tachycardia
7. High, narrow palate
8. Dental crowding

Diagnostic evaluation

1. Physical exam
2. Family history
3. Echocardiogram to evaluate for aortic root dilatation
4. Dilated eye exam (to exclude MFS)

Management

Cardiovascular: (recommend management by a skilled cardiologist)

A. Normal aortic root size:

Repeat echocardiogram every 2–3 years until adult height reached. If no dilatation present, repeat echocardiogram if cardiovascular symptoms develop or when a major increase in physical activity is planned. If dilatation present, follow as described in the next section.

B. Aortic root dilation:

Echocardiogram every 6 months if diameter is >4.5 cm in an adult or rate of increase in size is >0.5 cm/year. Annual echocardiogram for root diameter <4.5 cm in an adult and rate of increase is <0.5 cm/year

C. Bone Density:

Encourage calcium and vitamin D supplementation
Low-impact weight-bearing exercise
Order DXA scan for height loss greater than one inch

D. Gastrointestinal:

Gastritis and reflux may require a proton pump inhibitor, H-2 blocker, and sucralfate.
Delayed gastric emptying may require a promotility agent
Irritable bowel is treated with antispasmodics, antidiarrheals, and laxatives as needed

E. Musculoskeletal:

Low-resistance exercise is recommended to improve joint stability by increasing muscle tone. Physical therapy for myofascial release is often necessary to facilitate participation in low-resistance exercise. A pain management specialist is a crucial participant in the care of a patient with EDS hypermobile type with chronic pain. Due to a general decrease in the degree of stabilization and pain reduction and the duration of improvement as compared with those without EDS hypermobile type, orthopedic surgery should be delayed, if possible, in favor of physical therapy and bracing.
Vitamin C is a cofactor for cross-linking of collagen fibrils and may improve hypermobility

FAMILIAL THORACIC AORTIC ANEURYSM AND DISSECTION (FAA)

Some families show an autosomal dominant predisposition to developing aneurysms in the thoracic aorta whereas others develop dilatation anywhere in the aorta. The larger the dilatation, the greater the risk of dissection. In other families, a risk of dissection exists with minimal or no dilatation. Several genetic

loci have been mapped in families with aortic aneurysm, but most of the genes are yet to be identified. *TGFBR2* mutations have been reported in a few families. Two splicing and one missense mutation in the *MYH11* gene have been described in two families with FAA and patent ductus arteriosus.[15] Occasionally, patients with aortic aneurysm will have deformity of the thoracic cage (scoliosis, pectus excavatum). These skeletal changes are also seen in people with bicuspid aortic valve sequence, which raises the question of whether patients with only ascending aortic aneurysms are simply failing to express the bicuspid valve.

Diagnostic evaluation[16]

1. Physical examination
2. Family history
3. Echocardiogram
4. Dilated eye exam (to exclude MFS)
5. Consider gene sequencing
6. Imaging of the vasculature, including the cerebral vasculature, for those with a genetic mutation that predisposes to diffuse arteriopathy

Diagnostic criteria

Autosomal dominant family history of dilation or dissection of the aortic root, ascending aorta, or descending aorta in the absence of major criteria for the diagnosis of MFS or another connective tissue disorder.

Management (recommend management by a skilled cardiologist)

Aortic dissection is rare in early childhood, but aortic dilation may be present. In most adults, the risk of aortic dissection or rupture becomes significant at diameters >5.0 cm. However, mutations in some genes (*TGFBR1* and *-2*) predispose to dissection at smaller and even normal aortic diameters. Persons with mutations in *MYH11, SMAD3,* and *ACTA2* should be considered for repair with a diameter between 4.5–5.0 cm.

Annual echocardiograms for individuals with small aortic dimensions and slow rate of increase of the dilation in the absence of a *TGFBR* mutation

Echocardiograms at least every 6 months if the root exceeds 4.5 cm in an adult, the rate of aortic growth exceeds 0.5 cm/year, or significant aortic regurgitation occurs.

Imaging of the entire aorta every 2–3 years

β-blockade for aortic root dilation. No randomized controlled trial of angiotensin receptor blockade is planned.

Prophylactic surgical repair if the rate of dilation approaches 1 cm/year, if there is progression of aortic regurgitation, if the diameter approaches 5 cm in those with a mutation known to predispose to earlier dissection, when the diameter is 5.0 cm in those with bicuspid aortic valve, and for a diameter of 5.0–5.5 cm for all others.

LDS

The features include characteristic facial features, craniosynostosis, bifid uvula or cleft palate, tortuosity of the aorta and its branches, aortic dilatation and dissection, and joint hypermobility.[5] Patients have had mutations in one or another of the receptors for TGFβ. When this condition is suspected, echocardiography must be augmented by a CT or magnetic resonance angiogram of the thorax, abdomen, and pelvis. Because dissection tends to occur at smaller aortic diameters than in MFS, earlier prophylactic aortic root repair is indicated. A variant of LDS strongly resembles the vascular form of Ehlers–Danlos syndrome, especially in terms of thin skin.

Diagnostic evaluation

1. Physical exam
2. Family history
3. Echocardiogram
4. Dilated eye exam (to exclude MFS)
5. Magnetic resonance angiography of the head, neck, thorax, abdomen, and pelvis
6. *TGFBR1* and *TGFBR2* gene sequencing

Diagnostic criteria

In a patient found to have consistent facial features, bifid uvula, and arterial tortuosity, the diagnosis can be confirmed with *TGFBR* testing. Tortuosity can sometimes be isolated (e.g., found only in the head and neck), requiring the magnetic resonance angiography of the head, neck, thorax, abdomen, and pelvis for a complete evaluation.

Management (recommend management by a skilled cardiologist)

Craniosynostosis is treated in the standard manners

Annual echocardiogram if no aortic root dilation

Echocardiogram at least every 6 months if aortic root dilation is detected

Annual magnetic resonance angiography of the head, neck, thorax, abdomen, and pelvis

β-blockade. There is no randomized controlled trial of angiotensin receptor blockade under way.

Aortic dilation and dissection in childhood is a feature of LDS, but not for all patients with a mutation in *TGFBR1* or *TGFBR2*. There is a low threshold for prophylactic aortic grafting in LDS. If the progression is rapid, aortic root replacement is recommended at 2.0 cm.[17]

CCA, BEALS SYNDROME

This condition shows congenital contractures of the elongated digits, elbows, and knees that can improve with physical therapy. Scoliosis develops during childhood and can become severe. The helix of the ear shows overfolding. There is no ectopia lentis. There is a Marfan-like habitus. Originally, the cardiovascular system was said to be unaffected, but first MVP and more recently aortic dilatation have been described. Every patient thought to have CCA should have echocardiography, perhaps on a regular basis, at least during childhood and adolescence. Congenital contractures, typically of the digits and

elbows, also occur in MFS. Some "crumpling" of the ear is also not uncommon in the general population. Therefore, diagnosis of CCA can be difficult. The role of examining the *FBN2* locus for mutations is unclear.

Diagnostic evaluation

1. Physical exam
2. Family history
3. Echocardiogram
4. Dilated eye exam (to exclude MFS)
5. Consider *FBN2* gene sequencing

Diagnostic criteria

Individuals with CCA typically have a marfanoid habitus; flexion contractures of multiple joints including elbows, hips, knees, and fingers; kyphoscoliosis; muscular hypoplasia; and abnormal pinnae ("crumpled" outer helices).[18]

Management

Physical therapy for joint manifestations and surgical release of severe contractures

Bracing and/or surgical correction of kyphoscoliosis

Echocardiogram every 2 years until adult height reached. If no dilation present, repeat if symptomatic or when a major increase in physical activity is planned.

Annual evaluation by physical exam for scoliosis until adult height reached

Aortic root dilation is treated as described above MFS

MASS PHENOTYPE

The acronym stands for mitral valve prolapse and myopia, an aortic root that may be at the upper limits of normal in caliber, striae atrophicae, and skeletal features reminiscent of, but less severe than, MFS.[19] A few patients designated as having MASS have had mutations in *FBN1*, but most do not. This condition is termed a "phenotype" because there are undoubtedly multiple causes. Importantly, almost all patients diagnosed as having MASS over a decade showed no progressive aortic root dilatation or aortic dissection (Pyeritz unpublished).

This diagnosis cannot be made with certainty in the absence of a multigenerational family history documenting no progression of aortic root dilation. Therefore, a child who appears to have the MASS phenotype without a family history should be followed as described above for MFS.

FAMILIAL ARTERIAL TORTUOSITY SYNDROME

This condition was first described as a severe condition of infancy. Recently, a pleiotropic condition of adults that shows autosomal recessive inheritance was described.[6] Patients have marked tortuosity of the aorta and its branches, and there is a predisposition to dissection. They also have telangiectases of the cheeks, lax skin, high palate, and joint laxity. When this condition is suspected, echocardiography must be augmented by a CT or magnetic resonance angiogram of the thorax, abdomen, and pelvis. Mutations in a nuclear glucose transporter encoded by the *SLC2A10* gene have been found.[20]

Monitoring guidelines are the same as those described above for LDS.

MVP

In some families, MVP occurs as an autosomal dominant trait, either isolated or in association with an asthenic habitus. Because the pathogenesis of MVP is varied, the valvular abnormality is seen in a number of other hereditary syndromes, including dilated cardiomyopathy, myotonic dystrophy, fragile-X syndrome, and some in this Appendix, including MASS, various EDS types, bicuspid aortic valve, and Stickler syndrome.

A child with MVP and an asthenic habitus should have aortic root measurements taken when MVP monitoring echocardiograms are done. If dilation of the aortic root is found, the child should be followed as described above for MFS.

FAMILIAL ECTOPIA LENTIS

Some families show variable skeletal manifestations of MFS along with ectopia lentis. Initially, these cases were said not to have aortic dilatation, but subsequently some have developed this feature and are more appropriately said to have MFS. Mutations near the 3′-end of *FBN1* have been associated with this autosomal dominant condition. If ectopia lentis and 3′ mutation are observed, follow the cardiovascular system as described above for MFS.

BICUSPID AORTIC VALVE SEQUENCE

Left-sided flow defects show more familial predisposition than any other general class of congenital heart disease other than conotruncal defects. The causes are unknown, but a defect of the neural crest is suspected. The features that show variable expression in families with this sequence are congenital bicuspid aortic valve, dilatation of the ascending aorta (not usually the sinuses of Valsalva), aortic coarctation, MVP, and thoracic-cage deformity. Heterozygosity for mutations in *NOTCH1* has been reported in a few families and in 4% of sporadic cases of bicuspid aortic valve.[21]

FAMILIAL TALL STATURE

One family distinguished by pectus excavatum and tall stature in the proband and tall stature in the relatives had a mutation in *FBN1*. No one had cardiovascular problems. There has been no systematic survey of disproportionately tall individuals for other features of MFS or mutations in *FBN1*.

FAMILIAL PECTUS EXCAVATUM

Pectus excavatum occurs as an autosomal dominant trait in occasional families. The literature is silent on whether pectus carinatum or combined anterior chest defects also occur, probably because ascertainment has typically been through surgeons consulted to repair the excavatum defects. There has been no systematic survey for other features of MFS or mutations in *FBN1*.

FAMILIAL SCOLIOSIS

"Idiopathic" scoliosis clearly shows familial predispositions.[22,23] There has been no systematic survey for other features of MFS or mutations in *FBN1*.

STICKLER SYNDROME

Occasionally, the person with Stickler syndrome will be considered initially as having MFS or another connective tissue disorder. The features that overlap include retrognathia, high-grade myopia and retinal detachment, and MVP.

DISCLOSURE

The author declares no conflict of interest.

REFERENCES

1. DePaepe A, Deitz HC, Devereux RB, Hennekam R, et al. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996;62:417–426.
2. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010;47:476–485.
3. Beighton P, de Paepe A, Danks D, et al. International nosology of heritable disorders of connective tissue, Berlin, 1986. *Am J Med Genet* 1988;29:581–594.
4. Roman MJ, Devereux RB, Kramer-Fox R, et al. Two-dimensional echocardiographic aortic root dimensions in normal children and adults. *Am J Cardiol* 1989;64:507–512.
5. Loeys BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet* 2005;37:275–281.
6. Loeys BL, Schwarze U, Holm T, et al. Aneurysm syndromes caused by mutations in TGF-β receptor. *N Engl J Med* 2006;355:788–798.
7. Pyeritz RE. Marfan syndrome and related disorders. In: Rimoin DL, Conner JM, Pyeritz RE, Korf BR (eds). *Principles and Practice of Medical Genetics*, 5th edn. Churchill Livingstone: Philadelphia, 2007; 3579–3624.
8. Chow K, Pyeritz RE, Litt HI. Abdominal visceral findings in patients with Marfan syndrome. *Genet Med* 2007;9:208–212.
9. Dietz HC, Loeys B, Carta L, et al. Recent progress towards a molecular understanding of Marfan syndrome. *Am J Med Genet C Semin Med Genet* 2005;139:4–9.
10. Keane MG, Pyeritz RE. Medical management of Marfan syndrome. *Circulation* 2008;117:2802–2813.
11. Lacro RV, Dietz HC, Wruck LM, et al. Rationale and design of a randomized clinical trial of beta blocker therapy (atenolol) vs. angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome. *Am Heart J* 2007;154:624–631.
12. Wenstrup RJ, Meyer RA, Lyle JS, et al. Prevalence of aortic root dilation in the Ehlers-Danlos syndrome. *Genet Med* 2002;4:112–117.
13. Levy HP (updated 14 December 2010). Ehlers–Danlos syndrome, hypermobility type. In: *GeneReviews at GeneTests: Medical Genetics Information Resource* (database online). Copyright, University of Washington, Seattle. 1997–2006. http://www.ncbi.nlm.nih.gov/books/NBK1279/. Accessed 29 November 2011.
14. Beighton P, Solomon L, Soskolne CL. Articular mobility in an African population. *Ann Rhem Dis* 1973;32:413–418.
15. Zhu L, Vranckx R, Van Kien PK, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. *Nat Genet* 2006;38:343–349.
16. Milewicz DM, Regaldo E (updated 11 January 2011). *Thoracic aortic aneurysms and aortic dissections*. In: *GeneReviews at GeneTests: Medical Genetics Information Resource* (database online). Copyright, University of Washington, Seattle. 1997–2006. http://www.ncbi.nlm.nih.gov/books/NBK1120/. Accessed 29 November 2011.
17. Loeys BL, Dietz HC (updated 29 April 2008). Loeys–Dietz syndrome. In: *GeneReviews at GeneTests: Medical Genetics Information Resource* (database online). Copyright, University of Washington, Seattle. 1997–2006. http://www.ncbi.nlm.nih.gov/books/NBK1130/. Accessed 29 November 2011.
18. Godfrey M (updated 4 May 2007). Congenital contractural arachnodactyly. In: *GeneReviews at GeneTests: Medical Genetics Information Resource* (database online). Copyright, University of Washington, Seattle. 1997–2006. http://www.ncbi.nlm.nih.gov/books/NBK1386/. Accessed 29 November 2011.
19. Glesby MJ, Pyeritz RE. Association of mitral valve prolapse and systemic abnormalities of connective tissue: a phenotypic continuum. *JAMA* 1989;262:523–528.
20. Coucke PJ, Willaert A, Wessels MW, et al. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. *Nat Gene* 2006;38:452–457.
21. Mohamed SA, Aherrahrou Z, Liptau H, et al. Novel missense mutations (p.T596M and p.P1797H) on NOTCH1 in patients with bicuspid aortic valve. *Biochem Biophys Res Commun* 2006;345:1460–1465.
22. Pyeritz RE. Common disorders of connective tissue. In: King RA, Rotter JI, Motulsky AG (eds). *The Genetic Basis of Common Diseases*, 2nd edn. Oxford University Press: New York, 2002:638–645.
23. Miller NH. Idiopathic scoliosis: cracking the genetic code and what does it mean? *J Pediatr Orthop* 2011;31:S49–52.

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1. Introduction

The term “thoracic aortic disease” encompasses a broad range of degenerative, structural, acquired, genetic-based, and traumatic disease states and presentations. According to the Centers for Disease Control and Prevention death certificate data, diseases of the aorta and its branches account for 43 000 to 47 000 deaths annually in the United States. The precise number of deaths attributable to thoracic aortic diseases is unclear. However, autopsy studies suggest that the presentation of thoracic aortic disease is often death due to aortic dissection (AoD) and rupture, and these deaths account for twice as

many deaths as attributed to ruptured abdominal aortic aneurysms (AAAs). This guideline includes diseases involving any or all parts of the thoracic aorta with the exception of aortic valve diseases and includes the abdominal aorta when contiguous thoracic aortic diseases are present.

Table 1. Applying Classification of Recommendations and Level of Evidence

ESTIMATE OF CERTAINTY (PRECISION) OF TREATMENT EFFECT	SIZE OF TREATMENT EFFECT	
	CLASS I *Benefit >>> Risk* Procedure/Treatment SHOULD be performed/ administered	**CLASS IIA** *Benefit >> Risk* *Additional studies with focused objectives needed* IT IS REASONABLE to perform procedure/administer treatment
LEVEL A Multiple populations evaluated* Data derived from multiple randomized clinical trials or meta-analyses	■ Recommendation that procedure or treatment is useful/effective ■ Sufficient evidence from multiple randomized trials or meta-analyses	■ Recommendation in favor of treatment or procedure being useful/effective ■ Some conflicting evidence from multiple randomized trials or meta-analyses
LEVEL B Limited populations evaluated* Data derived from a single randomized trial or nonrandomized studies	■ Recommendation that procedure or treatment is useful/effective ■ Limited evidence from single randomized trial or nonrandomized studies	■ Recommendation in favor of treatment or procedure being useful/effective ■ Some conflicting evidence from single randomized trial or nonrandomized studies
LEVEL C Very limited populations evaluated* Only consensus opinion of experts, case studies, or standard of care	■ Recommendation that procedure or treatment is useful/effective ■ Only expert opinion, case studies, or standard of care	■ Recommendation in favor of treatment or procedure being useful/effective ■ Only diverging expert opinion, case studies, or standard of care
Suggested phrases for writing recommendations†	should is recommended is indicated is useful/effective/beneficial	is reasonable can be useful/effective/beneficial is probably recommended or indicated

Class IIb *Benefit ≥ Risk* *Additional studies with broad objectives needed; additional registry data would be helpful* Procedure/Treatment MAY BE CONSIDERED	Class III *Risk ≥ Benefit* Procedure/Treatment should NOT be performed/administered SINCE IT IS NOT HELPFUL AND MAY BE HARMFUL
■ Recommendation's usefulness/efficacy less well established ■ Greater conflicting evidence from multiple randomized trials or meta-analyses	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Sufficient evidence from multiple randomized trials or meta-analyses
■ Recommendation's usefulness/efficacy less well established ■ Greater conflicting evidence from single randomized trial or nonrandomized studies	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Limited evidence from single randomized trial or nonrandomized studies
■ Recommendation's usefulness/efficacy less well established ■ Only diverging expert opinion, case studies, or standard of care	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Only expert opinion, case studies, or standard of care
may/might be considered may/might be reasonable usefulness/effectiveness is unknown /unclear/uncertain or not well established	is not recommended is not indicated should not is not useful/effective/beneficial may be harmful

* Data available from clinical trials or registries about the usefulness/efficacy in different subpopulations, such as sex, age, history of diabetes, history of prior myocardial infarction, history of heart failure, and prior aspirin use. A recommendation with Level of Evidence B or C does not imply that the recommendation is weak. Many important clinical questions addressed in the guidelines do not lend themselves to clinical trials. Even though randomized trials are not available, there may be a very clear clinical consensus that a particular test or therapy is useful or effective.

† In 2003, the ACCF/AHA Task Force on Practice Guidelines developed a list of suggested phrases to use when writing recommendations. All guideline recommendations have been written in full sentences that express a complete thought, such that a recommendation, even if separated and presented apart from the rest of the document (including headings above sets of recommendations), would still convey the full intent of the recommendation. It is hoped that this will increase readers' comprehension of the guidelines and will allow queries at the individual recommendation level.

2. Critical Issues

As the writing committee developed this guideline, several critical issues emerged:

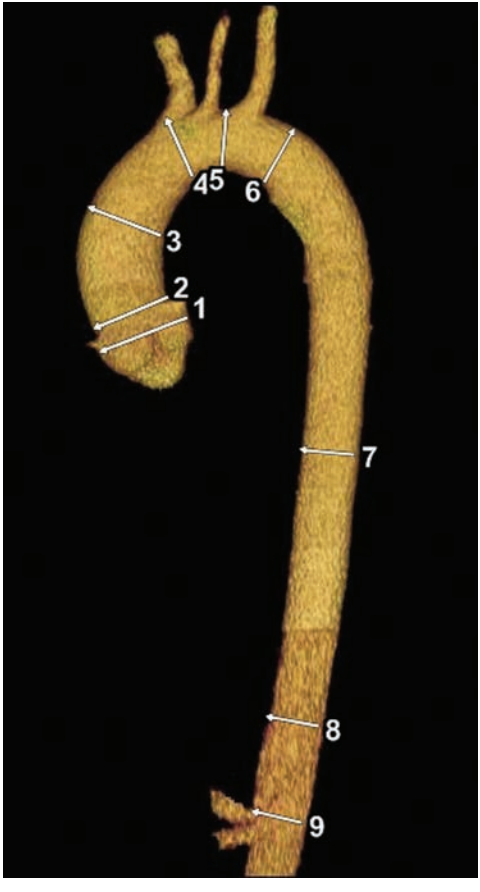
- Thoracic aortic diseases are usually asymptomatic and not easily detectable until an acute and often catastrophic complication occurs. Imaging of the thoracic aorta with computed tomographic imaging (CT), magnetic resonance imaging (MR), or in some cases, echocardiographic examination is the only method to detect thoracic aortic diseases
- Radiologic imaging technologies have improved in terms of accuracy of detection of thoracic aortic disease. However risks associated with repeated radiation exposure, as well as contrast medium–related toxicity have also been recognized. The writing committee therefore formulated recommendations on a standard reporting format (Section 3) as well as surveillance schedules (Section 21).
- Imaging for asymptomatic patients at high risk based on history or associated disease is expensive and not always covered by payers.
- For many thoracic aortic diseases, results of treatment for stable, often asymptomatic, but high-risk conditions are far better than the results of treatment required for acute and often catastrophic disease presentations. Thus, the identification and treatment of patients at risk for acute and

catastrophic disease presentations (eg, thoracic AoD and thoracic aneurysm rupture) prior to such an occurrence are paramount to eliminating the high morbidity and mortality associated with acute presentations.

■ Patients with acute AoD are subject to missed or delayed detection of this catastrophic disease state. Many present with atypical symptoms and findings, making diagnosis even more difficult. Awareness of the varied and complex nature of thoracic aortic disease presentations has been lacking, especially for acute AoD. Risk factors and clinical presentation clues are noted in Section 7. The collaboration of multiple medical specialties for this guideline will provide opportunities to raise the level of awareness among all medical specialties.

■ There is rapidly accumulating evidence that genetic alterations or mutations predispose some individuals to aortic diseases (see Sections 4-6). Therefore, identification of the genetic alterations leading to these aortic diseases has the potential for early identification of individuals at risk. In addition, biochemical abnormalities involved in the progression of aortic disease are being identified through studies of patients' aortic samples and animal models of the disease. The biochemical alterations identified in the aortic tissue have the potential to serve as biomarkers for aortic disease. Understanding the molecular pathogenesis may lead to targeted therapy to prevent aortic disease. Medical and gene-based treatments are beginning to show promise for reducing or delaying catastrophic complications of thoracic aortic diseases.

Figure 1. Normal Anatomy of the Thoracoabdominal Aorta.



Anatomic Location	
1.	Aortic sinuses of Valsalva
2.	Sinotubular junction
3.	Mid ascending aorta (midpoint in length between Nos. 2 and 4)
4.	Proximal aortic arch (aorta at the origin of the innominate artery)
5.	Mid aortic arch (between left common carotid and subclavian arteries)
6.	Proximal descending thoracic aorta (begins at the isthmus, approximately 2 cm distal to left subclavian artery)
7.	Mid descending aorta (midpoint in length between Nos. 6 and 8)
8.	Aorta at diaphragm (2 cm above the celiac axis origin)
9.	Abdominal aorta at the celiac axis origin

Normal anatomy of the thoracoabdominal aorta with standard anatomic landmarks for reporting aortic diameter as illustrated on a volume-rendered CT image of the thoracic aorta. CT indicates computed tomographic imaging.

3. Recommendations for Aortic Imaging Techniques to Determine the Presence and Progression of Thoracic Aortic Disease

Class I

1. Measurements of aortic diameter should be taken at reproducible anatomic landmarks, perpendicular to the axis of blood flow, and reported in a clear and consistent format. *(LOE: C)*

2. For measurements taken by computed tomographic imaging or magnetic resonance imaging, the external diameter should be measured perpendicular to the axis of blood flow. For aortic root measurements, the widest diameter, typically at the mid-sinus level, should be used. *(LOE: C)*

3. For measurements taken by echocardiography, the internal diameter should be measured perpendicular to the axis of blood flow. For aortic root measurements the widest diameter, typically at the mid-sinus level, should be used. *(LOE: C)*

4. Abnormalities of aortic morphology should be recognized and reported separately even when aortic diameters are within normal limits. *(LOE: C)*

5. The finding of aortic dissection, aneurysm, traumatic injury and/or aortic rupture should be immediately communicated to the referring physician. *(LOE: C)*

6. Techniques to minimize episodic and cumulative radiation exposure should be utilized whenever possible. *(LOE: B)*

Class IIa

1. If clinical information is available, it can be useful to relate aortic diameter to the patient's age and body size. *(LOE: C)*

Table 2. Essential Elements of Aortic Imaging Reports

1. The location at which the aorta is abnormal.
2. The maximum diameter of any dilatation, measured from the external wall of the aorta, perpendicular to the axis of flow, and the length of the aorta that is abnormal.
3. For patients with presumed or documented genetic syndromes at risk for aortic root disease measurements of aortic valve, sinuses of Valsalva, sinotubular junction, and ascending aorta.
4. The presence of internal filling defects consistent with thrombus or atheroma.
5. The presence of IMH, PAU, and calcification.
6. Extension of aortic abnormality into branch vessels, including dissection and aneurysm, and secondary evidence of end-organ injury (eg, renal or bowel hypoperfusion
7. Evidence of aortic rupture, including periaortic and mediastinal hematoma, pericardial and pleural fluid, and contrast extravasation from the aortic lumen.
8. When a prior examination is available, direct image to image comparison to determine if there has been any increase in diameter.

IMH indicates intramural hematoma; and PAU, penetrating atherosclerotic ulcer.

Table 3. Normal Adult Thoracic Aortic Diameters

Thoracic Aorta	Range of Reported Mean (cm)	Reported SD (cm)	Assessment Method
Root (female)	3.50 to 3.72	0.38	CT
Root (male)	3.63 to 3.91	0.38	CT
Ascending (female, male)	2.86	NA	CXR
Mid-descending (female)	2.45 to 2.64	0.31	CT
Mid-descending (male)	2.39 to 2.98	0.31	CT
Diaphragmatic (female)	2.40 to 2.44	0.32	CT
Diaphragmatic (male)	2.43 to 2.69	0.27 to 0.40	CT, arteriography

CT indicates computed tomographic imaging; CXR, chest x-ray; and NA, not applicable.

Reprinted with permission from Johnston KW, Rutherford RB, Tilson MD, et al. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. J Vasc Surg. 1991;13:452–8.

4. Recommendations for Genetic Syndromes

Class I

1. An echocardiogram is recommended at the time of diagnosis of Marfan syndrome to determine the aortic root and ascending aortic diameters and 6 months thereafter to determine the rate of enlargement of the aorta. *(LOE: C)*

2. Annual imaging is recommended for patients with Marfan syndrome if stability of the aortic diameter is documented. If the maximal aortic diameter is 4.5 cm or greater, or if the aortic diameter shows significant growth from baseline, more frequent imaging should be considered. *(LOE: C)*

3. Patients with Loeys-Dietz syndrome or a confirmed genetic mutation known to predispose to aortic aneurysms and aortic dissections *(TGFBR1, TGFBR2, FBN1, ACTA2,* or *MYH11)* should undergo complete aortic imaging at initial diagnosis and 6 months thereafter to establish if enlargement is occurring. *(LOE: C)*

4. Patients with Loeys-Dietz syndrome should have yearly magnetic resonance imaging from the cerebrovascular circulation to the pelvis. *(LOE: B)*

5. Patients with Turner syndrome should undergo imaging of the heart and aorta for evidence of bicuspid aortic valve, coarctation of the aorta, or dilatation of the ascending thoracic aorta. If initial imaging is normal and there are no risk factors for aortic dissection, repeat imaging should be performed every 5 to 10 years or if otherwise clinically indicated. If abnormalities exist, annual imaging or follow-up imaging should be done. *(LOE: C)*

Class IIa

1. It is reasonable to consider surgical repair of the aorta in all adult patients with Loeys-Dietz syndrome or a confirmed *TGFBR1* or *TGFBR2* mutation and an aortic diameter of 4.2 cm or greater by transesophageal echocardiogram (internal diameter) or 4.4 to 4.6 cm or greater by computed tomographic imaging and/or magnetic resonance imaging (external diameter). *(LOE: C)*

2. For women with Marfan syndrome contemplating pregnancy, it is reasonable to prophylactically replace the aortic root and ascending aorta if the diameter exceeds 4.0 cm. *(LOE: C)*

3. If the maximal cross-sectional area in square centimeters of the ascending aorta or root divided by the patient's height in meters exceeds a ratio of 10, surgical repair is reasonable because shorter patients have dissection at a smaller size and 15% of patients with Marfan syndrome have dissection at a size smaller than 5.0 cm. *(LOE: C)*

Class IIb

1. In patients with Turner syndrome with additional risk factors, including bicuspid aortic valve, coarctation of the aorta, and/or hypertension, and in patients who attempt to become pregnant or who become pregnant, it may be reasonable to perform imaging of the heart and aorta to help determine the risk of aortic dissection. *(LOE: C)*

Table 4. Gene Defects Associated With Familial Thoracic Aortic Aneurysm and Dissection

Defective Gene Leading to Familial Thoracic Aortic Aneurysms and Dissection	Contribution to Familial Thoracic Aortic Aneurysms and Dissection	Associated Clinical Features	Comments on Aortic Disease
TGFBR2 mutations	4%	Thin, translucent skin Arterial or aortic tortuosity Aneurysm of arteries	Multiple aortic dissections documented at aortic diameters <5.0 cm
MYH11 mutations	1%	Patent ductus arteriosus	Patient with documented dissection at 4.5 cm
ACTA2 mutations	14%	Livedo reticularis Iris flocculi Patent ductus arteriosus Bicuspid aortic valve	Two of 13 patients with documented dissections <5.0 cm

ACTA2 indicates actin, alpha 2, smooth muscle aorta; *MYH11*, smooth muscle specific beta-myosin heavy chain; and *TGFBR2*, transforming growth factor-beta receptor type II.

Table 5. Genetic Syndromes Associated With Thoracic Aortic Aneurysm

Genetic Syndrome	Common Clinical Features	Genetic Defect
Marfan syndrome	Skeletal features (see text) Ectopia lentis Dural ectasia	*FBN1* mutations*
Loeys-Dietz syndrome	Bifid uvula or cleft palate Arterial tortuosity Hypertelorism Skeletal features similar to MFS Craniosynostosis Aneurysms and dissections of other arteries	*TGFBR2* or *TGFBR1* mutations
Ehlers-Danlos syndrome, vascular form	Thin, translucent skin Gastrointestinal rupture Rupture of the gravid uterus Rupture of medium-sized to large arteries	*COL3A1* mutations
Turner syndrome	Short stature Primary amenorrhea Bicuspid aortic valve Aortic coarctation Webbed neck, low-set ears, low hairline, broad chest	45,X karyotype

* The defective gene at a second locus for MFS is TGFBR2 but the clinical phenotype as MFS is debated.

AoD indicates aortic dissection; *COL3A1*, type III collagen; CT, computed tomographic imaging; *FBN1*, fibrillin 1; MFS, Marfan syndrome; MR, magnetic resonance imaging; TEE, transesophageal echocardiogram; *TGFBR1*, transforming growth factor-beta receptor type I; and *TGFBR2*, transforming growth factor-beta receptor type II.

and Dissection

Diagnostic Test	Comments on Aortic Disease
Ghent diagnostic criteria DNA for sequencing	Surgical repair when the aorta reaches 5.0 cm unless there is a family history of AoD at <5.0 cm, a rapidly expanding aneurysm or presence or significant aortic valve regurgitation
DNA for sequencing	Surgical repair recommended at an aortic diameter of ≥4.2 cm by TEE (internal diameter) or 4.4 to ≥4.6 cm by CT and/or MR (external diameter)
DNA for sequencing Dermal fibroblasts for analysis of type III collagen	Surgical repair is complicated by friable tissues Noninvasive imaging recommended
Blood (cells) for karyotype analysis	AoD risk is increased in patients with bicuspid aortic valve, aortic coarctation, hypertension, or pregnancy

5. Recommendations for Familial Thoracic Aortic Aneurysms and Dissections

Class I

1. Aortic imaging is recommended for first-degree relatives of patients with thoracic aortic aneurysm and/or dissection to identify those with asymptomatic disease. *(LOE: B)*

2. If the mutant gene (*FBN1, TGFBR1, TGFBR2, COL3A1, ACTA2, MYH11*) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing. Then, only the relatives with the genetic mutation should undergo aortic imaging. *(LOE: C)*

Class IIa

1. If one or more first-degree relatives of a patient with known thoracic aortic aneurysm and/or dissection are found to have thoracic aortic dilatation, aneurysm, or dissection, then imaging of second-degree relatives is reasonable. *(LOE: B)*

2. Sequencing of the *ACTA2* gene is reasonable in patients with a family history of thoracic aortic aneurysms and/or dissections to determine if *ACTA2* mutations are responsible for the inherited predisposition. *(LOE: B)*

Class IIb

1. Sequencing of other genes known to cause familial thoracic aortic aneurysms and/or dissection (*TGFBR1, TGFBR2, MYH11*) may be considered in patients with a family history and clinical features associated with mutations in these genes. *(LOE: B)*

2. If one or more first-degree relatives of a patient with known thoracic aortic aneurysm and/or dissection are found to have thoracic aortic dilatation, aneurysm, or dissection, then referral to a geneticist may be considered. *(LOE: C)*

6. Recommendations for Bicuspid Aortic Valve and Associated Congenital Variants in Adults

Class I

1. First-degree relatives of patients with a bicuspid aortic valve, premature onset of thoracic aortic disease with minimal risk factors, and/or a familial form of thoracic aortic aneurysm and dissection should be evaluated for the presence of a bicuspid aortic valve and asymptomatic thoracic aortic disease. *(LOE: C)*

2. All patients with a bicuspid aortic valve should have both the aortic root and ascending thoracic aorta evaluated for evidence of aortic dilatation. *(LOE: B)*

7. Recommendations for Estimation of Pretest Risk of Thoracic Aortic Dissection

Class I

1. Providers should routinely evaluate any patient presenting with complaints that may represent acute thoracic aortic dissection to establish a pretest risk of disease that can then be used to guide diagnostic decisions. This process should include specific questions about medical history, family history, and pain features as well as a focused examination to identify findings that are associated with aortic dissection, including:

a. High-risk conditions and historical features *(LOE: B)*:

- Marfan syndrome, Loeys-Dietz syndrome, vascular Ehlers-Danlos syndrome, Turner syndrome, or other connective tissue disease.
- Patients with mutations in genes known to predispose to thoracic aortic aneurysms and dissection, such as *FBN1, TGFBR1, TGFBR2, ACTA2,* and *MYH11*.
- Family history of aortic dissection or thoracic aortic aneurysm.
- Known aortic valve disease.
- Recent aortic manipulation (surgical or catheter-based).
- Known thoracic aortic aneurysm.

b. High-risk chest, back or abdominal pain features *(LOE: B)*:
   - Pain that is abrupt or instantaneous in onset.
   - Pain that is severe in intensity.
   - Pain that has a ripping, tearing, stabbing, or sharp quality.

c. High-risk examination features *(LOE: B)*:
   - Pulse deficit.
   - Systolic blood pressure limb differential greater than 20 mm Hg.
   - Focal neurologic deficit.
   - Murmur of aortic regurgitation (new).

2. Patients presenting with sudden onset of severe chest, back and/or abdominal pain, particularly those less than 40 years of age, should be questioned about a history and examined for physical features of Marfan syndrome, Loeys-Dietz syndrome, vascular Ehlers-Danlos syndrome, Turner syndrome, or other connective tissue disorder associated with thoracic aortic disease. (LOE: B)

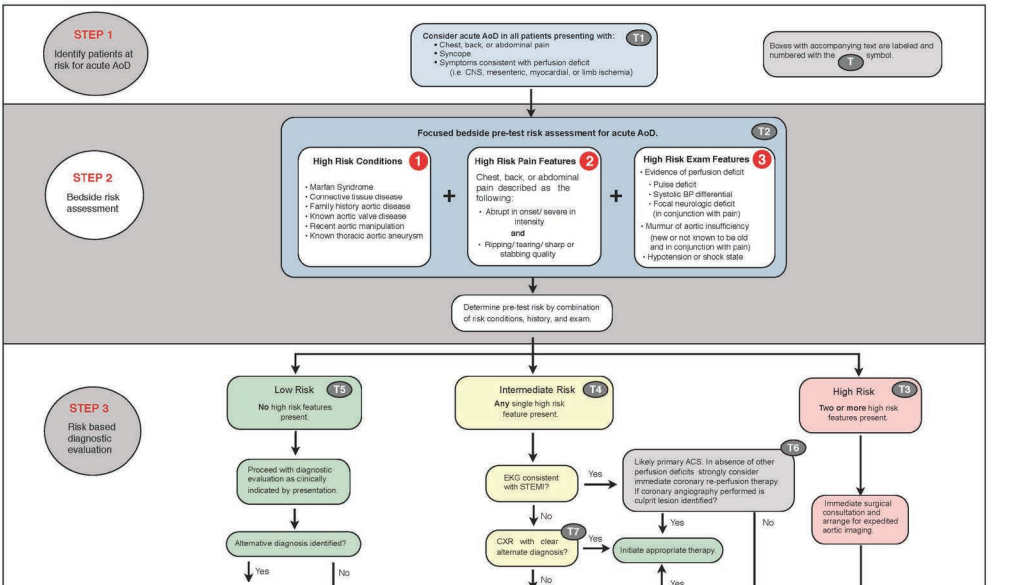
3. Patients presenting with sudden onset of severe chest, back, and/or abdominal pain should be questioned about a history of aortic pathology in immediate family members as there is a strong familial component to acute thoracic aortic disease. *(LOE: B)*

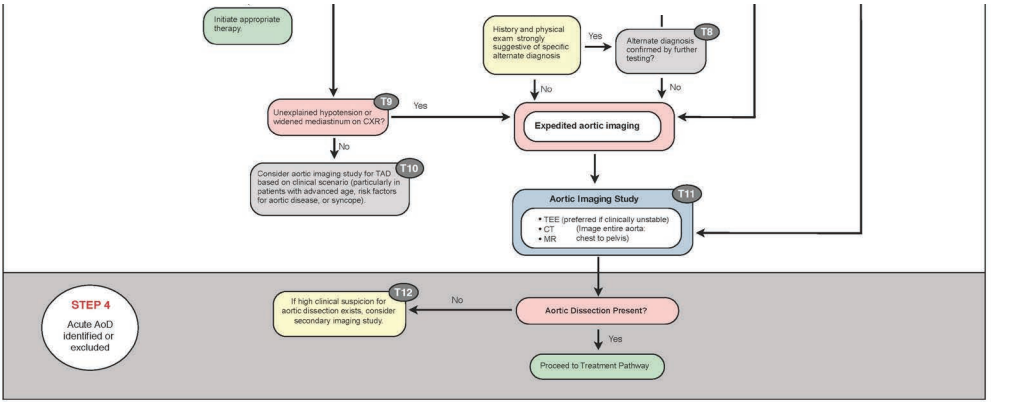
4. Patients presenting with sudden onset of severe chest, back and/or abdominal pain should be questioned about recent aortic manipulation (surgical or catheter-based) or a known history of aortic valvular disease, as these factors predispose to acute aortic dissection. *(LOE: C)*

5. In patients with suspected or confirmed aortic dissection who have experienced a syncopal episode, a focused examination should be performed to identify associated neurologic injury or the presence of pericardial tamponade. *(LOE: C)*

6. All patients presenting with acute neurologic complaints should be questioned about the presence of chest, back, and/or abdominal pain and checked for peripheral pulse deficits as patients with dissection-related neurologic pathology are less likely to report thoracic pain than the typical aortic dissection patient. *(LOE: C)*

Figure 2. AoD Evaluation Pathway.



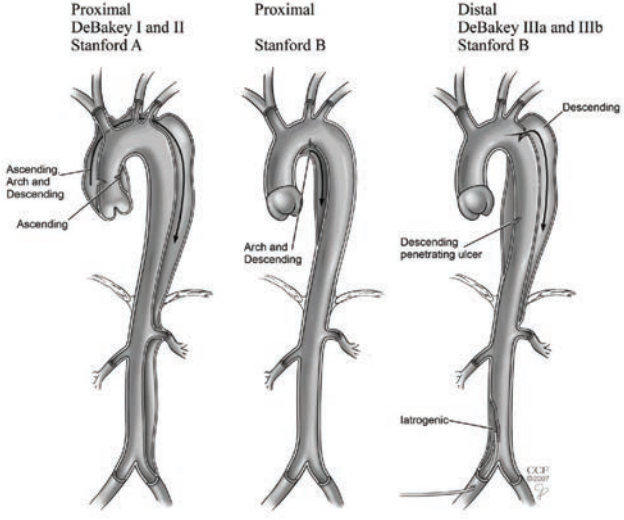


ACS indicates acute coronary syndrome; AoD, aortic dissection; BP, blood pressure; CNS, central nervous system; CT, computed tomographic imaging; CXR, chest x-ray; EKG, electrocardiogram; MR, magnetic resonance imaging; STEMI, ST-elevated myocardial infarction; TAD; thoracic aortic disease; and TEE, transesophageal echocardiogram.

Table 6. Risk Factors for Development of Thoracic Aortic Dissection

Conditions Associated With Increased Aortic Wall Stress
Hypertension, particularly if uncontrolled
Pheochromocytoma
Cocaine or other stimulant use
Weight lifting or other Valsalva maneuver
Trauma
Deceleration or torsional injury (eg, motor vehicle crash, fall)
Coarctation of the aorta
Conditions Associated With Aortic Media Abnormalities
Genetic
Marfan syndrome
Ehlers-Danlos syndrome, vascular form
Bicuspid aortic valve (including prior aortic valve replacement)
Turner syndrome
Loeys-Dietz syndrome
Familial thoracic aortic aneurysm and dissection syndrome
Inflammatory vasculitides
Takayasu arteritis
Giant cell arteritis
Behçet arteritis
Other
Pregnancy
Polycystic kidney disease
Chronic corticosteroid or immunosuppression agent administration
Infections involving the aortic wall either from bacteremia or extension of adjacent infection

Figure 3. Aortic Dissection Classification: DeBakey and Stanford Classifications.



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8. Initial Evaluation and Management of Acute Thoracic Aortic Disease

8.1. Recommendations for Screening Tests

Class I

1. An electrocardiogram should be obtained on all patients who present with symptoms that may represent acute thoracic aortic dissection.

a. Given the relative infrequency of dissection-related coronary artery occlusion, the presence of ST-segment elevation suggestive of myocardial infarction should be treated as a primary cardiac event without delay for definitive aortic imaging unless the patient is at high risk for aortic dissection. *(LOE: B)*

2. The role of chest x-ray in the evaluation of possible thoracic aortic disease should be directed by the patient's pretest risk of disease as follows.

a. Intermediate risk: Chest x-ray should be performed on all intermediate-risk patients, as it may establish a clear alternate diagnosis that will obviate the need for definitive aortic imaging. *(LOE: C)*

b. Low risk: Chest x-ray should be performed on all low-risk patients, as it may either establish an alternative diagnosis or demonstrate findings that are suggestive of thoracic aortic disease, indicating the need for urgent definitive aortic imaging. *(LOE: C)*

3. Urgent and definitive imaging of the aorta using transesophageal echocardiogram, computed tomographic imaging, or magnetic resonance imaging is recommended to identify or exclude thoracic aortic dissection in patients at high risk for the disease by initial screening. *(LOE: B)*

Class III

1. A negative chest x-ray should not delay definitive aortic imaging in patients determined to be high risk for aortic dissection by initial screening. *(LOE: C)*

8.2. Recommendations for Diagnostic Imaging Studies

Class I

1. Selection of a specific imaging modality to identify or exclude aortic dissection should be based on patient variables and institutional capabilities, including immediate availability. *(LOE: C)*

2. If a high clinical suspicion exists for acute aortic dissection but initial aortic imaging is negative, a second imaging study should be obtained. *(LOE: C)*

8.3. Recommendations for Initial Management (see Figure 4)

Class I

1. Initial management of thoracic aortic dissection should be directed at decreasing aortic wall stress by controlling heart rate and blood pressure as follows:

a. In the absence of contraindications, intravenous beta blockade should be initiated and titrated to a target heart rate of 60 beats per minute or less. *(LOE: C)*

b. In patients with clear contraindications to beta blockade, nondihydropyridine calcium channel–blocking agents should be utilized as an alternative for rate control. *(LOE: C)*

c. If systolic blood pressures remain greater than 120 mm Hg after adequate heart rate control has been obtained, then angiotensin-converting enzyme inhibitors and/or other vasodilators should be administered intravenously to further reduce blood pressure that maintains adequate end-organ perfusion. *(LOE: C)*

d. Beta blockers should be used cautiously in the setting of acute aortic regurgitation because they will block the compensatory tachycardia. *(LOE: C)*

Class III

1. Vasodilator therapy should not be initiated prior to rate control so as to avoid associated reflex tachycardia that may increase aortic wall stress, leading to propagation or expansion of a thoracic aortic dissection. *(LOE: C)*

8.4. Recommendations for Definitive Management (see Figures 3 and 5)

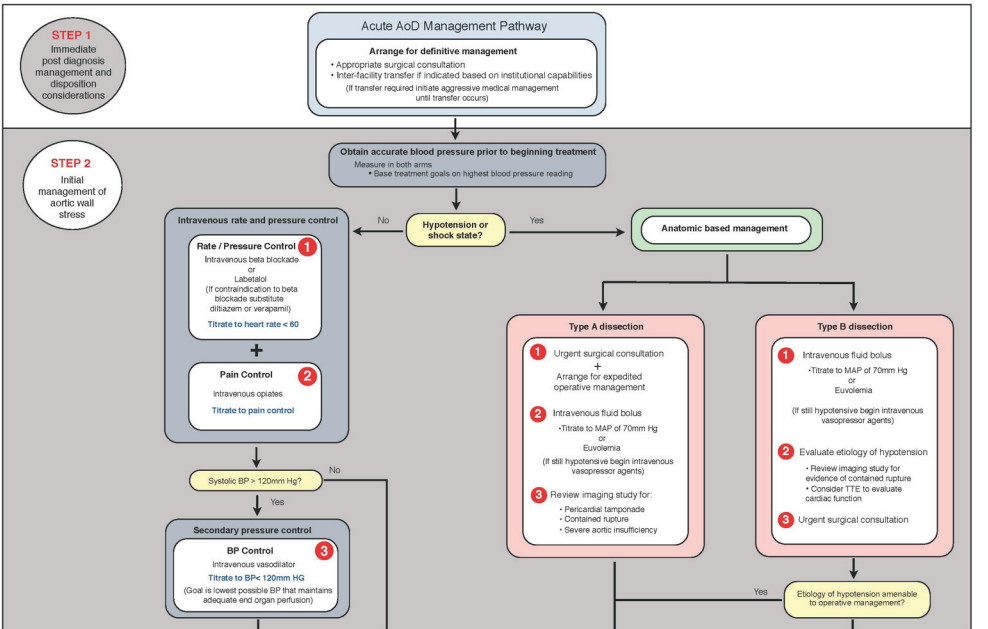
Class I

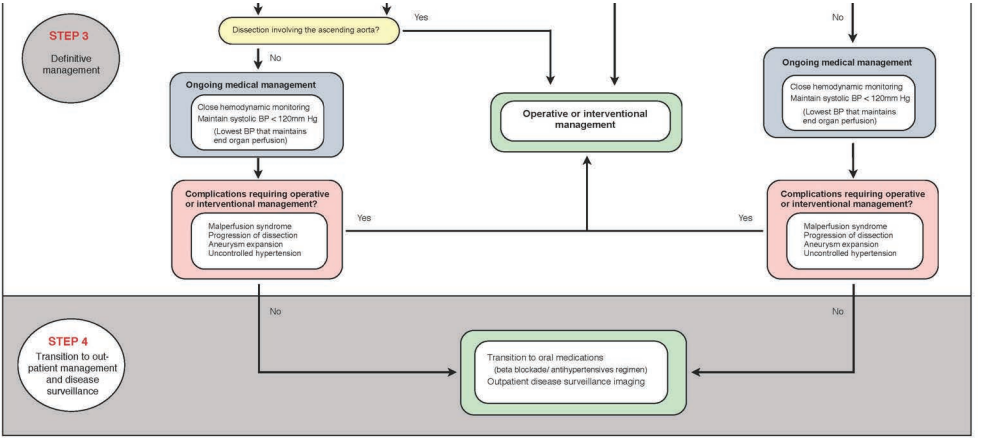
1. Urgent surgical consultation should be obtained for all patients diagnosed with thoracic aortic dissection regardless of the anatomic location (ascending versus descending) as soon as the diagnosis is made or highly suspected. *(LOE: C)*

2. Acute thoracic aortic dissection involving the ascending aorta should be urgently evaluated for emergent surgical repair because of the high risk of associated life-threatening complications such as rupture. *(LOE: B)*

3. Acute thoracic aortic dissection involving the descending aorta should be managed medically unless life-threatening complications develop (ie, malperfusion syndrome, progression of dissection, enlarging aneurysm, inability to control blood pressure or symptoms). *(LOE: B)*

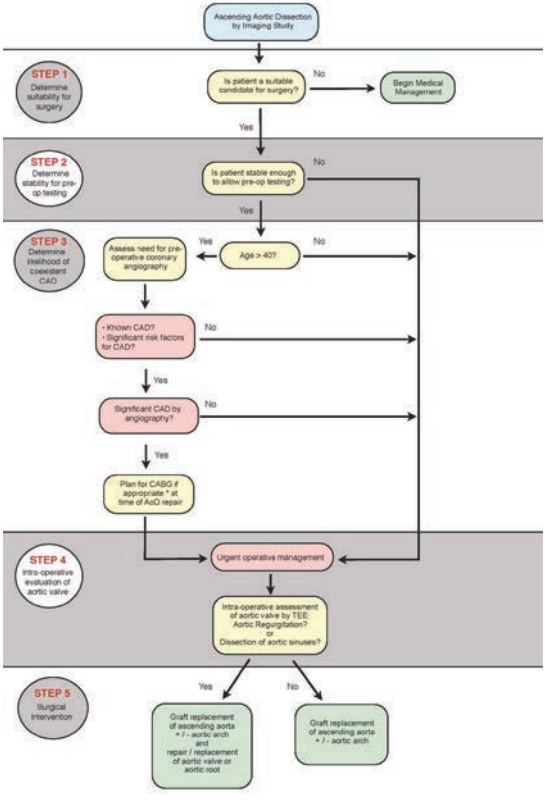
Figure 4. Acute AoD Management Pathway.





AoD indicates aortic dissection; BP, blood pressure; MAP, mean arterial pressure; and TTE, transthoracic echocardiogram.

Figure 5. Acute Surgical Management Pathway for AoD.



*Addition of 'if appropriate' based on Patel MR, Dehmer GJ, Hirshfeld JW, et al. ACCF/SCAI/STS/AATS/AHA/ASNC 2009 Appropriateness Criteria for Coronary Revascularization. J Am Coll Cardiol. 2009;53:530–53.

AoD indicates aortic dissection; CABG, coronary artery bypass graft surgery; CAD, coronary artery disease; TAD, thoracic aortic disease; and TEE, transesophageal echocardiogram.

9. Recommendation for Surgical Intervention for Acute Thoracic Aortic Dissection

Class I

1. For patients with ascending thoracic aortic dissection, all aneurysmal aorta and the proximal extent of the dissection should be resected. A partially dissected aortic root may be repaired with aortic valve resuspension. Extensive dissection of the aortic root should be treated with aortic root replacement with a composite graft or with a valve sparing root replacement. If a DeBakey Type II dissection is present, the entire dissected aorta should be replaced. *(LOE: C)*

10. Recommendation for Intramural Hematoma Without Intimal Defect

Class IIa

1. It is reasonable to treat intramural hematoma similar to aortic dissection in the corresponding segment of the aorta. *(LOE: C)*

11. Recommendation for History and Physical Examination for Thoracic Aortic Disease

Class I

1. For patients presenting with a history of acute cardiac and noncardiac symptoms associated with a significant likelihood of thoracic aortic disease, the clinician should perform a focused physical examination, including a careful and complete search for arterial perfusion differentials in both upper and lower extremities, evidence of visceral ischemia, focal neurologic deficits, a murmur of aortic regurgitation, bruits, and findings compatible with possible cardiac tamponade. *(LOE: C)*

12. Recommendation for Medical Treatment of Patients With Thoracic Aortic Diseases

Class I

1. Stringent control of hypertension, lipid profile optimization, smoking cessation, and other atherosclerosis risk-reduction measures should be instituted for patients with small aneurysms not requiring surgery, as well as for patients who are not considered surgical or stent graft candidates. *(LOE: C)*

12.1. Recommendations for Blood Pressure Control

Class I

1. Antihypertensive therapy should be administered to hypertensive patients with thoracic aortic diseases to achieve a goal of less than 140/90 mm Hg (patients without diabetes) or less than 130/80 mm Hg (patients with diabetes or chronic renal disease) to reduce the risk of stroke, myocardial infarction, heart failure, and cardiovascular death. *(LOE: B)*

2. Beta adrenergic–blocking drugs should be administered to all patients with Marfan syndrome and aortic aneurysm to reduce the rate of aortic dilatation unless contraindicated. *(LOE: B)*

Class IIa

1. For patients with thoracic aortic aneurysm, it is reasonable to reduce blood pressure with beta blockers and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers to the lowest point patients can tolerate without adverse effects. *(LOE: B)*

2. An angiotensin receptor blocker (losartan) is reasonable for patients with Marfan syndrome, to reduce the rate of aortic dilatation unless contraindicated. *(LOE: B)*

13. Recommendations for Asymptomatic Patients With Ascending Aortic Aneurysm (see Figures 6 and 7)

Class I

1. Asymptomatic patients with degenerative thoracic aneurysm, chronic aortic dissection, intramural hematoma, penetrating atherosclerotic ulcer, mycotic aneurysm, or pseudoaneurysm, who are otherwise suitable candidates and for whom the ascending aorta or aortic sinus diameter is 5.5 cm or greater should be evaluated for surgical repair. *(LOE: C)*

2. Patients with Marfan syndrome or other genetically mediated disorders (vascular Ehlers-Danlos syndrome, Turner syndrome, bicuspid aortic valve, or familial thoracic aortic aneurysm and dissection) should undergo elective operation at smaller diameters (4.0 to 5.0 cm depending on the condition; see Section 4) to avoid acute dissection or rupture. *(LOE: C)*

3. Patients with a growth rate of more than 0.5 cm/y in an aorta that is less than 5.5 cm in diameter should be considered for operation. *(LOE: C)*

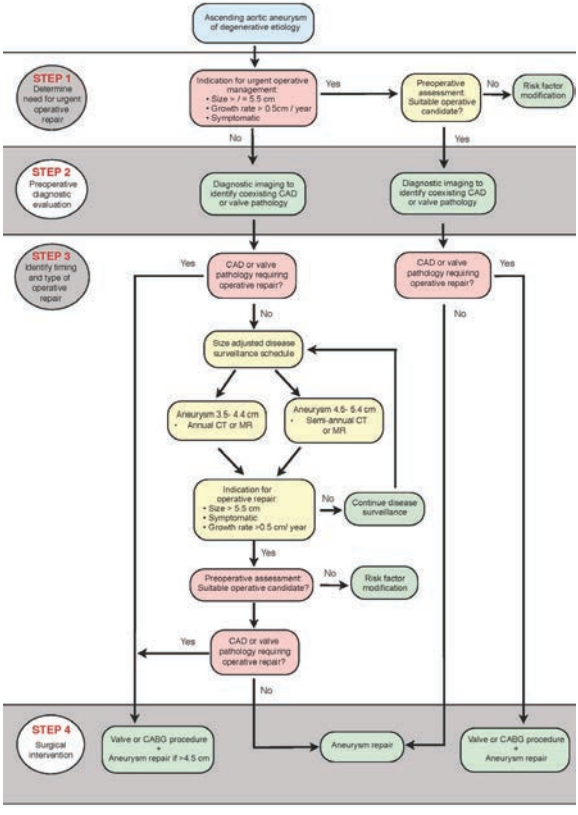
4. Patients undergoing aortic valve repair or replacement and who have an ascending aorta or aortic root of greater than 4.5 cm should be considered for concomitant repair of the aortic root or replacement of the ascending aorta. *(LOE: C)*

Class IIa

1. Elective aortic replacement is reasonable for patients with Marfan syndrome, other genetic diseases, or bicuspid aortic valves, when the ratio of maximal ascending or aortic root area ($\prod r^2$) in cm^2 divided by the patient's height in meters exceeds 10. *(LOE: C)*

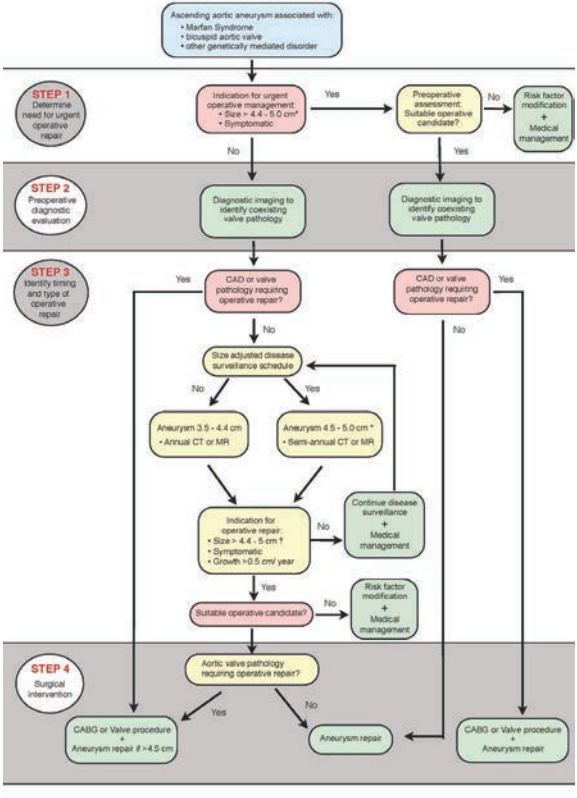
2. It is reasonable for patients with Loeys-Dietz syndrome or a confirmed *TGFBR1* or *TGFBR2* mutation to undergo aortic repair when the aortic diameter reaches 4.2 cm or greater by transesophageal echocardiogram (internal diameter) or 4.4 to 4.6 cm or greater by computed tomographic imaging and/or magnetic resonance imaging (external diameter). *(LOE: C)*

Figure 6. Ascending Aortic Aneurysm of Degenerative Etiology.



CABG indicates coronary artery bypass graft surgery; CAD, coronary artery disease; CT, computed tomographic imaging; and MR, magnetic resonance imaging.

Figure 7. Ascending Aortic Aneurysms Associated With Genetic Disorder.



*Depends on specific genetic condition. †See Recommendations for Asymptomatic Patients With Ascending Aortic Aneurysm (Section 13), and Recommendations for Bicuspid Aortic Valve and Associated Congenital Variants in Adults (Section 6). CABG indicates coronary artery bypass graft surgery; CAD, coronary artery disease; CT, computed tomographic imaging; and MR, magnetic resonance imaging.

14. Recommendation for Symptomatic Patients With Thoracic Aortic Aneurysm

Class I

1. Patients with symptoms suggestive of expansion of a thoracic aneurysm should be evaluated for prompt surgical intervention unless life expectancy from comorbid conditions is limited or quality of life is substantially impaired. *(LOE: C)*

15. Recommendations for Open Surgery for Ascending Aortic Aneurysm

Class I

1. Separate valve and ascending aortic replacement are recommended in patients without significant aortic root dilatation, in elderly patients, or in young patients with minimal dilatation who have aortic valve disease. *(LOE: C)*

2. Patients with Marfan, Loeys-Dietz, and Ehlers-Danlos syndromes and other patients with dilatation of the aortic root and sinuses of Valsalva should undergo excision of the sinuses in combination with a modified David reimplantation operation if technically feasible or, if not, root replacement with valved graft conduit. *(LOE: B)*

16. Recommendations for Aortic Arch Aneurysms

Class IIa

1. For thoracic aortic aneurysms also involving the proximal aortic arch, partial arch replacement together with ascending aorta repair using right subclavian/axillary artery inflow and hypothermic circulatory arrest is reasonable. *(LOE: B)*

2. Replacement of the entire aortic arch is reasonable for acute dissection when the arch is aneurysmal or there is extensive aortic arch destruction and leakage. *(LOE: B)*

3. Replacement of the entire aortic arch is reasonable for aneurysms of the entire arch, for chronic dissection when the arch is enlarged, and for distal arch aneurysms that also involve the proximal descending thoracic aorta, usually with the elephant trunk procedure. *(LOE: B)*

4. For patients with low operative risk in whom an isolated degenerative or atherosclerotic aneurysm of the aortic arch is present, operative treatment is reasonable for asymptomatic patients when the diameter of the arch exceeds 5.5 cm. *(LOE: B)*

5. For patients with isolated aortic arch aneurysms less than 4.0 cm in diameter, it is reasonable to reimage using computed tomographic imaging or magnetic resonance imaging, at 12-month intervals, to detect enlargement of the aneurysm. *(LOE: C)*

6. For patients with isolated aortic arch aneurysms 4.0 cm or greater in diameter, it is reasonable to reimage using computed tomographic imaging or magnetic resonance imaging, at 6-month intervals, to detect enlargement of the aneurysm. *(LOE: C)*

17. Recommendations for Descending Thoracic Aorta and Thoracoabdominal Aortic Aneurysms

Class I

1. For patients with chronic dissection, particularly if associated with a connective tissue disorder, but without significant comorbid disease, and a descending thoracic aortic diameter exceeding 5.5 cm, open repair is recommended. *(LOE: B)*

2. For patients with degenerative or traumatic aneurysms of the descending thoracic aorta exceeding 5.5 cm, saccular aneurysms, or postoperative pseudoaneurysms, endovascular stent grafting should be strongly considered when feasible. *(LOE: B)*

3. For patients with thoracoabdominal aneurysms, in whom endovascular stent graft options are limited and surgical morbidity is elevated, elective surgery is recommended if the aortic diameter exceeds 6.0 cm, or less if a connective tissue disorder such as Marfan or Loeys-Dietz syndrome is present. *(LOE: C)*

4. For patients with thoracoabdominal aneurysms and with end-organ ischemia or significant stenosis from atherosclerotic visceral artery disease, an additional revascularization procedure is recommended. *(LOE: B)*

Table 7. Summary of Society of Thoracic Surgeons Recommendations for Thoracic Stent Graft Insertion

Entity/Subgroup	Classification	Level of Evidence
Penetrating ulcer/intramural hematoma		
Asymptomatic	III	C
Symptomatic	IIa	C
Acute traumatic	I	B
Chronic traumatic	IIa	C
Acute Type B dissection		
Ischemia	I	A
No ischemia	IIb	C
Subacute dissection	IIb	B
Chronic dissection	IIb	B
Degenerative descending		
>5.5 cm, comorbidity	IIa	B
>5.5 cm, no comorbidity	IIb	C
<5.5 cm	III	C
Arch		
Reasonable open risk	III	A
Severe comorbidity	IIb	C
Thoracoabdominal/Severe comorbidity	IIb	C

Reprinted from Svensson LG, Kouchoukos NT, Miller DC, et al. Expert consensus document on the treatment of descending thoracic aortic disease using endovascular stent-grafts. Ann Thorac Surg. 2008;85:S1–41.

18. Recommendations for Counseling and Management of Chronic Aortic Diseases in Pregnancy

Class I

1. Women with Marfan syndrome and aortic dilatation, as well as patients without Marfan syndrome who have known aortic disease, should be counseled about the risk of aortic dissection as well as the heritable nature of the disease prior to pregnancy. *(LOE: C)*

2. For pregnant women with known thoracic aortic dilatation or a familial or genetic predisposition for aortic dissection, strict blood pressure control, specifically to prevent Stage II hypertension, is recommended. *(LOE: C)*

3. For all pregnant women with known aortic root or ascending aortic dilatation, monthly or bimonthly echocardiographic measurements of the ascending aortic dimensions are recommended to detect aortic expansion until birth. *(LOE: C)*

4. For imaging of pregnant women with aortic arch, descending, or abdominal aortic dilatation, magnetic resonance imaging (without gadolinium) is recommended over computed tomographic imaging to avoid exposing both the mother and fetus to ionizing radiation. Transesophageal echocardiogram is an option for imaging of the thoracic aorta. (LOE: C)

5. Pregnant women with aortic aneurysms should be delivered where cardiothoracic surgery is available. *(LOE: C)*

Class IIa

1. Fetal delivery via cesarean section is reasonable for patients with significant aortic enlargement, dissection, or severe aortic valve regurgitation. *(LOE: C)*

Class IIb

1. If progressive aortic dilatation and/or advancing aortic valve regurgitation are documented, prophylactic surgery may be considered. *(LOE: C)*

19. Recommendations for Aortic Arch and Thoracic Aortic Atheroma and Atheroembolic Disease

Class IIa	**1.** Treatment with a statin is a reasonable option for patients with aortic arch atheroma to reduce the risk of stroke. *(LOE: C)*
Class IIb	**1.** Oral anticoagulation therapy with warfarin (INR 2.0 to 3.0) or antiplatelet therapy may be considered in stroke patients with aortic arch atheroma 4.0 mm or greater to prevent recurrent stroke. *(LOE: C)*

20. Periprocedural and Perioperative Management

20.1. Recommendations for Brain Protection During Ascending Aortic and Transverse Aortic Arch Surgery

Class I	**1.** A brain protection strategy to prevent stroke and preserve cognitive function should be a key element of the surgical, anesthetic, and perfusion techniques used to accomplish repairs of the ascending aorta and transverse aortic arch. *(LOE: B)*
Class IIa	**1.** Deep hypothermic circulatory arrest, selective antegrade brain perfusion, and retrograde brain perfusion are techniques that alone or in combination are reasonable to minimize brain injury during surgical repairs of the ascending aorta and transverse aortic arch. Institutional experience is an important factor in selecting these techniques. *(LOE: B)*

Class III	**1.** Perioperative brain hyperthermia is not recommended in repairs of the ascending aortic and transverse aortic arch as it is probably injurious to the brain. *(LOE: B)*

20.2. Recommendations for Spinal Cord Protection During Descending Aortic Open Surgical and Endovascular Repairs

Class I	**1.** Cerebrospinal fluid drainage is recommended as a spinal cord protective strategy in open and endovascular thoracic aortic repair for patients at high risk of spinal cord ischemic injury. *(LOE: B)*
Class IIa	**1.** Spinal cord perfusion pressure optimization using techniques, such as proximal aortic pressure maintenance and distal aortic perfusion, is reasonable as an integral part of the surgical, anesthetic, and perfusion strategy in open and endovascular thoracic aortic repair patients at high risk of spinal cord ischemic injury. Institutional experience is an important factor in selecting these techniques. *(LOE: B)* **2.** Moderate systemic hypothermia is reasonable for protection of the spinal cord during open repairs of the descending thoracic aorta. *(LOE: B)*

Class IIb

1. Adjunctive techniques to increase the tolerance of the spinal cord to impaired perfusion may be considered during open and endovascular thoracic aortic repair for patients at high risk of spinal cord injury. These include distal perfusion, epidural irrigation with hypothermic solutions, high-dose systemic glucocorticoids, osmotic diuresis with mannitol, intrathecal papaverine, and cellular metabolic suppression with anesthetic agents. *(LOE: B)*

2. Neurophysiological monitoring of the spinal cord (somatosensory evoked potentials or motor evoked potentials) may be considered as a strategy to detect spinal cord ischemia and to guide reimplantation of intercostal arteries and/or hemodynamic optimization to prevent or treat spinal cord ischemia. *(LOE: B)*

21. Recommendations for Surveillance of Thoracic Aortic Disease or Previously Repaired Patients

Class IIa

1. Computed tomographic imaging or magnetic resonance imaging of the thoracic aorta is reasonable after a Type A or B aortic dissection or after prophylactic repair of the aortic root/ascending aorta. *(LOE: C)*

2. Computed tomographic imaging or magnetic resonance imaging of the aorta is reasonable at 1, 3, 6, and 12 months postdissection and, if stable, annually thereafter so that any threatening enlargement can be detected in a timely fashion. *(LOE: C)*

3. When following patients with imaging, utilization of the same modality at the same institution is reasonable, so that similar images of matching anatomic segments can be compared side by side. *(LOE: C)*

4. If a thoracic aortic aneurysm is only moderate in size and remains relatively stable over time, magnetic resonance imaging instead of computed tomographic imaging is reasonable to minimize the patient's radiation exposure. *(LOE: C)*

5. Surveillance imaging similar to classic aortic dissection is reasonable in patients with intramural hematoma. *(LOE: C)*

Table 8. Suggested Follow-up of Aortic Pathologies After Repair or Treatment

Pathology	Interval	Study
Acute dissection	Before discharge, 1 mo, 6 mo, yearly	CT or MR, chest plus abdomen TTE
Chronic dissection	Before discharge, 1 y, 2 to 3 y	CT or MR, chest plus abdomen TTE
Aortic root repair	Before discharge, yearly	TTE
AVR plus ascending	Before discharge, yearly	TTE
Aortic arch	Before discharge, 1 y, 2 to 3 y	CT or MR, chest plus abdomen
Thoracic aortic stent	Before discharge, 1 mo, 2 mo, 6 mo, yearly Or 30 days*	CXR, CT, chest plus abdomen
Acute IMH/PAU	Before discharge, 1 mo, 3 mo, 6 mo, yearly	CT or MR, chest plus abdomen

*US Food and Drug Administration stent graft studies usually required before discharge or at 30-day CT scan to detect endovascular leaks. If there is concern about a leak, a predischarge study is recommended; however, the risk of renal injury should be borne in mind. All patients should be receiving beta blockers after surgery or medically managed aortic dissection, if tolerated. Adapted from Erbel R, Alfonso F, Boileau C, et al. Diagnosis and management of aortic dissection. Eur Heart J. 2001;22:1642–81.

AVR indicates aortic valve replacement; CT, computed tomographic imaging; CXR, chest X-ray; IMH, intramural hematoma; MR, magnetic resonance imaging; PAU, penetrating atherosclerotic ulcer; and TTE, transthoracic echocardiography.

22. Recommendation for Employment and Lifestyle in Patients With Thoracic Aortic Disease

Class IIa

1. For patients with a current thoracic aortic aneurysm or dissection, or previously repaired aortic dissection, employment and lifestyle restrictions are reasonable, including the avoidance of strenuous lifting, pushing or straining that would require a Valsalva maneuver. *(LOE: C)*

Canadian Journal of Cardiology 30 (2014) 577–589

Position Statement

Canadian Cardiovascular Society Position Statement on the Management of Thoracic Aortic Disease

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ABSTRACT

This Canadian Cardiovascular Society position statement aims to provide succinct perspectives on key issues in the management of thoracic aortic disease (TAD). This document is not a comprehensive overview of TAD and important elements of the epidemiology, presentation, diagnosis, and management of acute aortic syndromes are deliberately not discussed; readers are referred to the 2010 guidelines published by the American Heart Association, American College of Cardiology, American Association for Thoracic Surgery, and other stakeholders. Rather, this document is a practical guide for clinicians managing adult patients with TAD. Topics covered include size thresholds for surgical intervention, emerging thera-

RÉSUMÉ

Cet énoncé de position de la Société canadienne de cardiologie a pour but de donner des perspectives succinctes sur les aspects clés de la prise en charge de la maladie de l'aorte thoracique (MAT). Ce document n'est pas un aperçu complet de la MAT, et les éléments importants de l'épidémiologie, du tableau clinique, du diagnostic et de la prise en charge des syndromes aortiques aigus ne sont délibérément pas discutés; les lecteurs sont invités à consulter les lignes directrices de 2010 publiées par l'American Heart Association, l'American College of Cardiology, l'American Association for Thoracic Surgery et les autres parties prenantes. Ce document est plutôt un guide pratique pour les cliniciens

Thoracic Aortic Aneurysm

Size thresholds for elective thoracic aortic intervention

Thoracic aortic aneurysms are largely asymptomatic until a sudden and catastrophic event, including aortic rupture or dissection, occurs, and is rapidly fatal in a large proportion of patients.[1,2] Elective intervention on the thoracic aorta carries a much lower risk of mortality and morbidity, and prophylactic aortic surgery can be life-saving.

The decision to perform aortic intervention is a balance between risks of natural history of the disease vs the risk of the surgical intervention itself, and any additional long-term risks

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The disclosure information of the authors and reviewers is available from the CCS on the following websites: www.ccs.ca and/or www.ccsguidelineprograms.ca.

This statement was developed following a thorough consideration of medical literature and the best available evidence and clinical experience. It represents the consensus of a Canadian panel comprised of multidisciplinary experts on this topic with a mandate to formulate disease-specific recommendations. These recommendations are aimed to provide a reasonable and practical approach to care for specialists and allied health professionals obliged with the duty of bestowing optimal care to patients and families, and can be subject to change as scientific knowledge and technology advance and as practice patterns evolve. The statement is not intended to be a substitute for physicians using their individual judgement in managing clinical care in consultation with the patient, with appropriate regard to all the individual circumstances of the patient, diagnostic and treatment options available and available resources. Adherence to these recommendations will not necessarily produce successful outcomes in every case.

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Special Report: Exome Sequencing for Clinical Diagnosis of Patients with Suspected Genetic Disorders

Assessment Program
Volume 28, No. 3
August 2013

Executive Summary

Background

There are different clinical diagnostic scenarios for which traditional Sanger DNA sequencing technology is clinically available. Its success as a diagnostic tool depends on the accuracy and completeness of the differential diagnosis, as well as the number and locations of mutations responsible for the observed disease. Single-gene disorders are diseases that result from a mutation in one gene that has a large effect on phenotype. A clinical focus of this Special Report is the scenario of an undiagnosed, suspected single-gene disorder that features multiple congenital anomalies. Disorders (often early developmental) that present with multiple anomalies suggest a genetic etiology. They are often clinically difficult to diagnose due to nonspecific presentation, potentially overlapping differential diagnoses (clinical heterogeneity) or a rare disorder, and lack of a clear diagnostic testing path. The search for a clinical diagnosis may result in a long (months to years) diagnostic odyssey comprising several specialist consults and many different individual molecular, cytogenetic and biochemical tests, as well as other types of diagnostic procedures. A relatively unbiased sequencing application that examines most genes without requiring prior knowledge of potential diagnoses would be advantageous.

Single-gene disorders are inherited in various patterns (e.g., autosomal dominant, autosomal recessive, X-linked) and are affected by penetrance (proportion of individuals with the disease-related mutation who have the disease phenotype at all) and by expressivity (variable phenotypes that can occur in different individuals with the same genetic mutation). Disorders arising from a mutation in a single gene may be caused by exactly the same mutation in all individuals with the condition, or may be caused by any one of many different mutations in the same gene (allelic heterogeneity). In contrast, a large proportion of heritable disorders are genetically heterogeneous; clinically indistinguishable or overlapping phenotypes may be caused by mutations in different genes (locus heterogeneity). The number of genes involved can range from just a few to hundreds. For many of these genetically heterogeneous disorders, the full complement of causal genes is not yet known.

Determining genetic causality for disease and establishing a molecular diagnosis in clinical practice is challenging; in recent years, molecular technologies have strikingly increased gene discovery and understanding the causes of single-gene disorders among patients with suspected, but previously undiagnosed, genetic disorders. The value of molecular diagnosis in these patients can be viewed from a clinical perspective similar to other diagnostic tests; it is also commonly addressed from perspectives of clinical and personal utility, as well as a societal view. A molecular diagnosis can: confirm a suspected or established clinical diagnosis; inform prognosis; aid in selecting treatment, surveillance or preventive options; reveal mode of inheritance; identify carrier/risk status of family members; and guide research regarding new therapy or patient management. Available guidelines are currently focused on molecular diagnosis, distinguishing among phenotypically overlapping possible conditions, and on referral for genetic consultation.

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Clinical Policy Bulletin:
Genetic Testing
Number: 0140

Policy

Aetna considers genetic testing medically necessary to establish a molecular diagnosis of an inheritable disease when *all* of the following are met:

- The member displays clinical features, or is at direct risk of inheriting the mutation in question (pre-symptomatic); *and*
- The result of the test will directly impact the treatment being delivered to the member; *and*
- After history, physical examination, pedigree analysis, genetic counseling, and completion of conventional diagnostic studies, a definitive diagnosis remains uncertain, and one of the following diagnoses is suspected (this list is not all-inclusive):

Achondroplasia (FGFR3) Albinism Alpha-1 antitrypsin deficiency (SERPINA1) Alpha thalassemia/Hb Bart hydrops fetalis syndrome/HbH disease** (HBA1/HBA2, alpha globin 1 and alpha globulin 2) Angelman syndrome (GABRA, SNRPN Beta thalassemia** (beta globin) Bloom syndrome (BLM) CADASIL (see below) Canavan disease (ASPA (aspartoacylase A)) Charcot-Marie Tooth disease (PMP-22) Classical lissencephaly Congenital adrenal hyperplasia/21 hydroxylase deficiency (CYP21A2)* Congenital amegakaryocytic thrombocytopenia Congenital central hypoventilation syndrome (PHOX2B) Congenital muscular dystrophy type 1C (MDC1C) (FKRP (Fukutin related protein)) Crouzon syndrome (FGFR2, FGFR3) Cystic fibrosis (CFTR) (see below) Dentatorubral-pallidoluysian atrophy Duchenne/Becker muscular dystrophy (dystrophin) Dysferlin myopathy Ehlers-Danlos syndrome Emery-Dreifuss muscular dystrophy (EDMD1, 2, and 3) Fabry disease	Hereditary polyposis coli (APC) Hereditary spastic paraplegia 3 (SPG3A) and 4 (SPG4, SPAST) Huntington's disease (HTT, HD (Huntington)) Hypochondroplasia (FGFR3) Hypertrophic cardiomyopathy (see below) Jackson-Weiss syndrome (FGFR2)Kallmann syndrome (FGFR1) Kennedy disease (SBMA) Leber hereditary optic neuropathy (LHON) Leigh Syndrome and NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa) Long QT syndrome (see below) Limb girdle muscular dystrophy (LGMD1, LGMD2) (FKRP (Fukutin related protein)) Malignant hyperthermia (RYR1) Maple syrup urine disease (branched-chain keto acid dehydrogenase E1) Marfan's syndrome (TGFBR1, TGFBR2) McArdle's disease Medium chain acyl coA dehydrogenase deficiency (ACADM) Medullary thyroid carcinoma MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) (MTTL1, tRNAleu) Mucolipidosis type IV (MCOLN1, mucolipin 1) Mucopolysaccharidoses type 1 (MPS-1)

Policy History
Last Review: 08/01/2014
Effective: 05/31/1996
Next Review: 02/12/2015
Review History
Definitions
Additional Information
Clinical Policy Bulletin Notes

Factor V Leiden mutation (F5 (Factor V))	Muenke syndrome (FGFR3)
Factor XIII deficiency, congenital (F13 (Factor XIII beta globulin))	Multiple endocrine neoplasia type 1
Familial adenomatous polyposis coli (APC) (see below)	Muscle-Eye-Brain disease (POMGNT1)
Familial dysautonomia (IKBKAP)	MYH-associated polyposis (MYH) (see below)
Familial hypocalciuric hypercalcemia (see below)	Myoclonic epilepsy (MERRF) (MTTK (tRNAlys))
Familial Mediterranean fever (MEFV)	Myotonic dystrophy (DMPK, ZNF-9)
Fanconi anemia (FANCC, FANCD)	Neimann-Pick disease, type A (SMPD1, sphingomyelin phosphodiesterase)
Facioscapulohumeral muscular dystrophy (FSHMD1A)	Nephrotic syndrome, congenital (NPHS1, NPHS2)
Fragile X syndrome, FRAXA (FMR1) (see below)	Neurofibromatosis type 1 (NF1, neurofibromin)
Friedreich's ataxia (FRDA (frataxin))	Neurofibromatosis type 2 (Merlin)
Galactosemia (GALT)	Neutropenia, congenital cyclic
Gaucher disease (GBA (acid beta glucosidase))	Phenylketonuria (PAH)
Gitelman's syndrome	Pfeiffer syndrome (FGFR1)
Hemoglobin E thalassemia **	Prader-Willi-Angelman syndrome (SNRPN, GABRA5, NIPA1, UBE3A, ANCR, GABRA)
Hemoglobin S and/or C **	Primary dystonia (TOR1A (DYT1))
Hemophilia A/VWF (F8 (Factor VIII))	Prothrombin (F2 (Factor II, 20210G> A mutation))
Hemophilia B (F9 (Factor IX))	Pyruvate kinase deficiency (PKD)
Hereditary amyloidosis (TTR variants)	Retinoblastoma (Rh)
Hereditary deafness (GJB2 (Connexin-26, Connexin-32))	Rett syndrome (FOXG1, MECP2)
Hereditary hemorrhagic telangiectasia (HHT)	Saethre-Chotzen syndrome (TWIST, FGFR2)
Hereditary hemochromatosis (HFE) (see below)	SHOX-related short stature (see below)
Hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome (fumarate hydratase (FH) gene)	Smith-Lemli-Opitz syndrome
Hereditary neuropathy with liability to pressure palsies (HNPP)	Spinal muscular atrophy (SMN1, SMN2)
Hereditary non-polyposis colorectal cancer (HNPCC) (MLH1, MSH2, MSH6. MSI) (see below)	Spinocerebellar ataxia (SCA types 1, 2, 3 (MJD), 6 (CACNA1A), 7, 8, 10, 17 and DRPLA)
Hereditary pancreatitis (PRSS1) (see below)	Tay-Sachs disease (HEXA (hexosaminidase A))
Hereditary paraganglioma (SDHD, SDHB)	Thanatophoric dysplasia (FGFR3)
	Von Gierke disease (G6PC, Glycogen storage disease, Type 1a)
	Von Hippel-Lindau syndrome (VHL)
	Walker-Warburg syndrome (POMGNT1)
	22q11 deletion syndromes (DCGR (CATCH-22))

* Medically necessary if results of the adrenocortical profile following cosyntropin stimulation test are equivocal or for purposes of genetic counseling.

** Electrophoresis is the appropriate initial laboratory test for individuals judged to be at-risk for a hemoglobin disorder.

In the absence of specific information regarding advances in the knowledge of mutation characteristics for a particular disorder, the current literature indicates that genetic tests for inherited disease need only be conducted once per lifetime of the member.

Note: Genetic testing of Aetna members is excluded from coverage under Aetna's benefit plans if the testing is performed primarily for the medical management of other family members who are not covered under an Aetna benefit plan. In these circumstances, the insurance carrier for the family members who are not covered by Aetna should be contacted regarding coverage of genetic testing. Occasionally, genetic testing of tissue samples from other family members who are not covered by Aetna may be required to provide the medical information necessary for the proper medical care of an Aetna member. Aetna covers genetic testing for heritable disorders in non-Aetna members when *all* of the following conditions are met:

- The information is needed to adequately assess risk in the Aetna member; *and*
- The information will be used in the immediate care plan of the Aetna member; *and*

The non-Aetna member's benefit plan, if any, will not cover the test (a copy of the denial letter*** from the non-Aetna member's benefit plan must be provided).

*** Aetna may also request a copy of the certificate of coverage from the non-member's health insurance plan if: (i) the denial letter from the non-member's insurance carrier fails to specify the basis for non-coverage; (ii) the denial is based on a specific plan exclusion; or (iii) the genetic test is denied by the non-member's insurance carrier as not medically necessary and the medical information provided to Aetna does not make clear why testing would not be of significant medical benefit to the non-member.

Medical Necessity Criteria for Specific Genetic Tests:

1. Adenosis polyposis coli (APC):

   Aetna considers adenosis polyposis coli (APC) genetic testing medically necessary for either of the following indications:

   1. Members with greater than 20 colonic polyps; *or*
   2. Members with 1st-degree relatives (i.e., siblings, parents, and offspring) diagnosed with familial adenomatous polyposis (FAP) or with a documented APC mutation. The specific APC mutation should be identified in the affected 1st-degree relative with FAP prior to testing the member, if feasible. Full sequence APC genetic testing is considered medically necessary only when it is not possible to determine the family mutation first.

   Aetna considers APC genetic testing experimental and investigational for all other indications (e.g., desmoid tumor) because its effectiveness for indications other than the ones listed above has not been established.

2. CADASIL:

   Aetna considers DNA testing for CADASIL medically necessary for *either* of the following indications:

   1. Pre-symptomatic individuals where there is a family history consistent with an autosomal dominant pattern of inheritance and there is a known mutation in an affected member of the family; *or*
   2. Symptomatic individuals who have a family history consistent with an autosomal dominant pattern of inheritance of this condition (clinical signs and symptoms of CADASIL include stroke, cognitive defects and/or dementia, migraine, and psychiatric disturbances).

   Aetna considers CADASIL genetic testing experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

3. Catecholaminergic polymorphic ventricular tachycardia (CPVT):

   Aetna considers genetic testing for CPVT medically necessary for the following indications:

   1. Children or young adults (less than 40 years of age) with a 1st degree relative with a clinical diagnosis of CPVT, or a 1st or 2nd degree relative with a defined CPVT mutation; or
   2. Persons who display exercise- or emotion-induced PVT or ventricular fibrillation, occurring in a structurally normal heart.

4. Cystic fibrosis:

   Aetna considers genetic carrier testing for cystic fibrosis medically necessary for members in any of the following groups:

   1. Couples seeking prenatal care; *or*
   2. Couples who are planning a pregnancy; *or*
   3. Persons with a family history of cystic fibrosis; *or*
   4. Persons with a 1st degree relative identified as a cystic fibrosis carrier; *or*
   5. Reproductive partners of persons with cystic fibrosis.

   Aetna considers genetic carrier testing for cystic fibrosis experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

   Aetna considers a core panel of 25 mutations that are recommended by the American College of Medical Genetics (ACMG) medically necessary for cystic fibrosis genetic testing. The standard CF transmembrane regulator (CFTR)mutation panel is as follows (Available at: http://www.acmg.net/):

ΔF508	ΔI507	G542X	G551D	W1282X	N1303K
R553X	621+1G→T	R117H	1717-1G→A	A455E	R560T
R1162X	G85E	R334W	R347P	711+1G→T	1898+1G→A
		3849+10kbC→	2789+5G→		

2184delA	1078delT	T	A	3659delC	I148T
3120+1G→ A					

Aetna considers experimental and investigational screening for cystic fibrosis mutations that extend beyond the standard mutation panel recommended by the ACMG.

5. Factor V Leiden:

   Aetna considers Factor V Leiden genetic testing medically necessary for members with *any* of the following indications:

   1. Age less than 50, any venous thrombosis; *or*
   2. Myocardial infarction in female smokers under age of 50; *or*
   3. Recurrent venous thrombosis; *or*
   4. Relatives of individuals with venous thrombosis under age of 50; *or*
   5. Venous thrombosis and a strong family history of thrombotic disease; *or*
   6. Venous thrombosis in pregnant women or women taking oral contraceptives; *or*
   7. Venous thrombosis in unusual sites (such as hepatic, mesenteric, and cerebral veins).

   Aetna considers Factor V Leiden genetic testing experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established .

   Aetna considers Factor V HR2 allele DNA mutation analysis experimental and investigational because its effectiveness has not been established.

6. Familial nephrotic syndrome (NPHS1, NPHS2):

   1. Aetna considers genetic testing for an NPHS1 mutation medically necessary for children with congenital nephrotic syndrome (nephrotic syndrome appearing within the first month of life) who are of Finnish descent or who have a family history of congenital nephrotic syndrome. Genetic testing for NPHS1 mutations are considered experimental and investigational for screening other persons with nephrotic syndrome and for all other indications because its effectiveness for other indications other has not been established.
   2. Aetna considers genetic testing for an NPHS2 mutation medically necessary for children with steroid resistant nephrotic syndrome (SRNS) and for children who have a family history of SRNS. Genetic testing for NPHS2 is considered experimental and investigational for persons with steroid-responsive nephrotic syndrome and for all other indications because its effectiveness for indications other than the ones listed above has not been established.

   Aetna considers genetic testing for familial nephrotic syndrome experimental and investigational for all other indications.

7. Fragile X:

   Aetna considers genetic testing for fragile X syndrome medically necessary for members in any of the following risk categories where the results of the test will affect a member's clinical management or reproductive decisions:

   1. Individuals with mental retardation, developmental delay, or autism; *or*
   2. Individuals planning a pregnancy who have *either* of the following:

      1. A family history of fragile X syndrome, *or*
      2. A family history of undiagnosed mental retardation; *or*

   3. Fetuses of known carrier mothers. Prenatal testing of a fetus by amniocentesis or chorionic villus sampling is indicated following a positive Fragile X carrier test in the mother.

   Aetna considers Fragile X DNA testing medically necessary for members with a negative cytogenetic test for fragile X if they have any physical or behavioral characteristics of fragile X syndrome and have a family history of fragile X syndrome or undiagnosed mental retardation.

   Aetna considers Fragile X DNA testing medically necessary for members with a phenotype that is not typical for fragile X syndrome who have a cytogenetic test that is positive for fragile X.

   Aetna considers population-based fragile X syndrome screening of individuals who are not in any of the above-listed risk categories experimental and investigational because its effectiveness for indications other than the ones listed above has not been established.

8. Hereditary hemochromatosis:

   Aetna considers genetic testing for HFE gene mutations medically necessary for persons who meet all of the following criteria:

1. Member who has symptoms consistent with iron overload; and
2. Member who has 2 consecutive transferrin saturations of 45 % or more.

Aetna considers genetic testing for HFE gene mutations medically necessary for 1st degree relatives of persons homozygous for HFE gene mutations. Genetic testing for hereditary hemochromatosis is considered experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

9. Hereditary non-polyposis colorectal cancer (HNPCC):

    1. Aetna considers genetic testing for HNPCC (MLH1, MSH2, MSH6, PMS2 sequence analysis) medically necessary for members who meet any one of the following criteria:

        1. Member meets Amsterdam II criteria or revised Bethesda guidelines (see appendix); *or*
        2. Member is diagnosed with endometrial cancer before age 50 years; *or*
        3. Member has a 1st- or 2nd-degree relative with a disease confirmed to be caused by a HNPCC mutation (genes MLH1, MSH2, MSH6, PMS2) upon testing of the 1st- or 2nd-degree relative.

    2. Aetna considers microsatellite instability (MSI) testing or immunohistochemical (IHC) analysis of tumors medically necessary as an initial test in persons with colorectal and/or endometrial cancer who meet the revised Bethesda criteria (see appendix) in order to identify those persons who should proceed with HNPCC mutation analysis.

    See also CPB 0516 - Colorectal Cancer Screening.

10. Hereditary pancreatitis (PRSS1):

    Aetna considers genetic testing for hereditary pancreatitis (PRSS1 mutation) medically necessary in symptomatic persons with any of the following indications:

    1. A family history of pancreatitis in a 1st-degree (parent, sibling, child) or 2nd-degree (aunt, uncle, grandparent) relative; *or*
    2. An unexplained episode of documented pancreatitis occurring in a child that has required hospitalization, and where there is significant concern that hereditary pancreatitis should be excluded; *or*
    3. Recurrent (2 or more separate, documented episodes with hyper-amylasemia) attacks of acute pancreatitis for which there is no explanation (anatomical anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gallstones, alcohol, drugs, hyperlipidemia, etc.); *or*
    4. Unexplained (idiopathic) chronic pancreatitis.

    This policy is based upon guidelines from the Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, the Midwest Multi-Center Pancreatic Study Group and the International Association of Pancreatology (Ellis et al, 2001).

    Aetna considers genetic testing for hereditary pancreatitis experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

11. Long QT syndrome:

    Aetna considers genetic testing for long QT syndrome medically necessary for either of the following:

    1. Persons with a prolonged QT interval on resting electrocardiogram (a corrected QT interval (QTc) of 470 msec or more in males and 480 msec or more in females) without an identifiable external cause for QTc prolongation (such as heart failure, bradycardia, electrolyte imbalances, certain medications and other medical conditions); *or*
    2. Persons with 1st-degree relatives (siblings, parents, offspring) with a defined LQT mutation, or long QT syndrome in sudden death (1st or 2nd degree) close relatives.

    Aetna considers genetic testing for long QT syndrome experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

12. Malignant Hyperthermia Susceptibility:

    Aetna considers genetic testing for malignant hyperthermia susceptibility (MHS) medically necessary for either of the following indications:

    1. screening clinically confirmed MHS patients for variants in the RYR1 gene that are considered causative for MH by the European Malignant Hyperthermia Group (EMHG) to facilitate predictive testing in at-risk relatives.
    2. screening at-risk relatives of patients with clinically confirmed MHS for a known familial variant in the RYR1 gene that is considered causative for MH by the EMHG.

    Aetna considers genetic testing for malignant hyperthermia susceptibility (MHS) experimental and investigational for all other indications.

Aetna considers genetic testing for central core disease (CCD) experimental and investigational because there is inadequate evidence in the peer-reviewed published literature regarding its effectiveness.

13. MYH-associated polyposis:

    Aetna considers testing for MYH mutations medically necessary for the following indications:

    1. Asymptomatic siblings of individuals with known MYH polyposis; *or*
    2. Individuals with personal history of adenomatous polyposis who have negative APC mutation testing and a negative family history for adenomatous polyposis; *or*
    3. Individuals with personal history of adenomatous polyposis whose family history is consistent with recessive inheritance (i.e., family history is positive only for sibling(s)).

    Aetna considers MYH mutations testing experimental and investigational for members of a polyposis family with clear autosomal dominant inheritance, or for any other indications because its effectiveness for indications other than the ones listed above has not been established.

14. Primary dystonia (DYT1):

    Aetna considers genetic testing for DYT1 medically necessary for the following indications:

    1. Parents of children with an established DYT1 mutation, for purposes of family planning; *or*
    2. Persons with onset of primary dystonia other than focal cranial-cervical dystonia after age 30 years who have a affected relative with early onset (before 30 years); *or*
    3. Persons with primary dystonia with onset before age 30 years.

    Aetna considers DYT-1 testing experimental and investigational for all other indications, including the following because its effectiveness for indications other than the ones listed above has not been established:

    1. Asymptomatic individuals (other than parents of affected children), including those with affected family members (genetic testing for dystonia (DYT-1) is not sufficient to make a diagnosis of dystonia unless clinical features show dystonia); *or*
    2. Persons with onset of symptoms after age 30 years who either have focal cranial-cervical dystonia; *or*
    3. Persons with onset of symptoms after age 30 years who have no affected relative with early onset dystonia.

    This policy is adapted from guidelines from the European Federation of Neurological Societies.

15. Spinal Muscular Atrophy

    Aetna considers genetic testing for spinal muscular atrophy (SMA) medically necessary for the following indications:

    1. the diagnosis of persons with hypotonia and muscle weakness who are suspected of having spinal muscular atrophy; or
    2. the identification of SMN1 deletion carriers in the families of persons with SMA; or
    3. the prenatal diagnosis or preimplantation genetic diagnosis of SMA in the pregnancy of two known carriers

    Aetna considers genetic testing for spinal muscular atrophy (SMA) experimental and investigational for the identification of SMN1 deletion carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

16. SHOX-related short stature:

    Aetna considers genetic testing for SHOX-related short stature medically necessary for children and adolescents with any of the following features:

    1. Above-average body mass index (BMI); *or*
    2. Cubitus valgus (increased carrying angle); *or*
    3. Dislocation of the ulna at the elbow; *or*
    4. Increased sitting height/height ratio; *or*
    5. Madelung deformity of the forearm; *or*
    6. Muscular hypertrophy; *or*
    7. Reduced arm span/height ratio; *or*
    8. Short or bowed forearm.

    Aetna considers genetic testing for SHOX-related short stature experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

17. Hypertrophic cardiomyopathy (HCM):

    Aetna considers genetic testing for HCM medically necessary for individuals who are at-risk for development of HCM, defined as having a close (1st or 2nd degree) relative with established HCM, when there is a known pathogenic gene mutation present in an affected relative. Aetna considers genetic testing for HCM experimental and investigational for all

other indications because its effectiveness for indications other than the one listed above has not been established.

18. Thoracic aortic aneurysms and dissections (TAAD)

    Aetna considers genetic testing for thoracic aortic aneurysms and dissections (TAAD) medically necessary for asymptomatic blood relatives of persons with genetically confirmed TAAD. Genetic testing for thoracic aortic aneurysms and dissections (TAAD) is considered experimental and investigational for any other indication, including but not limited to patients clinically diagnosed with TAAD, with a positive family history of the disorder, and for whom a genetic syndrome has been excluded.

19. Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C)

    Genetic testing for Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C) is considered medically necessary for the following indications:

    1. Testing for sequence variants in the DSG2, DSP, and PKP2 genes in probands with ITF-confirmed ARVD/C to facilitate genetic screening for ARVD/C in at-risk relatives; or
    2. Testing for a known familial sequence variant in the DSG2, DSP, and PKP2 genes for at-risk relatives of probands with ITF-confirmed ARVD/C, who are either asymptomatic or have ARVD/C symptoms that fails to meet the ITF diagnostic criteria.

    Genetic testing for ARVD/C is considered experimental and investigational for all other indications.

20. Osteogenesis imperfecta

    Genetic testing for COL1A1 and COL1A2 gene sequencing in the management of osteogenesis imperfecta types I to IV, is considered medically necessary for the following indications:

    1. Genetic testing for sequence variants in COL1A1/2 to confirm the presence of mosaicism in the asymptomatic parent of a child with OI caused by sequence variants in COL1A1/2 for reproductive decision making purposes; or
    2. Preimplantation genetic diagnosis or prenatal diagnosis for sequence variants in COL1A1/2 in couples in which 1 or both members have OI caused by sequence variants in COL1A1/2.

    Genetic testing for COL1A1 and COL1A2 gene sequencing is considered experimental and investigational in any other circumstances, including, but not limited to:

    1. testing for sequence variants in COL1A1/2 to confirm diagnosis of OI when clinical and radiological examination and family history provide adequate information for diagnosis of OI.
    2. Genetic testing for sequence variants in COL1A1/2 for the diagnosis of OI when clinical and radiological examination and family history provide inadequate information for diagnosis of OI.
    3. Genetic testing for sequence variants in COL1A1/2 in children diagnosed with OI to aid in reproductive planning for unaffected couples seeking to have additional children.

    Genetic testing for COLA1A/2 is considered experimental and investigational for all other indications.

21. Neurofibromatosis

    Genetic testing for neurofibromatosis is considered medically necessary for persons who meet all of the following criteria:

    - Displays a sign of or has clinical features of the NF; and
    - Has a 50% risk of inheriting NF (pre-symptomatic); and
    - A definitive diagnosis remains uncertain despite a complete family/personal history, physical examination and conventional diagnostic studies; and
    - Confirmation of the diagnosis will impact treatment.

    Genetic testing for neurofibromatosis is considered experimental and investigational for all other indications.

22. Marfan syndrome

    Aetna considers genetic testing for Marfan syndrome (MFS) medically necessary for the following indications:

    1. the use of FBN1 gene testing to facilitate the diagnosis of Marfan syndrome in patients who do not fulfill the Ghent diagnostic criteria but have at least one major feature of the condition; or
    2. the use of Marfan syndrome gene testing in patients fulfilling the Ghent diagnostic criteria for the purpose of obtaining information for reproductive decision making or facilitating the diagnosis of Marfan syndrome in at-risk relatives; or.
    3. the use of Marfan syndrome gene testing in the evaluation of at-risk relatives of patients carrying known disease-causing variants; or
    4. the prenatal diagnosis or PGD of Marfan syndrome in the offspring of patients with known disease-causing variants.

    Genetic testing for Marfan syndrome (MFS) is considered experimental and investigational for any other indications, including but not limited to:

1. the use of FBN1 gene testing in the diagnostic evaluation of Marfan syndrome in patients exhibiting only minor features of the condition, according to the Ghent diagnostic criteria.
2. the use of TGFBR2 gene testing to facilitate the diagnosis of Marfan syndrome in patients testing negative for FBN1 gene variants
3. the use of TGFBR1 gene testing to facilitate the diagnosis of Marfan syndrome in patients testing negative for FBN1 and TGFBR2 gene variants.
4. the use of Marfan syndrome gene testing in patients fulfilling the Ghent diagnostic criteria who will not be using the information for reproductive decision making or facilitating the diagnosis of Marfan syndrome in at-risk relatives.

23. Cadherin-1 for hereditary diffuse gastric cancer

Cadherin-1 (e-cadherin, CDH1) for hereditary diffuse gastric cancer (HDGC) is considered medically necessary for persons who meet the following criteria:

1. Individuals with HDGC for the purpose of identifying a CDH1 gene sequence variant that may be used to screen at-risk first- and second-degree relatives; or
2. Presymptomatic testing of first- and second-degree at-risk relatives for a known familial variant in the CDH1 gene.

Cadherin 1 testing is considered experimental and investigational for confirming the clinical diagnosis of HDGC and for other indications because there is inadequate evidence in the peer-reviewed published clinical literature regarding its effectiveness.

24. Maturity Onset Diabetes of the Young (MODY)

Genetic testing for maturity-onset diabetes of the young (MODY) is considered medically necessary for the diagnosis of MODY2 or MODY3 in persons with hyperglycemia or non-insulin-dependent diabetes who have a family history of abnormal glucose metabolism in at least 2 consecutive generations, with the patient or ≥ 1 family members diagnosed before age 25.

Genetic testing for maturity-onset diabetes of the young (MODY) is considered experimental and investigational for all other indications.

25. Huntington disease

Aetna considers genetic testing for Huntington disease (HD) medically necessary for either of the following indications:

1. predictive testing for CAG repeat length in asymptomatic individuals from families in which there is a history of HD to define risk of transmission; or
2. prenatal testing for CAG repeat length in fetuses from families in which there is a history of HD.

Genetic testing for Huntington disease is considered experimental and investigational for indications other than those listed above.

26. Familial hypocalciuric hypercalcemia:

Aetna considers familial hypocalciuric hypercalcemia (FHH) medically necessary in any of the following:

1. Atypical cases where no family members are available for testing; *or*
2. Families with familial isolated hyperparathyroidism; *or*
3. Infants or children under 10 years of age in whom neonatal hyperparathyroidism, neonatal severe hyperparathyroidism, and FHH are the commonest causes of parathyroid hormone-dependent hypercalcemia; *or*
4. Individuals with overlap in the calcium/creatinine (Ca/Cr) clearance ratio, namely between 0.01 and 0.02; *or*
5. Individuals with the phenotype of FHH whose parents are both normocalcemic (i.e., FHH possibly caused by a de novo CaSR mutation).

27. PTEN Gene Testing

Based upon guidelines from the National Comprehensive Cancer Network, PTEN gene testing is considered medically necessary in individuals with a suspected or known clinical diagnosis of Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome (BRR), or a known family history of a PTEN mutation who meet any of the following criteria:

1. A relative with a known deleterious PTEN gene mutation; *or*
2. Personal history of any of the following:

    1. Bannayan RIley-Ruvalcaba syndrome; *or*
    2. Adult Lhermitte-Duclos disease (LDD); *or*
    3. Autism-spectrum disorder and macrocephaly; *or*
    4. At least 2 biopsy-proven trichilemmomas; *or*
    5. Macrocephaly plus one other major criteria*; *or*
    6. Three major criteria* without macrocephaly; *or*

7. One major and at least three minor criteria*; *or*
8. Four or more minor criteria*.

3. Family history of both of the following:

    1. At-risk relative (includes 1st-degree relative or more distant relatives if the 1st degree relative is unavailable or unwilling to be tested) with a clinical diagnosis of Cowden syndrome or BRR (no previous genetic testing); *and*
    2. One major or two minor criteria* in the at-risk relative.

* Criteria for PTEN genetic testing purposes are:

Major:

- Breast cancer
- Mucocutaneous lesions
    - One biopsy-proven trichilemmoma
    - Multiple palmoplantar keratoses
    - Multi-focal or extensive oral mucosal papillomatosis
    - Multiple cutaneous facial papules (often verrucous)
    - Macular pigmentation of glans penis
- Macrocephaly (97th percentile or greater; 58 cm in adult women, 60 cm in adult men)
- Endometrial cancer
- Non-medullary thyroid cancer
- Multiple GI hamartomas or ganglioneuromas

Minor:

- Other thyroid lesions (e.g., adenoma, nodule(s), goiter)
- Mental retardation (IQ < 75)
- Autism spectrum disorder
- Single gastrointestinal hamartoma or ganglioneuroma
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- Renal cell carcinoma
- Uterine fibroids.

1. Li-Fraumeni syndrome (TP53 gene)

    TP53 gene testing is considered medically necessary for individuals with a suspected or known clinical diagnosis of Li-Fraumeni syndrome (LFS) or Li-Fraumeni-Like syndrome, or a known family history of a TP53 mutation. Testing is considered medically necessary in individuals whose medical and/or family history is consistent with any of these:

    1. A relative with a known deleterious TP53 gene mutation; *or*
    2. A diagnosis of classic Li-Fraumeni syndrome, defined by all of the following:

        1. Diagnosis of sarcoma before the age of 45 years; *and*
        2. A parent, child, or full sibling diagnosed with cancer before the age of 45 years; *and*
        3. An additional 1st- or 2nd-degree relative in the same lineage with cancer diagnosed before age 45 years, or a sarcoma at any age; *or*

    3. Persons meeting Chompret criteria:

        1. Persons with a tumor from the LFS tumor spectrum (e.g., soft tissue sarcoma, osteosarcoma, brain tumor, breast cancer, adrenocortical carcinoma, leukemia, lung bronchoalveolar cancer) before age 46 years AND at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) before the age of 56 years or with multiple primaries at any age; *or*
        2. Persons with multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum with the initial cancer occurring before the age of 46 years; *or*
        3. Individuals with adrenocortical carcinoma or choroid plexus carcinoma at any age of onset, regardless of family history; *or*

    4. A diagnosis of breast cancer before age 30 years with a negative BRCA1/2 test especially if there is a family history of sarcoma, brain tumor, or adrenocortical carcinoma.

    **** Cancers associated with Li-Fraumeni syndrome include but are not limited to premenopausal breast cancer,

bone and soft tissue sarcomas, acute leukemia, brain tumor, adrenocortical carcinoma, choroid plexus carcinoma, colon cancer, and early onset of other adenocarcinomas or other childhood cancers. Note that Ewing sarcoma is less likely to be related to Li-Fraumeni as compared to other sarcomas.

2. Peutz-Jeghers syndrome

Based on NCCN guidelines, STK11 (LKB1) gene testing may be considered for individuals with a suspected or known clinical diagnosis of Peutz-Jeghers syndrome, or a known family history of a STK11 (LKB1) mutation. Testing may be considered for individuals whose medical and/or family history is consistent with any of the following:

1. A relative with a known deleterious STK11 (LKB1) gene mutation; *or*
2. A clinical diagnosis of PJS based on at least 2 of the following features:

    1. At least 2 PJS-type hamartomatous polyps of the small intestine;
    2. Mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers;
    3. A family history of PJS.

3. Ashkenazi Jewish Testing Panel

Aetna considers medically necessary preconception or prenatal carrier screening for couples of Ashkenazi Jewish ancestry with a panel of genetic tests recommended by the American College of Medical Genetics (ACMG):

1. Tay Sachs disease
2. Canavan disease
3. Cystic fibrosis
4. Familial dysautonomia
5. Bloom syndrome
6. Fanconi anemia
7. Niemann-Pick disease
8. Gaucher disease
9. Mucolipidosis IV

If only one partner is of Ashkenazi Jewish ancestry, then testing of that partner is considered medically necessary. Testing of the other partner is considered medically necessary only if the result of testing of the Ashkenazi Jewish partner is positive.

Aetna considers genetic testing experimental and investigational for any of the following:

1. Age-related macular degeneration
2. Brugada syndrome
3. Choroidal neovascularization (e.g., Retnagene)
4. Congenital stationary night blindness
5. Coronary artery disease
6. Costello syndrome (HRAS gene)
7. Diamond-Blackfan anemia
8. Dilated cardiomyopathy [CMD1A]
9. Epidermolytic hyperkeratosis
10. Familial Alzheimer disease
11. Familial amyotrophic lateral sclerosis (SOD1 mutation)
12. Familial cold urticaria/familial cold autoinflammatory syndrome
13. Familial partial lipodystrophy [FPLD2])
14. Genetic testing panels for nonsyndromic hereditary hearing loss (OtoScope, OtoGenome, OtoSeq)
15. Glioblastoma multiforme
16. Hemiplegic migraine (HM)
17. Hemophilia C (F11 (Factor XI))
18. Heterotaxy
19. Klippel-Feil syndrome
20. Lactose intolerance
21. Legius syndrome (SPRED1 gene)
22. Malignant melanoma
23. May-Hegglin anomaly
24. Mccune-Albright syndrome
25. Mowat-Wilson syndrome (ZEB2 gene)
26. Myoclonus-dystonia (epsilon-sarcoglycan gene (SCGE) deletion analysis)
27. Migrainous vertigo
28. Narcolepsy
29. Oculopharyngeal muscular dystrophy (OPMD) (PABPN1 gene)
30. Parkinson disease
31. Polycystic kidney disease
32. Prostate cancer

33. Seizure disorders (e.g., creatine transporter 1 sequencing for testing parents of individuals with seizures; GABRG2 mutations and SCN1A deletion test for infantile febrile seizures; Generalized epilepsy with febrile seizures plus (GEFS+))
34. Sleep-walking
35. Townes-Brocks syndrome (SALL1 gene)
36. Type 2 diabetes (other than MODY)
37. Very long chain acylCoA dehydrogenase deficiency (VLCADD)
38. von Willebrand factor gene testing.

4. Aetna considers the following tests experimental and investigational:

- EpiSEEK test for epilepsy/seizures
- Exome sequencing
- Home genetic tests
- OncoVue breast cancer risk test
- POLG1 for mitochondrial recessive ataxia syndrome
- SF3B1 and TET2 mutation for myelodysplastic syndrome

See also CPB 0189 - Genetic Counseling, CPB 0227 BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy, CPB 0282 - Noninvasive Down Syndrome Screening, CPB 0348 - Recurrent Pregnancy Loss, CPB 0349 - Alzheimer's Disease, CPB 0464 - Serum Marker Screening for Down Syndrome, and CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing.

Background

According to the American College of Medical Genetics (ACMG), an important issue in genetic testing is defining the scope of informed consent. The obligation to counsel and obtain consent is inherent in the clinician-patient and investigator-subject relationships. In the case of most genetic tests, the patient or subject should be informed that the test might yield information regarding a carrier or disease state that requires difficult choices regarding their current or future health, insurance coverage, career, marriage, or reproductive options. The objective of informed consent is to preserve the individual's right to decide whether to have a genetic test. This right includes the right of refusal should the individual decide the potential harm (stigmatization or undesired choices) outweighs the potential benefits.

DNA-based mutation analysis is not covered for routine carrier testing for the diagnosis of Tay-Sachs and Sandhoff disease. Under accepted guidelines, diagnosis is primarily accomplished through biochemical assessment of serum, leukocyte, or platelet hexosaminidase A and B levels. The literature states that mutation analysis is appropriate for individuals with persistently inconclusive enzyme-based results and to exclude pseudo-deficiency (non-disease related) mutations in carrier couples.

Testing of a member who is at substantial familial risk *for* being a heterozygote (carrier) for a particular detectable mutation that is recognized to be attributable to a specific genetic disorder is only covered for the purpose of prenatal counseling under plans with this benefit (see CPB 0189 - Genetic Counseling).

Confirmation by molecular analysis of inborn errors of metabolism by traditional screening methodologies (e.g., Guthrie microbiologic assays) is covered. Rigorous clinical evaluation should precede diagnostic molecular testing.

In many instances, reliable mutation analysis requires accurate determination of specific allelic variations in a proband (affected individual in a family) before subsequent carrier testing in other at-risk family members can be accurately performed. Coverage of testing for individuals who are not Aetna members is not provided, except under the limited circumstances outlined in the policy section above.

Hereditary non-polyposis colon cancer

Hereditary non-polyposis colon cancer ([HNPCC], Lynch syndrome) is one of the most common cancer predisposition syndromes affecting 1 in 200 individuals and accounting for 13 to 15 % of all colon cancer. HNPCC is defined clinically by early-onset colon carcinoma and by the presence of other cancers such as endometrial, gastric, urinary tract and ovarian found in at least 3 first-degree relatives. Two genes have been identified as being primary responsible for this syndrome: hMLH1 at chromosome band 3p21 accounts for 30 % of HNPCC2,3 and hMLH2 or FCC at chromosome band 2p22 which together with hMLH1 accounts for 90 % of HNPCC.

Unlike other genetic disorders that are easily diagnosed, the diagnosis of HNPCC relies on a very strongly positive family history of colon cancer. Specifically, several organizations have defined criteria that must be met to make the diagnosis of HNPCC.

Although HNPCC lacks strict clinical distinctions that can be used to make the diagnosis, and therefore diagnosis is based on the strong family history, genetic testing is now available to study patient's DNA for mutations to one of the mismatch repair genes. A mutation to one of these genes is a characteristic feature and confirms the diagnosis of HNPCC. Identifying individuals with this disease and performing screening colonoscopies on affected persons may help reduce colon cancer mortality.

Microsatellite instability (MSI) is found in the colorectal cancer DNA (but not in the adjacent normal colorectal mucosa) of most individuals with germline mismatch repair gene mutations. In combination with immunohistochemistry for MSH2 and MLH1,

MSI testing using the Bethesda markers should be performed on the tumor tissue of individuals putatively affected with HNPCC. A result of MSI-high in tumor DNA usually leads to consideration of germline testing for mutations in the MSH2 and MLH1 genes. Individuals with MSI-low or microsatellite stable (MSS) results are unlikely to harbor mismatch repair gene mutations, and further genetic testing is usually not pursued.

HNPCC is caused by germline mutation of the DNA mismatch repair genes. Over 95 % of HNPCC patients have mutations in either MLH1 or MSH2. As a result, sequencing for mismatch repair gene mutations in suspected HNPCC families is usually limited to MLH1 and MSH2 and sometimes MSH6 and PMS2. In general, MSH6 and PMS2 sequence analysis is performed in persons meeting aforementioned criteria for genetic testing for HNPCC, and who do not have mutations in either the MLH1 or MSH2 genes. In addition, single site MSH6 or PMS2 testing may be appropriate for testing family members of persons with HNPCC with an identified MSH6 or PMS2 gene mutation.

HNPCC is a relatively rare disease, which makes screening the entire populace burdensome and ineffective. The incidence of this disease, even among the families of patients with colon cancer, is too small to make screening effective. (See also CPB 0189 - Genetic Counseling and CPB 0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy).

Familial adenomatous polyposis (FAP)

Familial adenomatous polyposis (FAP) is caused by mutation of the adenomatous polyposis coli (APC) gene. According to guidelines from the American Gastroenterological Association (AGA, 2001), adenomatous polyposis coli gene testing is indicated to confirm the diagnosis of familial adenomatous polyposis, provide pre-symptomatic testing for at-risk members (1st degree relatives 10 years or older of an affected patient), confirm the diagnosis of attenuated familial adenomatous polyposis in those with more than 20 adenomas, and test those 10 years or older at risk for attenuated FAP.

The AGA guidelines state that germline testing should first be performed on an affected member of the family to establish a detectable mutation in the pedigree. If a mutation is found in an affected family member, then genetic testing of at-risk members will provide true positive or negative results. The AGA guidelines state that, if a pedigree mutation is not identified, further testing of at-risk relatives should be suspended because the gene test will not be conclusive: a negative result could be a false negative because testing is not capable of detecting a mutation even if present. When an affected family member is not available for evaluation, starting the test process with at-risk family members can provide only positive or inconclusive results. In this circumstance, a true negative test result for an at-risk individual can only be obtained if another at-risk family member tests positive for a mutation.

MYH-associated polyposis

MYH is a DNA repair gene that corrects DNA base pair mismatch errors in the genetic code before replication. Mutation of the MYH gene may result in colon cancer. In this regard, the MYH gene has been found to be significantly involved in colon cancer, both in cases where there is a clear family history of the disease, as well as in cases without any sign of a hereditary cause.

The National Comprehensive Cancer Network (NCCN)'s practice guidelines on colorectal cancer screening (2006) recommended testing for MYH mutations for individuals with personal history of adenomatous polyposis (more than 10 adenomas, or more than 15 cumulative adenomas in 10 years) either consistent with recessive inheritance or with adenomatous polyposis with negative adenomatous polyposis coli (APC) mutation testing. The guideline noted that when polyposis is present in a single person with negative family history, de novo APC mutation should be tested; if negative, testing for MYH should follow. When family history is positive only for a sibling, recessive inheritance should be considered and MYH testing should be done first. In a polyposis family with clear autosomal dominant inheritance, and absence of APC mutation, MYH testing is unlikely to be informative. Members in such family are treated according to the polyposis phenotype, including classical or attenuated FAP.

Factor V Leiden mutation

Factor V Leiden mutation is the most common hereditary blood coagulation disorder in the United States. It is present in 5 % of the Caucasian population and 1.2 % of the African-American population. Factor V Leiden increases the risk of venous thrombosis 3 to 8 fold for heterozygous individuals and 30 to 140 fold for homozygous individuals. Factor V Leiden mutation has been associated with the following complications:

- Cerebrovascular accident and myocardial infarction
- Deep venous thrombosis
- Gallbladder dysfunction
- Preeclampsia and/or eclampsia
- Pulmonary embolism
- Unexplained miscarriage
- Venous thrombosis.

According to the American College of Medical Genetics, Factor V Leiden genetic testing is indicated in the following patients:

- Age less than 50, any venous thrombosis; or
- Myocardial infarction in female smokers under age 50; or
- Recurrent venous thrombosis; or
- Relatives of individuals with venous thrombosis under age 50; or
- Venous thrombosis and a strong family history of thrombotic disease; or
- Venous thrombosis in pregnant women or women taking oral contraceptives; or

- Venous thrombosis in unusual sites (such as hepatic, mesenteric, and cerebral veins).

The ACMG does not recommend random screening of the general population for factor V Leiden. Routine testing is also not recommended for patients with a personal or family history of arterial thrombotic disorders (e.g., acute coronary syndromes or stroke) except for the special situation of myocardial infarction in young female smokers. According to the ACMG, testing may be worthwhile for young patients (less than 50 years of age) who develop acute arterial thrombosis in the absence of other risk factors for atherosclerotic arterial occlusive disease. The ACMG does not recommend prenatal testing or routine newborn screening for factor V Leiden mutation.

The ACMG does not recommend general screening for factor V Leiden mutation before administration of oral contraceptives. The ACMG recommends targeted testing prior to oral contraceptive use in women with a personal or family history of venous thrombosis.

Factor V Leiden screening of asymptomatic individuals with other recognized environmental risk factors, such as surgery, trauma, paralysis, and malignancy is not necessary or recommended by the ACMG, since all such individuals should receive appropriate medical prophylaxis for thrombosis regardless of carrier status. When Factor V Leiden testing is indicated, the ACMG recommends either direct DNA-based genotyping or factor V Leiden-specific functional assay (e.g., activated protein C (APC) resistance). Patients who test positive by a functional assay should then be further studied with the DNA test for confirmation and to distinguish heterozygotes from homozygotes. According to the ACMG, patients testing positive for factor V Leiden or APC resistance should be considered for molecular genetic testing for prothrombin 20210A, the most common thrombophilia with overlapping phenotype for which testing is easily and readily available. The prothrombin 20210A mutation is the second most common inherited clotting abnormality, occurring in 2 % of the general population. It is only a mild risk factor for thrombosis, but may potentiate other risk factors (such as Factor V Leiden, oral contraceptives, surgery, trauma, etc.).

A factor V gene haplotype (HR2) defined by the R2 polymorphism (A4070G) may confer mild APC resistance and interact with the factor V Leiden mutation to produce a more severe APC resistance phenotype (Bernardi et al, 1997; de Visser et al, 2000; Mingozzi et al, 2003). In one study, co-inheritance of the HR2 haplotype increased the risk of venous thromboembolism associated with factor V Leiden by approximately 3-fold (Faioni et al, 1999). However, double heterozygosity for factor V Leiden and the R2 polymorphism was not associated with a significantly higher risk of early or late pregnancy loss than a heterozygous factor V Leiden mutation alone (Zammiti et al, 2006). Whether the HR2 haplotype alone is an independent thrombotic risk factor is still unclear. Several studies have suggested that the HR2 haplotype is associated with a 2-fold increase in risk of venous thromboembolism (Alhenc-Gelas et al, 1999; Jadaon and Dashti, 2005). In contrast, other studies (de Visser 2000; Luddington et al, 2000; Dindagur et al, 2006) found no significant increase in thrombotic risk (GeneTests, University of Washington, Seattle, 2007).

CADASIL

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare, genetically inherited, congenital vascular disease of the brain that causes strokes, subcortical dementia, migraine-like headaches, and psychiatric disturbances. CADASIL is very debilitating and symptoms usually surface around the age of 45. Although CADASIL can be treated with surgery to repair the defective blood vessels, patients often die by the age of 65. The exact incidence of CADASIL in the United States is unknown.

DNA testing for CADASIL is appropriate for symptomatic patients who have a family history consistent with an autosomal dominant pattern of inheritance of this condition. Clinical signs and symptoms of CADASIL include stroke, cognitive defects and/or dementia, migraine, and psychiatric disturbances. DNA testing is also indicated for pre-symptomatic patients where there is a family history consistent with an autosomal dominant pattern of inheritance and there is a known mutation in an affected member of the family. This policy is consistent with guidelines on CADASIL genetic testing from the European Federation of Neurological Societies.

Cystic fibrosis

Cystic fibrosis is the most common potentially fatal autosomal recessive disease in the United States. It is characterized by chronic progressive disease of the respiratory system, malabsorption due to pancreatic insufficiency, increased loss of sodium and chloride in sweat, and male infertility as a consequence of atresia of the vas deferens. Pulmonary disease is the most common cause of mortality and morbidity in individuals with CF. The incidence of this disease ranges from 1:500 in Amish (Ohio) to 1:90,000 in Hawaiian Orientals, and is estimated to be 1:2,500 newborns of European ancestry. It occurs less frequently in people with other ethnic and racial backgrounds. About 1:25 persons of European ancestry is a carrier (or heterozygote), possessing one normal and one abnormal CF gene. Because of recent advances in clinical management of CF, babies born today are expected to live well into middle age.

Currently, the most frequently employed test for CF is the quantitative pilocarpine iontophoresis sweat test. Sweat chloride is more reliable than sweat sodium for diagnostic purposes with a sensitivity of 98 % and a specificity of 83 %. However, this test can not detect CF carriers because the electrolyte content of sweat is normal in heterozygotes (Wallach, 1991). The gene for CF (cystic fibrosis trans-membrane conductance regulator, CFTR) was cloned, and the principal mutant gene in white people (DF508) was characterized in 1989. This mutation is due to a 3-base-pair deletion that results in the loss of a phenylalanine at position 508 from the 1,480-amino acid coding region (Riordan et al, 1989). This mutation is found in approximately 70 % of carriers of European ancestry, but the relative frequency varies from 30 % in Ashkenazi Jews to 88 % in Danes (Cutting et al, 1992). Available evidence indicates that CFTR functions as a chloride channel, although it may also serve other functions. Since then, more than 200 CF mutations have been described. Five of the most common mutations (DF508, G542X, F551D, R553X, N1303K) constitute approximately 85 % of the alleles in the United States (Elias et al, 1991). Thus, screening

procedures that test for these 5 mutations will detect approximately 85 % of CF carriers. The genetic screening test for CF is usually based on mouthwash samples collected by agitating sucrose or saline in the mouth. The DNA of these cells are amplified, digested, and subjected to separation techniques that identify 3 to 5 common mutations.

A National Institutes of Health consensus panel (1997) recommended that genetic testing for CF should be offered to adults with a positive family history of CF, to partners of people with the disease, to couples currently planning a pregnancy, and to couples seeking prenatal testing. However, the panel did not recommend genetic testing of CF to the general public or to newborn infants.

The American College of Obstetricians and Gynecologists (2001) has issued similar recommendations on genetic carrier testing for CF. ACOG recommends that obstetricians should offer CF screening to:

- Couples in whom one or both members are white and who are planning a pregnancy or seeking prenatal care;
- Individuals with a family history of CF; and
- Reproductive partners of people who have CF.

ACOG also recommends that screening should be made available to couples in other racial and ethnic groups. To date, over 900 mutations in the CF gene have been identified. As it is impractical to test for every known mutation, the ACMG Accreditation of Genetic Services Committee has compiled a standard screening panel of 25 CF mutations, which represents the standard panel that ACMG recommends for screening in the U.S. population (Grody et al, 2001). This 25-mutation panel incorporates all CF-causing mutations with an allele frequency of greater than or equal to 0.1 % in the general U.S. population, including mutation subsets shown to be sufficiently predominant in certain ethnic groups, such as Ashkenazi Jews and African Americans. This standard panel of mutations is intended to provide the greatest pan-ethnic detectability that can practically be performed.

The ACOG's update on carrier screening for CF (2011) provided the following recommendations.

- If a patient has been screened previously, CF screening results should be documented but the test should not be repeated.
- Complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening.

Fragile X syndrome

Fragile X syndrome is the most common cause of inherited mental retardation, seen in approximately one in 1,200 males and one in 2,500 females. Phenotypic abnormalities associated with Fragile X syndrome include mental retardation, autistic behaviors, characteristic narrow face with large jaw, and speech and language disorders. Fragile X syndrome was originally thought to be transmitted in an X-linked recessive manner; however, the inheritance pattern of fragile X syndrome has been shown to be much more complex.

Standard chromosomal analysis does not consistently demonstrate the cytogenetic abnormality in patients with fragile X syndrome, and molecular diagnostic techniques (DNA testing) have become the diagnostic procedure of choice for fragile X syndrome.

Aetna's policy on coverage of fragile X genetic testing is based on guidelines fromm the ACMG (1994) and the ACOG (1995).

Lactose intolerance

Lactase-phlorizin hydrolase, which hydrolyzes lactose, the major carbohydrate in milk, plays a critical role in the nutrition of the mammalian neonate (Montgomery et al, 1991). Lactose intolerance in adult humans is common, usually due to low levels of small intestinal lactase. Low lactase levels result from either intestinal injury or (in the majority of the world's adult population) alterations in the genetic expression of lactase. Although the mechanism of decreased lactase levels has been the subject of intensive investigation, no consensus has yet emerged.

The LactoTYPE Test (Prometheus Laboratories) is a blood test that is intended to identify patients with genetic-based lactose intolerance. According to the manufacturer, this test provides a more definitive diagnosis and scientific explanation for patients with persistent symptoms.

There is insufficient evidence that the assessment of the genetic etiology of lactose intolerance would affect the management of patients such that clinical outcomes are improved. Current guidelines on the management of lactose intolerance do not indicate that genetic testing is indicated (NHS, 2005; National Public Health Service for Wales, 2005).

Long QT syndrome

Voltage-gated sodium channels are transmembrane proteins that produce the ionic current responsible for the rising phase of the cardiac action potential and play an important role in the initiation, propagation, and maintenance of normal cardiac rhythm. Inherited mutations in the sodium channel alpha-subunit gene (SCN5A), the gene encoding the pore-forming subunit of the cardiac sodium channel, have been associated with distinct cardiac rhythm syndromes such as the congenital long QT3 syndrome (LQT3), Brugada syndrome, isolated conduction disease, sudden unexpected nocturnal death syndrome (SUNDS), and sudden infant death syndrome (SIDS). Electrophysiological characterization of heterologously expressed mutant sodium channels have revealed gating defects that, in many cases, can explain the distinct phenotype associated with the rhythm disorder.

The long QT syndrome (LQTS) is a familial disease characterized by an abnormally prolonged QT interval and, usually, by stress-mediated life-threatening ventricular arrhythmias (Priori et al, 2001). Characteristically, the first clinical manifestations of

LQTS tend to appear during childhood or in teenagers. Two variants of LQTS have been described: a rare recessive form with congenital deafness (Jervell and Lange-Nielsen syndrome, J-LN), and a more frequent autosomal dominant form (Romano-Ward syndrome, RW). Five genes encoding subunits of cardiac ion channels have been associated to LQTS and genotype-phenotype correlation has been identified. Of the 5 genetic variants of LQTS currently identified, LQT1 and LQT2 subtypes involve 2 genes, KCNQ1 and HERG, which encode major potassium currents. LQT3 involves SCN5A, the gene encoding the cardiac sodium current. LQT5 and LQT6 are rare subtypes also involving the major potassium currents.

The principal diagnostic and phenotypic hallmark of LQTS is abnormal prolongation of ventricular repolarization, measured as lengthening of the QT interval on the 12-lead ECG (Maron et al, 1998). This is usually most easily identified in lead II or V1, V3, or V5, but all 12 leads should be examined and the longest QT interval used; care should also be taken to exclude the U wave from the QT measurement.

LQT3 appears to be the most malignant variant and may be the one less effectively managed by beta blockers. LQT1 and LQT2 have a higher frequency of syncopal events but their lethality is lower and the protection afforded by beta-blockers, particularly in LQT1, is much higher. The Jervell and Lange-Nielsen recessive variant is associated with very early clinical manifestations and a poorer prognosis than the Romano-Ward autosomal dominant form. The presence of syndactyly seems to represent a different genetic variant of LQTS also associated with a poor prognosis.

Guidelines on sudden cardiac death from the European College of Cardiology (Priori et al, 2001) state that identification of specific genetic variants of LQTS are useful in risk stratification. The clinical variants presenting association of the cardiac phenotype with syndactyly or with deafness (Jervell and Lange-Nielsen syndrome) have a more severe prognosis. Genetic defects on the cardiac sodium channel gene (SCN5A) are also associated with higher risk of sudden cardiac death. In addition, identification of specific genetic variants may help in suggesting behavioral changes likely to reduce risk. LQT1 patients are at very high risk during exercise, particularly swimming. LQT2 patients are quite sensitive to loud noises, especially when they are asleep or resting.

Genetic testing for LQTS may be indicated in persons with close relatives that have a defined mutation. Genetic testing may also be indicated in individuals with a prolonged QT interval on resting electrocardiogram (a corrected QT interval (QTc) of 470 msec or more in males and 480 msec or more in females) without an identifiable external cause for QTc prolongation. Common external causes of QTc prolongation are listed in the table below.

Table: Common External Causes of Prolongation of QTc Interval
Bradycardia
Heart disease (heart failure, ischemia)
Hypocalcemia
Hypomagnesemia
Hypokalemia
Hypothyroidism
Antiarrhythmic medications (quinidine, procainamide, amiodarone, sotalol, and dofetilide)
Tricyclic and tetracyclic antidepressants (e.g., amitriptyline)
Erythromycin
Cisapride
Pimozide
Thioridazine

Genetic testing for long QT syndrome has not been evaluated in patients who present with a borderline QT interval, suspicious symptoms (e.g., syncope), and no relevant family history (Roden, 2008). In these patients, the incidence of false positive and false negative results and their implications for management remain unknown.

Genetic testing may also be necessary in person with long QT syndrome in sudden death close relatives.

Brugada syndrome

Brugada syndrome is an inherited condition comprising a specific EKG abnormality and an associated risk of ventricular fibrillation and sudden death in the setting of a structurally normal heart. Brugada syndrome is characterized by ST-segment abnormalities on EKG and a high risk of ventricular arrhythmias and sudden death. Brugada syndrome presents primarily during adulthood but age at diagnosis ranges from 2 days to 85 years. Clinical presentations may also include sudden infant death syndrome and sudden unexpected nocturnal death syndrome, a typical presentation in individuals from Southeast Asia.

Brugada et al (2005) reported that Brugada syndrome and LQTS are both due to mutations in genes encoding ion channels and that the genetic abnormalities causing Brugada syndrome have been linked to mutations in the ion channel gene SCN5A. Brugada stated that the syndrome has been identified only recently but an analysis of data from published studies indicates that the disease is responsible for 4 to 12 % of unexpected sudden deaths, and up to 50 % of all sudden death in patients with an apparently normal heart. Brugada explained that Brugada syndrome is a clinical diagnosis based on syncopal or sudden death episodes in patients with a structurally normal heart and a characteristic ECG pattern. The ECG shows ST segment elevation in the primordial leads V1-V3, with a morphology of the QRS complex resembling a right bundle branch block; this pattern may also be caused by J point elevation. When ST elevation is the most prominent feature, the pattern is called "coved-type". When the most prominent feature is J point elevation, without ST elevation the pattern is called "saddle-type". Brugada pointed out that it is important to exclude other causes of ST segment elevation before making the diagnosis of Brugada syndrome. Brugada syndrome is inherited in an autonomic dominant manner with variable penetrance. Most individuals diagnosed with Brugada syndrome have an affected parent. The proportion of cases caused by de novo mutations is estimated at 1 %. Each child of an individual with Brugada syndrome has a 50 % chance of inheriting the mutation. According to Brugada, antiarrhythmic drugs do not prevent sudden death in symptomatic or asymptomatic individuals with Brugada syndrome and that implantation of an automatic cardioverter-defibrillator is the only currently proven effective therapy.

To date the great majority of identified disease-causing mutations have been located in the SCN5A gene encoding the a subunit of the human cardiac voltage-gated sodium channel but such mutations can be identified in, at most, 30 % of affected people. Moreover, a positive genetic test adds little or nothing to the clinical management of such a person (HRUK, 2007). The identification of an SCN5A mutation does, of course, allow screening of family members but the usefulness of genetic screening may be less than for other familial syndromes, however, given that the routine 12-lead EKG (with or without provocative drug testing) appears to be a relatively effective method of screening for the condition.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium in which a portion of the myocardium is hypertrophied without any obvious cause; it is among the most common genetically transmitted cardiovascular diseases.

The genetic abnormalities that cause HCM are heterogeneous. Hypertrophic cardiomyopathy is most commonly due to a mutation in one of 9 genes that results in a mutated protein in the sarcomere. Some of the genes responsible for HCM have not yet been identified, and among those genes that have been identified, the spectrum of possible disease-causing mutations is incomplete. As a result, a thorough evaluation of known genes requires extensive DNA sequencing, which is onerous for routine clinical testing. Less rigorous methods (such as selective sequencing) reduces the likelihood of identifying the responsible mutation.

Population studies have demonstrated that some patients are compound heterozygotes (inheriting 2 different mutations within a single HCM gene), double heterozygotes (inheriting mutations in 2 HCM genes), or homozygotes (inheriting the same mutation from both parents). To be certain of detecting such genotypes, sequencing of candidate genes would need to continue in a given patient even after a single mutation was identified.

In many persons with HCM mutations, the disease can be mild and the symptoms absent or minimal. In addition, phenotypic expression of HCM can be influenced by factors other than the basic genetic defect, and the clinical consequences of the genetic defect can vary. There is sufficient heterogeneity in the clinical manifestations of a given gene mutation that, even when a patient's mutation is known, his or her clinical course can not be predicted with any degree of certainty.

In addition, the prognostic impact of a given mutation may relate to a particular family and not to the population at large. Many families have their own "private" mutations and thus knowledge of the gene abnormalities can not be linked to experience from other families.

Family members with echocardiography evidence of HCM should be managed like other patients with HCM. In general, genetically affected but phenotypically normal family members should not be subjected to the same activity restriction as patients with HCM.

Bos and colleagues (2009) stated that over the past 20 years, the pathogenic basis for HCM, the most common heritable cardiovascular disease, has been studied extensively. Affecting about 1 in 500 persons, HCM is the most common cause of sudden cardiac death (SCD) among young athletes. In recent years, genomic medicine has been moving from the bench to the bedside throughout all medical disciplines including cardiology. Now, genomic medicine has entered clinical practice as it pertains to the evaluation and management of patients with HCM. The continuous research and discoveries of new HCM susceptibility genes, the growing amount of data from genotype-phenotype correlation studies, and the introduction of commercially available genetic tests for HCM make it essential that cardiologists understand the diagnostic, prognostic, and therapeutic implications of HCM genetic testing.

Hudecova et al (2009) noted that the clinical symptoms of HCM are partly dependent on mutations in affected sarcomere genes. Different mutations in the same gene can present as malign with a high-risk of SCD, while other mutations can be benign. The clinical symptomatology can also be influenced by other factors such as the presence of polymorphisms in other genes. Currently, the objective of intensive clinical research is to access the contribution of molecular genetic methods in HCM diagnostics as well as in risk stratification of SCD. It is expected that genetic analyses will have an important consequence in the screening of the relatives of HCM patients and also in the prenatal diagnostics and genetic counseling.

Shephard and Semsarian (2009) stated that genetic heart disorders are an important cause of SCD in the young. While pharmacotherapies have made some impact on the prevention of SCD, the introduction of implantable cardioverter-defibrillator

(ICD) therapy has been the single major advance in the prevention of SCD in the young. In addition, the awareness that most causes of SCD in the young are inherited, means family screening of relatives of young SCD victims allows identification of previously unrecognised at-risk individuals, thereby enabling prevention of SCD in relatives. The role of genetic testing, both in living affected individuals as well as in the setting of a "molecular autopsy", is emerging as a key factor in early diagnosis of an underlying cardiovascular genetic disorder.

The Heart Failure Society of America's practice guideline on "Genetic evaluation of cardiomyopathy" (Hershberger et al, 2009) stated that genetic testing is primarily indicated for risk assessment in at-risk relatives who have little or no clinical evidence of cardiovascular disease. Genetic testing for HCM should be considered for the one most clearly affected person in a family to facilitate family screening and management. Specific genes available for testing for HCM include MYH7, MYBPC3, TNNT2, TNN13, TPM1, ACTC, MYL2, and MYL3. MYH7 and MYBPC each accounts for 30 % to 40 % of mutations; TNNT2 for 10 % to 20 %. Genetic cause can be identified in 35 % to 45 % overall; up to 60 % to 65 % when the family history is positive.

The BlueCross BlueShield Association Technology Evaluation Center (TEC)'s assessement on genetic testing for predisposition to inherited HCM (2010) concluded that the use of genetic testing for inherited HCM meets the TEC criteria for individuals who are at-risk for development of HCM, defined as having a close relative with established HCM, when there is a known pathogenic gene mutation present in an affected relative. In order to inform and direct genetic testing for at-risk individuals, genetic testing should be initially performed in at least 1 close relative with definite HCM (index case) if possible. This testing is intended to document whether a known pathologic mutation is present in the family, and optimize the predictive value of predisposition testing for at-risk relatives. Due to the complexity of genetic testing for HCM and the potential for misinterpretation of results, the decision to test and the interpretation of test results should be performed by, or in consultation with an expert in the area of medical genetics and/or HCM.

The TEC assessment also concluded that genetic testing for inherited HCM does not meet the TEC criteria for predisposition testing in individuals who are at-risk for development of HCM, defined as having a close relative with established HCM, when there is no known pathogenic gene mutation present in an affected relative. This includes:

- Patients with a family history of HCM, with unknown genetic status of affected relatives; and
- Patients with a family history of HCM, when a pathogenic mutation has not been identified in affected relatives.

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)

Arrhythmogenic right ventricular dysplasia/cardiomyopathy is a condition characterized by progressive fibro-fatty replacement of the myocardium that predisposes individuals to ventricular tachycardia and sudden death. The prevalence of ARVD/C is estimated to be 1 case per 10,000 population. Familial occurrence with an autosomal dominant pattern of inheritance and variable penetrance has been demonstrated. Recessive variants have been reported. It is estimated that half of the individuals have a family history of ARVD/C and the remaining cases are new mutations.

Genetic testing has not been demonstrated to be necessary to establish the diagnosis of ARVD/C or determine its prognosis. Twelve-lead ECG and echocardiography can be used to identify affected relatives.

The genetic abnormalities that cause ARVD/C are heterogeneous. The genes frequently associated with ARVD/C are PKP2 (plakophilin-2), DSG2 (desmoglein-2), and DSP (desmoplakin). A significant proportion of ARVD/C cases have been reported with no linkage to known chromosomal loci; in one report, 50 % of families undergoing clinical and genetic screening did not show linkage with any known genetic loci (Corrado et al, 2000).

Most affected individuals live a normal lifestyle. Management of individuals with ARVD/C is complicated by incomplete information on the natural history of the disease and the variability of disease expression even within families. High-risk individuals with signs and symptoms of ARVD/C are treated with anti-arrhythmic medications and those at highest risk who have been resuscitated or who are unresponsive to or intolerant of anti-arrhythmic therapy may be considered for an ICD.

According to the Heart Failure Society of America's Practice Guideline on the genetic evaluation of cardiomyopathy (2009), the clinical utility for all genetic testing of cardiomyopathies remains to be defined. The guideline stated, "[b]ecause the genetic knowledge base of cardiomyopathy is still emerging, practitioners caring for patients and families with genetic cardiomyopathy are encouraged to consider research participation." The Multidisciplinary Study of Right Ventricular Dysplasia (North American registry) is a 5-year study funded by the National Institutes of Health to determine how the genes responsible for ARVD/C affect the onset, course, and severity of the disease. Enrollment in the study was completed in May 2008 and the study is currently in the follow-up period.

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal form of inherited arrhythmogenic disease characterized by adrenergically mediated polymorphic ventricular tachycardia (Liu et al, 2007). Mutations in the cardiac ryanodine receptor (RyR2) gene and the cardiac calsequestrin (CASQ2) gene are responsible for the autosomal dominant and recessive variants of CPVT, respectively. The clinical presentation encompasses exercise- or emotion-induced syncopal events and a distinctive pattern of reproducible, stress-related, bi-directional ventricular tachycardia in the absence of both structural heart disease and a prolonged QT interval.

CPVT typically begins in childhood or adolescence. The mortality rate in untreated individuals is 30 to 50 % by age 40 years. Clinical evaluation by exercise stress testing and Holter monitoring and genetic screening can facilitate early diagnosis. Beta-blockers are the most effective drugs for controlling arrhythmias in CPVT patients, yet about 30 % of patients with CPVT still experience cardiac arrhythmias on beta-blockers and eventually require an implantable cardioverter defibrillator. Liu et al

(2008) stated that molecular genetic screening of the genes encoding the cardiac RyR2 and CASQ2 is critical to confirm uncertain diagnosis of CPVT.

Katz et al (2009) noted that CPVT is a primary electrical myocardial disease characterized by exercise- and stress-related ventricular tachycardia manifested as syncope and sudden death. The disease has a heterogeneous genetic basis, with mutations in the cardiac RyR2 gene accounting for an autosomal-dominant form (CPVT1) in approximately 50 % and mutations in the cardiac CASQ2 gene accounting for an autosomal-recessive form (CPVT2) in up to 2 % of CPVT cases. Both RyR2 and calsequestrin are important participants in the cardiac cellular calcium homeostasis. These researchers reviewed the physiology of the cardiac calcium homeostasis, including the cardiac excitation contraction coupling and myocyte calcium cycling.

Although the clinical presentation of CPVT is similar in many respects to the LQTS, there are important differences that are relevant to genetic testing. CPVT appears to be a more malignant condition, as many people are asymptomatic before the index lethal event and the majority of cardiac events occur before 20 years of age. Affected people are advised to avoid exercise-related triggers and start prophylactic beta-blockers with dose titration guided by treadmill testing.

Genetic testing has been recommended in individuals with clinical features considered typical of CPVT following expert clinical assessment (HRUK, 2008). Clinically the condition is difficult to diagnose in asymptomatic family members as the ECG and echocardiogram are completely normal at rest. Exercise stress testing has been advised in family members in order to identify exercise-induced ventricular arrhythmias, but the sensitivity of this clinical test is unknown. Although the diagnostic yield from genetic testing is less than that for the LQTS (about 50 %) in patients with typical clinical features, a positive genetic test may be of value for the individual patient (given the prognostic implications) and for screening family members (given the difficulties in clinical screening methods) (HRUK, 2008). The RyR2 gene is large and a ''targeted'' approach is usually undertaken, in which only exons that have been previously implicated are examined.

The 2006 guidelines from the American College of Cardiology on management of patients with ventricular arrhythmias and the prevention of sudden cardiac death (Zipes et al, 2006) included the following recommendations for patients with CPVT:

- There is evidence and/or general agreement supporting the use of beta blockers for patients clinically diagnosed on the basis of spontaneous of documented stress-induced ventricular arrhythmias.
- There is evidence and/or general agreement supporting the use of an implantable ICD in combination with beta blockers for survivors of cardiac arrest who have a reasonable expectation of survival with a good functional capacity for more than 1 year.
- The weight of evidence and/or opinion supports the use of beta blockers in patients without clinical manifestations who are diagnosed in childhood based upon genetic analysis.
- The weight of evidence and/or opinion supports the use of an ICD in combination with beta blockers for patients with a history of syncope and/or sustained ventricular tachycardia while receiving beta blockers who have a reasonable expectation of survival with a good functional capacity for more than 1 year.
- The usefulness and/or efficacy of beta blockers is less well established in patients without clinical evidence of arrhythmias who are diagnosed in adulthood based upon genetic analysis.

Hereditary hemochromatosis

Hemochromatosis, a condition involving excess accumulation of iron, can lead to iron overload, which in turn can result in complications such as cirrhosis, diabetes, cardiomyopathy, and arthritis (Burke 1992; Hanson et al, 2001).

Hereditary hemochromatosis (HHC) is characterized by inappropriately increased iron absorption from the duodenum and upper intestine, with consequent deposition in various parenchymal organs, notably the liver, pancreas, joints, heart, pituitary gland and skin, with resultant end-organ damage (Limdi and Crampton, 2004). Clinical features may be non-specific and include lethargy and malaise, or reflect target organ damage and present with abnormal liver tests, cirrhosis, diabetes mellitus, arthropathy, cardiomyopathy, skin pigmentation and gonadal failure. Early recognition and treatment (phlebotomy) is essential to prevent irreversible complications such as cirrhosis and hepatocellular carcinoma.

HHC is an autosomal recessive condition associated with mutations of the HFE gene. Two of the 37 allelic variants of the HFE gene, C282Y and H63D, are significantly correlated with HHC. C282Y is the more severe mutation, and homozygosity for the C282Y genotype accounts for the majority of clinically penetrant cases. Hanson et al (2001) reported that homozygosity for the C282Y mutation has been found in 52 to 100 % of previous studies on clinically diagnosed index cases. Five percent of HHC probands were found by Hanson et al to be compound heterozygotes (C282Y/H63D), and 1.5 % were homozygous for the H63D mutation; 3.6 % were C282Y heterozygotes, and 5.2 % were H63D heterozygotes. In 7 % of cases, C282Y and H63D mutations were not present. In the general population, the frequency of the C282Y/C282Y genotype is 0.4 %.

HHC is a very common genetic defect in the Caucasian population. C282Y heterozygosity ranges from 9.2 % in Europeans to nil in Asian, Indian subcontinent, African, Middle Eastern, Australian and Asian populations (Hanson et al, 2001). The H63D carrier frequency is 22 % in European populations.

Accurate data on the penetrance of the different HFE genotypes are not available. But current data suggest that clinical disease does not develop in a substantial proportion of people with this genotype. Available data suggest that up to 38 % to 50 % of C282Y homozygotes may develop iron overload, with up to 10 % to 33 % eventually developing hemochromatosis-associated morbidity (Whitlock et al, 2006). A pooled analysis found that patients with the HFE genotypes C282Y/H63D and H63D/H63D are also at increased risk for iron overload, yet overall, disease is likely to develop in fewer than 1 % of people with these genotypes (Burke, 1992). Thus, DNA-based tests for hemochromatosis identify a genetic risk rather than the disease itself.

Environmental factors such as diet and exposure to alcohol or other hepatotoxins may modify the clinical outcome in patients with hemochromatosis, and variations in other genes affecting iron metabolism may also be a factor. As a result, the clinical condition of iron overload is most reliably diagnosed on the basis of biochemical evidence of excess body iron (Burke, 1992).

Whether it is beneficial to screen asymptomatic people for a genetic risk of iron overload is a matter of debate. To date, population screening for HHC is not recommended because of uncertainties about optimal screening strategies, optimal care for susceptible persons, laboratory standardization, and the potential for stigmatization or discrimination (Hanson et al, 2001; Whitlock et al, 2006). A systematic evidence review prepared for the U.S. Preventive Services Task Force concluded: "Research addressing genetic screening for hereditary hemochromatosis remains insufficient to confidently project the impact of, or estimate the benefit from, widespread or high-risk genetic screening for hereditary hemochromatosis" (Whitlock et al, 2006).

Familial nephrotic syndrome (NPHS1, NPHS2)

Nephrotic syndrome comes in 2 variants: (i) those sensitive to treatment with immunosuppressants (steroid-sensitive), and (ii) those resistant to immunosuppressants (steroid-resistant). Familial forms of nephrotic syndrome are steroid resistant (Niaudet, 2007). Mutations in two genes, NPHS1 and NPHS2, have been associated with a familial nephrotic syndrome. Mutations in the gene for podocin, called NPHS2, also known as familial focal glomerulosclerosis, are observed in patients with both familial and sporadic steroid-resistant nephrotic syndrome (SRNS).

Identifying children with nephrotic syndrome due to NPHS2 mutations can avoid unnecessary exposure to immunosuppressive therapy, because immunosuppressive therapy has not been shown to be effective in treating these children (Niaudet, 2007). Thus, authorities have recommended testing for such mutations in those with a familial history of steroid resistant nephrotic syndrome and children with steroid-resistant disease .

Some have suggested that, to avoid unnecessary exposure to steroid therapy, all children with a first episode of the nephrotic syndrome should be screened for NPHS2 mutations (Niaudet, 2007). However, given that over 85 % of children with idiopathic nephrotic syndrome are steroid-sensitive and only approximately 20 % of steroid-resistant patients have NPHS2 mutations, screening for abnormalities at this genetic locus would identify less than 5 % of all cases. However, screening a child with a first episode of the nephrotic syndrome with a familial history of steroid-resistant nephrotic syndrome has been recommended because they are at increased risk for having a NPHS2 gene mutation.

Mutations in the gene for nephrin, called NPHS1, cause the congenital nephrotic syndrome of Finnish type (CNF) (Niaudet, 2007). CNF is inherited as an autosomal recessive trait, with both sexes being involved equally. There are no manifestations of the disease in heterozygous individuals. Most infants with the CNF are born prematurely (35 to 38 weeks), with a low birth weight for gestational age. Edema is present at birth or appears during the first week of life in 50 % of cases. Severe nephrotic syndrome with marked ascites is always present by 3 months. End-stage renal failure usually occurs between 3 and 8 years of age. Prolonged survival is possible with aggressive supportive treatment, including dialysis and renal transplantation.

The nephrotic syndrome in CNF is always resistant to corticosteroids and immunosuppressive drugs, since this is not an immunologic disease (Niaudet, 2007). Furthermore these drugs may be harmful due to affected individuals' already high susceptibility to infection.

The CNF becomes manifest during early fetal life, beginning at the gestation age of 15 to 16 weeks. The initial symptom is fetal proteinuria, which leads to a more than 10-fold increase in the amniotic fluid alpha-fetoprotein (AFP) concentration (Niaudet, 2007). A parallel, but less important increase in the maternal plasma AFP level is observed. These changes are not specific, but they may permit the antenatal diagnosis of CNF in high risk families in which termination of the pregnancy might be considered. However, false positive results do occur, often leading to abortion of healthy fetuses.

Genetic linkage and haplotype analyses may diminish the risk of false positive results in informative families (Niaudet, 2007). The 4 major haplotypes, which cover 90 % of the CNF alleles in Finland, have been identified, resulting in a test with up to 95 % accuracy.

Authorities do not recommend screening for NPHS1 mutations for all children with the first episode of nephrotic syndrome, for the reasons noted above regarding NPHS2 mutation screening. However, genetic testing may be indicated for infants with congenital nephrotic syndrome (i.e., appearing within the first months of life) who are of Finnish descent and/or who have a family history that suggests a familial cause of congenital nephrotic syndrome. The primary purpose of this testing is for pregnancy planning. Detection of an NPHS1 mutation also has therapeutic implications, as such nephrotic syndrome is steroid resistant.

Primary dystonia (DYT-1)

Dystonia consists of repetitive, patterned, twisting, and sustained movements that may be either slow or rapid. Dystonic states are classified as primary, secondary, or psychogenic depending upon the cause (Jankovic, 2007). By definition, primary dystonia is associated with no other neurologic impairment, such as intellectual, pyramidal, cerebellar, or sensory deficits. Cerebral palsy is the most common cause of secondary dystonia.

Primary dystonia may be sporadic or inherited (Jankovic, 2007). Cases with onset in childhood usually are inherited in an autosomal dominant pattern. Many patients with hereditary dystonia have a mutation in the TOR1A (DYT1) gene that encodes the protein torsinA, an ATP-binding protein in the 9q34 locus. The role of torsinA in the pathogenesis of primary dystonia is unknown. DNA testing for the abnormal TOR1A gene can be performed on individuals with dystonia. The purpose of such testing is to help rule out secondary or psychogenic causes of dystonia, and for family planning purposes.

Malignant melanoma

An estimated 8 to 12 % of persons with melanoma have a family history of the disease, but not all of these individuals have hereditary melanoma (Tsao and Haluska, 2007). In some cases, the apparent familial inheritance pattern may be due to clustering of sporadic cases in families with common heavy sun exposure and susceptible skin type.

A melanoma susceptibility locus has been identified on chromosome 9p21; this has been designated CDKN2A (also known as MTS1 (multiple tumor suppressor 1)) (Tsao and Haluska, 2007). There is a variable rate of CDKN2A mutations in patients with hereditary melanoma. The risk of CDKN2A mutation varies from approximately 10 % for families with at least 2 relatives having melanoma, to more than 40 % for families having multiple affected 1st degree relatives spanning several generations.

Persons at increased risk of melanoma are managed with close clinical surveillance and education in risk-reduction behavior (e.g., sun avoidance, sunscreen use). It is unclear how CDKN2A genetic test information would alter clinical recommendations (Tsao and Haluska, 2007). The negative predictive value of a negative test for a CDKN2A mutation is also not established since many familial cases occur in the absence of CDKN2A mutations. It is estimated that the prevalence of CDKN2A mutation carriers is less than 1 % in high incidence populations. Thus, no mutations will be identifiable in the majority of families presenting to clinical geneticists.

The American Society of Clinical Oncology (ASCO) has issued a consensus report on the utility of genetic testing for cancer susceptibility (ASCO, 1996), and recommendations for the process of genetic testing were updated in 2003 (ASCO, 2003). The report notes that the sensitivity and specificity of the commercially available test for CDKN2A mutations are not fully known. Because of the difficulties with interpretation of the genetic tests, and because test results do not alter patient or family member management, ASCO recommends that CDKN2A testing be performed only in the context of a clinical trial.

The Scottish Intercollegiate Guidelines Network (SIGN, 2003) protocols on management of cutaneous melanoma reached similar conclusions, stating that "[g]enetic testing in familial or sporadic melanoma is not appropriate in a routine clinical setting and should only be undertaken in the context of appropriate research studies."

The Melanoma Genetics Consortium recommends that genetic testing for melanoma susceptibility should not be offered outside of a research setting (Kefford et al, 2002). They state that “[u]ntil further data become available, however, clinical evaluation of risk remains the gold standard for preventing melanoma. First-degree relatives of individuals at high risk should be engaged in the same programmes of melanoma prevention and surveillance irrespective of the results of any genetic testing.”

Charcot-Marie Tooth disease type 1A (PMP-22)

Charcot Marie Tooth disease, also known as peroneal muscular atrophy, progressive neural muscular atrophy, as well as hereditary motor and sensory neuropathy, is 1 of the 3 major types of hereditary neuropathy. With an estimated prevalence of at least 1:2,500 (autosomal dominance), CMT is one of the most common genetic neuromuscular disorders affecting approximately 125,000 persons in the United States. This hereditary peripheral neuropathy is genetically and clinically heterogeneous. It is usually inherited in an autosomal dominant manner, and occasionally in an autosomal recessive manner. Sporadic as well as X-linked cases have also been reported. In the X-linked recessive patterns, only males develop the disease, although females who inherit the defective gene can pass the disease onto their sons. In the X-linked dominant pattern, an affected mother can pass on the disorder to both sons and daughters, while an affected father can only pass it onto his daughters. The clinical manifestations can vary greatly in severity and age of onset. The clinical features may be so mild that they may be undetectable by patients, their families and physicians.

Charcot-Marie-Tooth disease is usually diagnosed by an extensive physical examination, assessing characteristic weakness in the foot, leg, and hand, as well as deformities and impaired function in walking and manual manipulation. The clinical diagnosis is then confirmed by electromyogram and nerve conduction velocity tests, and sometimes by biopsy of muscle and of sural cutaneous nerve. Since CMT is a hereditary disease, family history can also help to confirm the diagnosis. Based on studies of motor nerve conduction velocity, CMT can be further classified into 2 types: (i) CMT Type I -- slow conduction velocity (less than 40 meters/second for the median nerve or less than 15 meters/second for the peroneal nerve) which accounts for 70 % of all CMT cases, and (ii) CMT Type II -- normal or near normal nerve conduction velocity with decreased amplitude which accounts for the remaining 30 % of CMT cases. Charcot Marie Tooth Type I disease is a demyelinating neuropathy with hypertrophic changes in peripheral nerves, and has its onset usually during late childhood. On the other hand, CMT Type II is a non-demyelinating neuronal disorder without hypertrophic changes, and has its onset generally during adolescence.

Both CMT Types I and II are characterized by a slow degeneration of peripheral nerves and roots, resulting in distal muscle atrophy commencing in the lower extremities, and affecting the upper extremities several years later. Symptoms include foot drop or clubfoot, paresthesia in legs, sloping gait, later weakness and atrophy of hands, then arms, absence or reduction of deep tendon reflexes, and occasionally mild sensory loss. Charcot Marie Tooth disease is not a fatal disorder. It does not shorten the normal life expectancy of patients, and it does not affect them mentally. As stated earlier, there is a wide range of variation in the clinical manifestations of CMT -- the degree of severity can vary considerably from patient to patient, even among affected family members within the same generation. The condition can range from having no problems to having major difficulties in ambulation in early adult life, however, the latter is unusual. Most patients are able to ambulate and have gainful employment until old age. Currently, there is no specific treatment for this disease. Management of the majority of patients with CMT disease consists of supportive care with emphasis on proper bracing, foot care, physical therapy and occupational counseling. For example, the legs and shoes can be fitted with light braces and springs, respectively, to overcome foot drop. If foot drop is severe and the disease has become stationary, the ankle can be stabilized by arthrodeses.

The underlying genetic basis for CMT Type I has been characterized. A point mutation in the PMP22 gene which encodes a peripheral myelin protein with an apparent molecular weight of 22,000 or a DNA duplication of a specific region 5 megabases) including the PMP22 gene in the proximal short arm of chromosome 17 (band 17p11.2-p12) has been identified in 70 % of clinically diagnosed patients --- CMT Type IA. Thus, patients with CMT Type IA represent approximately 50 % of all CMT cases. Other CMT Type I patients (CMT Type IB) exhibit an abnormality (Duffy locus) in the proximal long arm of chromosome number 1 (band 1q21-22). Presently, no test is available for the dominant CMTIB gene on chromosome 1. On the other hand, a CMT Type IA DNA test is available commercially. The test is accomplished through a blood sample analysis -- DNAs are extracted from leukocytes of patients and pulsed-field gel electrophoresis is employed to isolate large segments of DNA encompassing CMTIA duplication-specific junction fragments which are then detected by hybridization with aCMTIA duplication-specific probe (CMTIA-REP). This probe identifies the homologous regions that flank the CMTIA duplication monomer unit.

A positive CMTIA DNA test means the presence of a 500 kilobases CMTIA duplication specific junction fragment, and is diagnostic for CMT Type IA. A negative CMT Type IA means the absence of the CMTIA duplication specific junction fragment, and does not rule out a diagnosis of CMT disease. This is because patients with CMT Type IA represent approximately 50 % of all CMT cases. The value of this molecular test in family planning is questionable because of its relatively low detection rate and its inability to predict the severity of the disease. Moreover, it is likely that there are undiscovered CMTI genes since there are dominant CMTI pedigrees who do not have abnormalities at the known chromosome 1 and 17 locations (CMT Type IC). In addition, other investigators have reported X-linked forms of CMTI at the region of Xq13-21, and Xq26.

Since CMT is not life-threatening, rarely severely disabling, and has no specific treatment, it is unclear how the results of this CMT Type I DNA test, which can not predict the severity of the disease, would affect family planning. Moreover, because of its low detection rate, the CMT Type I DNA test appears to be inferior to the conventional means of diagnosis through physical examination, family history, electromyography and nerve conduction velocity studies. Thus, the sole value of genetic testing for CMTIA is to establish the diagnosis and to distinguish this from other causes of neuropathy.

Familial amyotrophic lateral sclerosis (SOD1 Mutation)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease involving both the upper motor neurons (UMN) and lower motor neurons (LMN). UMN signs include hyperreflexia, extensor plantar response, increased muscle tone, and weakness in a topographical representation. LMN signs include weakness, muscle wasting, hyporeflexia, muscle cramps, and fasciculations. In the early stage of the disease, the clinical aspects of ALS can vary. Affected individuals typically present with asymmetric focal weakness of the extremities (stumbling or poor handgrip) or bulbar findings (dysarthria, dysphagia). Other findings include muscle fasciculations, muscle cramps, and lability of affect but not necessarily mood. Regardless of initial symptoms, atrophy and weakness eventually affect other muscles. Approximately 5,000 people in the U.S. are diagnosed with AML each year.

Most people with ALS have a form of the condition that is described as sporadic or non-inherited. The cause of sporadic ALS is largely unknown but probably involves a combination of genetic and environmental factors. About 10 % of people with ALS have a familial form of the condition, which is caused by an inherited genetic mutation, usually as an autosomal dominant trait. The mean age of onset of ALS in individuals with no known family history is 56 years and in familial ALS it is 46 years.

The diagnosis of ALS is based on clinical features, electrodiagnostic testing (EMG), and exclusion of other health conditions with related symptoms. At present, genetic testing in ALS has no value in making the diagnosis. The only genetic test currently available detects the SOD1 mutation. Since only 20 % of familial ALS patients will test positively for an SOD1 mutation, this test has limited value in genetic counseling.

Migrainous vertigo

Migrainous vertigo is a term used to describe episodic vertigo in patients with a history of migraines or with other clinical features of migraine. Approximately 20 to 33 % of migraine patients experience episodic vertigo. The underlying cause of migrainous vertigo is not very well understood. There are no confirmatory diagnostic tests or susceptible genes associated with migrainous vertigo. Other conditions, specifically Meniere's disease and structural and vascular brainstem disease, must be excluded (Black, 2006).

Prostate cancer

At this time, there are no susceptibility genes that have been unequivocally associated with prostate cancer predisposition. Genetic testing for prostate cancer is currently available only within the context of a research study. A special report on prostate cancer genetics by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2008) stated that single-nucleotide polymorphisms (SNPs) do not predict certainty of disease, nor do they clearly predict aggressive versus indolent disease. The report noted that, while the monitoring of high-risk men may improve outcomes, it is also possible that these could be offset by the harms of identifying and treating additional indolent disease.

Type 2 diabetes

Available evidence has shown that screening for a panel of gene variants associated with type 2 diabetes does not substantially improve prediction of risk for the disease than an assessment based on traditional risk factors. Available evidence suggests that both genetic and environmental factors play a role in the development of type 2diabetes. Recent genetic studies have identified 18 gene variants that appear to increase the risk for type 2 diabetes.

A study reported in the New England Journal of Medicine evaluated the potential utility of genetic screening in predicting future risk of type 2 diabetes (Meigs et al, 2008). The investigators analyzed records from the Framingham Offspring Study, which follows a group of adult children of participants of the original Framingham Heart study, to evaluate risk factors for the development of cardiovascular disease, including diabetes. Full genotype results for the 18 gene variants as well as clinical outcomes were available for 2,377 participants, 255 of whom developed type 2 diabetes during 28 years of follow-up. Each participant was assigned a genotype score, based on the number of risk-associated gene copies inherited. The investigators compared the predictive value of the genotype score to that of family history alone or of physiological risk factors. Overall, the genetic score was 17.7 among those who developed diabetes and 17.1 among those who did not. The investigators found that, while the genetic score did help predict who would develop diabetes, once other known risk factors were taken into consideration, it offered little additional predictive power. The investigators concluded that: "[t]he genotype score resulted in the appropriate risk reclassification of, at most, 4 % of the subjects, compared with risk estimates based on age, sex, blood lipids, body mass index, family history, and other standard risk factors." The investigators reported that "[o]ur findings underscore the view that identification of adverse phenotypic characteristics remains the cornerstone of approaches to predicting the risk of type 2 diabetes," the authors said.

A similar study among Swedish and Finnish patients, published in the same issue of the New England Journal of Medicine, also found only a small improvement in risk estimates when genetic factors were added to traditional risk factors (Lyssenko et al, 2008).

The OncoVue breast cancer risk test

The OncoVue breast cancer risk test (Intergenetics, Inc., Oklahoma City, OK) is a genetic-based breast cancer risk test that incorporates both individualized genetic-based single nucleotide polymorphisms (SNPs) and personal history measures to arrive at an estimate of a woman’s breast cancer risk at various stages in her life.

Cells that are collected from the inside of the cheek are analyzed using thousands of proprietary (Intergenetic, Inc.) combinations of multiple genes. The genetic information and the data from the medical history are combined to assign a numeric value that tells a woman's lifetime risk of developing breast cancer. Her OncoVue risk test will tell her if she is standard, moderate or high risk for developing breast cancer during each stage of her life.

OncoVue is based on an un-published case-controlled associative study that examined common genetic polymorphisms and medical history variables. Currently, 117 common polymorphisms (mostly SNPs) located in over 87 genes believed to alter breast cancer risk are examined. Most result in amino acid changes in the proteins encoded by the genes in which they occur. The medical history variables include answers to questions concerning women’s reproductive histories, family histories of cancer and a few other questions related to general health.

There are no published controlled studies on the OncoVue breast cancer risk test in the peer-reviewed medical literature.

Gail (2009) evaluated the value of adding SNP genotypes to a breast cancer risk model. Criteria that are based on 4 clinical or public health applications were used to compare the National Cancer Institute's Breast Cancer Risk Assessment Tool (BCRAT) with BCRATplus7, which includes 7 SNPs previously associated with breast cancer. Criteria included number of expected life-threatening events for the decision to take tamoxifen, expected decision losses (in units of the loss from giving a mammogram to a woman without detectable breast cancer) for the decision to have a mammogram, rates of risk re-classification, and number of lives saved by risk-based allocation of screening mammography. For all calculations, the following assumptions were made: Hardy-Weinberg equilibrium, linkage equilibrium across SNPs, additive effects of alleles at each locus, no interactions on the logistic scale among SNPs or with factors in BCRAT, and independence of SNPs from factors in BCRAT. Improvements in expected numbers of life-threatening events were only 0.07 % and 0.81 % for deciding whether to take tamoxifen to prevent breast cancer for women aged 50 to 59 and 40 to 49 years, respectively. For deciding whether to recommend screening mammograms to women aged 50 to 54 years, the reduction in expected losses was 0.86 % if the ideal breast cancer prevalence threshold for recommending mammography was that of women aged 50 to 54 years. Cross-classification of risks indicated that some women classified by BCRAT would have different classifications with BCRATplus7, which might be useful if BCRATplus7 was well calibrated. Improvements from BCRATplus7 were small for risk-based allocation of mammograms under costs constraints. The author reported that the gains from BCRATplus7 were small in the applications examined and that models with SNPs, such as BCRATplus7, have not been validated for calibration in independent cohort data. The author concluded that additional studies are needed to validate a model with SNPs and justify its use.

There is insufficient evidence on the effectiveness of the OncoVue breast cancer risk test in determining a woman’s breast cancer risk at various stages in her life.

The phosphatase and tensin homolog (PTEN) gene test

Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome is an autosomal dominant group of disorders with significant clinical overlap, most notably predisposition to hamartomatous polyposis of the gastro-intestinal tract. Laurent-Puig et al (2009) stated that the occurrence of KRAS mutation is predictive of non-response and shorter survival in patients treated by anti-epidermal growth factor receptor (anti-EGFR) antibody for metastatic colorectal cancer (mCRC), leading the European Medicine Agency to limit its use to patients with wild-type KRAS tumors. However, only 50 % of these patients will benefit from treatment, suggesting the need to identify additional biomarkers for cetuximab-based treatment efficacy. These investigators retrospectively collected tumors from 173 patients with mCRC. All but 1 patient received a cetuximab-based regimen as second-line or greater therapy. KRAS and BRAF status were assessed by allelic discrimination. EGFR amplification was assessed by chromogenic in situ hybridization and fluorescent in situ hybridization, and the expression of PTEN was assessed by immunochemistry. In patients with KRAS wild-type tumors (n = 116), BRAF mutations (n = 5) were

weakly associated with lack of response ($p = 0.063$) but were strongly associated with shorter progression-free survival ($p < 0.001$) and shorter overall survival (OS; $p < 0.001$). A high EGFR polysomy or an EGFR amplification was found in 17.7 % of the patients and was associated with response ($p = 0.015$). PTEN null expression was found in 19.9 % of the patients and was associated with shorter OS ($p = 0.013$). In multi-variate analysis, BRAF mutation and PTEN expression status were associated with OS. The authors concluded that BRAF status, EGFR amplification, and cytoplasmic expression of PTEN were associated with outcome measures in KRAS wild-type patients treated with a cetuximab-based regimen. They stated that more studies in clinical trial cohorts are needed to confirm the clinical utility of these markers.

Siena et al (2009) noted that the monoclonal antibodies panitumumab and cetuximab that target the EGFR have expanded the range of treatment options for mCRC. Initial evaluation of these agents as monotherapy in patients with EGFR-expressing chemotherapy-refractory tumors yielded response rates of approximately 10 %. The realization that detection of positive EGFR expression by immunostaining does not reliably predict clinical outcome of EGFR-targeted treatment has led to an intense search for alternative predictive biomarkers. Oncogenic activation of signaling pathways downstream of the EGFR, such as mutation of KRAS, BRAF, or PIK3CA oncogenes, or inactivation of the PTEN tumor suppressor gene is central to the progression of colorectal cancer. Tumor KRAS mutations, which may be present in 35 % to 45 % of patients with colorectal cancer, have emerged as an important predictive marker of resistance to panitumumab or cetuximab treatment. In addition, among colorectal tumors carrying wild-type KRAS, mutation of BRAF or PIK3CA or loss of PTEN expression may be associated with resistance to EGFR-targeted monoclonal antibody treatment, although these additional biomarkers require further validation before incorporation into clinical practice. Additional knowledge of the molecular basis for sensitivity or resistance to EGFR-targeted monoclonal antibodies will allow the development of new treatment algorithms to identify patients who are most likely to respond to treatment and could also provide rationale for combining therapies to overcome primary resistance. The use of KRAS mutations as a selection biomarker for anti-EGFR monoclonal antibody (e.g., panitumumab or cetuximab) treatment is the first major step toward individualized treatment for patients with mCRC.

Epsilon-sarcoglycan gene (SCGE) deletion analysis

Myoclonus-dystonia (M-D), an autosomal dominant inherited movement disorder, has been associated with mutations in the epsilon-sarcoglycan gene (SCGE) on 7q21. Raymond et al (2008) noted that M-D due to SGCE mutations is characterized by early onset myoclonic jerks, often associated with dystonia. Penetrance is influenced by parental sex, but other sex effects have not been established. In 42 affected individuals from 11 families with identified mutations, these researchers found that sex was highly associated with age at onset regardless of mutation type; the median age onset for girls was 5 years versus 8 years for boys ($p < 0.0097$). Moreover, the authors found no association between mutation type and phenotype.

Ritz et al (2009) stated that various mutations within the SGCE gene have been associated with M-D, but mutations are detected in only about 30 % of patients. The lack of stringent clinical inclusion criteria and limitations of mutation screens by direct sequencing might explain this observation. Eighty-six M-D index patients from the Dutch national referral center for M-D underwent neurological examination and were classified according to previously published criteria into definite, probable and possible M-D. Sequence analysis of the SGCE gene and screening for copy number variations were performed. In addition, screening was carried out for the 3 bp deletion in exon 5 of the DYT1 gene. Based on clinical examination, 24 definite, 23 probable and 39 possible M-D patients were detected. Thirteen of the 86 M-D index patients carried a SGCE mutation: 7 nonsense mutations, 2 splice site mutations, 3 missense mutations (2 within 1 patient) and 1 multi-exonic deletion. In the definite M-D group, 50 % carried an SGCE mutation and 1 single patient in the probable group (4 %). One possible M-D patient showed a 4 bp deletion in the DYT1 gene (c.934_937delAGAG). The authors concluded that mutation carriers were mainly identified in the definite M-D group. However, in 50 % of definite M-D cases, no mutation could be identified.

Home genetic tests

Walker (2010) stated that according to an undercover investigation by the Government Accountability Office (GAO), home genetic tests often provide incomplete or misleading information to consumers. For the GAO investigation, investigators purchased 10 tests each from 4 different direct-to-consumer genetic tests companies: 23andMe, deCode Genetics, Navigenics, and Pathway Genomics. Five saliva donors each sent 2 DNA samples to each company. In one sample, the donor used his or her real personal and medical information, and for the second sample, they developed faux identifying and medical information. The results, according to the GAO, were far from precise. For example, a donor was told by a company that he had a "below average" risk of developing hypertension, but a second company rated his risk as "average", while a third company, using DNA from the same donor, said the sample revealed an "above average" risk for hypertension. In some cases, the results conflicted with the donor's real medical condition. None of the genetic tests currently offered to consumers has undergone FDA pre-market review.

Familial Cold Autoinflammatory Syndrome

Familial cold autoinflammatory syndrome (FCAS), also known as familial cold urticaria (FCU), is an autosomal dominant condition characterized by rash, conjunctivitis, fever/chills and arthralgias elicited by exposure to cold -- sometimes temperatures below 22° C (72° F). It is rare and is estimated as having a prevalence of 1 per million people and mainly affects Americans and Europeans. Familial cold autoinflammatory syndrome is one of the cryopyrin-associated periodic syndromes (CAPS) caused by mutations in the CIAS1/NALP3 (also known as NLRP3) gene at location 1q44. Familial cold autoinflammatory syndrome shares symptoms, and should not be confused, with acquired cold urticaria, a more common condition mediated by different mechanisms that usually develop later in life and are rarely inherited. There is insufficient evidence to support the use of genetic testing in the management of patients with FCAS/FCU. UpToDate reviews on "Cold urticaria" (Maurer, 2011) and "Cryopyrin-associated periodic syndromes and related disorders" (Nigrovic, 2011) do not mention the use of genetic testing.

Santome Collazo et al (2010) noted that congenital adrenal hyperplasia (CAH) is not an infrequent genetic disorder for which mutation-based analysis for CYP21A2 gene is a useful tool. An UpToDate review on "Diagnosis of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency" (Merke, 2011) states that "[g]enetic testing also can be used to evaluate borderline cases. Genetic testing detects approximately 95 percent of mutant alleles". Furthermore, the Endocrine Society's clinical practice guideline on congenital adrenal hyperplasia (Speiser et al, 2010) suggested genotyping only when results of the adrenocortical profile following cosyntropin stimulation test are equivocal or for purposes of genetic counseling. The Task Force recommends that genetic counseling be given to parents at birth of a CAH child, and to adolescents at the transition to adult care.

Malignant Hyperthermia

Wappler (2010) stated that malignant hyperthermia (MH)-susceptible patients have an increased risk during anesthesia. The aim of this review was to present current knowledge about pathophysiology and triggers of MH as well as concepts for safe anesthesiological management of these patients. Trigger substances and mechanisms have been well-defined to date. Anesthesia can be safely performed with i.v. anesthetics, nitrous oxide, non-depolarizing muscle relaxants, local anesthetics as well as xenon. Attention must be directed to the preparation of the anesthetic machine because modern work-stations need longer cleansing times than their predecessors. Alternatively, activated charcoal might be beneficial for elimination of volatile anesthetics. Day case surgery can be performed in MH-susceptible patients, if all safety aspects are regarded. Whether there is an association between MH susceptibility and other disorders is still a matter of debate. The authors concluded that the incidence of MH is low, but the prevalence can be estimated as up to 1: 3,000. Because MH is potentially lethal, it is relevant to establish management concepts for peri-operative care in susceptible patients. This includes pre-operative genetic and in-vitro muscle contracture test (IVCT), preparation of the anesthetic work-station, use of non-triggering anesthetics, adequate monitoring, availability of sufficient quantities of dantrolene and appropriate post-operative care. Taking these items into account, anesthesia can be safely performed in susceptible patients.

Moreover, an UpToDate review on "Susceptibility to malignant hyperthermia" (Litman, 2011) states that "the contracture test is performed at specific centers around the world (four in the United States). Following testing, the referring physician receives a report indicating whether testing was positive, negative, or equivocal. Positive or equivocal results should be followed-up with genetic testing. Referral information can be found on the Malignant Hyperthermia Association of the United States (MHAUS) website". Genetic testing for MH is indicated in the following groups:

- Patients with a positive or equivocal contracture test to determine the presence of a specific mutation.
- Individuals with a positive genetic test for MH in a family member.
- Patients with a clinical history suspicious for MH (acute MH episode, masseter muscle rigidity, post-operative myoglobinuria, heat or exercise induce rhabdomyolysis) who are unable or unwilling to undergo contracture testing.

Sleep-Walking

Licis et al (2011) stated that sleep-walking is a common and highly heritable sleep disorder. However, inheritance patterns of sleep-walking are poorly understood and there have been no prior reports of genes or chromosomal localization of genes responsible for this disorder. These researchers described the inheritance pattern of sleep-walking in a 4-generation family and identified the chromosomal location of a gene responsible for sleep-walking in this family. A total of 9 affected and 13 unaffected family members of a single large family were interviewed and DNA samples collected. Parametric linkage analysis was performed. Sleep-walking was inherited as an autosomal dominant disorder with reduced penetrance in this family. Genome-wide multi-point parametric linkage analysis for sleep-walking revealed a maximum logarithm of the odds score of 3.44 at chromosome 20q12-q13.12 between 55.6 and 61.4 cM. The authors described the first genetic locus for sleep-walking at chromosome 20q12-q13.12; and concluded that sleep-walking may be transmitted as an autosomal dominant trait with reduced penetrance.

In an editorial that accompanied the afore-metnioned study, Dogu and Pressman (2011) noted that "[a]ccording to currently accepted evidence-based theories, the occurrence of sleepwalking requires genetic predisposition, priming factors such as severe sleep deprivation or stress, and, in addition, a proximal trigger factor such as noise or touch. These factors form the background for a “perfect storm,” all of which must occur before a sleepwalking episode will occur. Hereditary factors likely play an important role, with recessive and multifactorial inheritance patterns having been reported. A recent genetic study has shown that the HLADQB1*05 Ser74 variant is a major susceptibility factor for sleepwalking in familial cases, but this finding has yet to be replicated. Another study attempted to find a causal relationship between sleepwalking and sleep-disordered breathing in cosegregated families of both disorders. However, this study was limited by the absence of molecular data .... The current diagnosis of sleepwalking is based almost entirely on clinical history. There are no objective, independent means of confirming the diagnosis. Additionally, treatment of sleepwalking is symptomatic, aimed at suppressing arousal or reducing deep sleep. identification of causative genes may eventually permit development of an independent test and treatments aimed at the underlying causes of this disorder".

Choroidal Neovascularization

RetnaGene AMD (Sequenom Center for Molecular Medicine) is a laboratory developed genetic test to assess the risk of developing choroidal neovascularization (CNV), the wet form of age-related macular degeneration (AMD), a common eye disorder of the elderly that can lead to blindness. The test identifies at-risk Caucasians, age 60 and older. A report of the American Academy of Ophthalmology (Stone, et al., 2012) recommends avoidance of routine genetic testing for genetically complex disorders like age-related macular degeneration and late-onset primary open-angle glaucoma until specific treatment or surveillance strategies have been shown in one or more published clinical trials to be of benefit to individuals with specific disease-associated genotypes. The report recommends that, in the meantime, genotyping of such patients should be confined to

research studies. The report stated that complex disorders (e.g., age-related macular degeneration and glaucoma) tend to be more common in the population than monogenic diseases, and the presence of any one of the disease-associated variants is not highly predictive of the development of disease. The report stated that, in many cases, standard clinical diagnostic methods like biomicroscopy, ophthalmoscopy, tonography, and perimetry will be more accurate for assessing a patient's risk of vision loss from a complex disease than the assessment of a small number of genetic loci. The report said that genetic testing for complex diseases will become relevant to the routine practice of medicine as soon as clinical trials can demonstrate that patients with specific genotypes benefit from specific types of therapy or surveillance. The report concluded that, until such benefit can be demonstrated, the routine genetic testing of patients with complex eye diseases, or unaffected patients with a family history of such diseases, is not warranted.

Malignant Hyperthermia

Central core disease (CCD) also known as central core myopathy and Shy-Magee syndrome, is an inherited neuromuscular disorder characterized by central cores on muscle biopsy and clinical features of a congenital myopathy. Prevalence is unknown but the condition is probably more common than other congenital myopathies. CCD typically presents in infancy with hypotonia and motor developmental delay and is characterized by predominantly proximal weakness pronounced in the hip girdle; orthopedic complications are common and malignant hyperthermia susceptibility (MHS) is a frequent complication.

Malignant hyperthermia (MH) or malignant hyperpyrexia is a rare but severe pharmacogenetic disorder that occurs when patients undergoing anesthesia experience a hyperthermic reaction when exposed to certain anesthetic agents. Anesthetic agents that may trigger MH are desflurane, enflurane, halothane, isoflurane, sevoflurane, and suxamethonium chloride. MH usually occurs in the operating theater, but can occur at anytime during anesthesia and up to an hour after discontinuation.

CCD and MHS are allelic conditions both due to (predominantly dominant) mutations in the skeletal muscle ryanodine receptor (RYR1) gene, encoding the principal skeletal muscle sarcoplasmic reticulum calcium release channel (RyR1). Altered excitability and/or changes in calcium homeostasis within muscle cells due to mutation-induced conformational changes of the RyR protein are considered the main pathogenetic mechanism(s).

The diagnosis of CCD is based on the presence of suggestive clinical features and central cores on muscle biopsy; muscle MRI may show a characteristic pattern of selective muscle involvement and aid the diagnosis in cases with equivocal histopathological findings. Mutational analysis of the RYR1 gene may provide genetic confirmation of the diagnosis. Further evaluation of the underlying molecular mechanisms may provide the basis for future rational pharmacological treatment.

The reference standard test for establishing a clinical diagnosis of MHS is the caffeine halothane contracture test (CHCT) in the United States, and the in vitro contracture test (IVCT) in Europe and Australasia. The CHCT and IVCT are similar and measure the muscle contracture in the presence of the anesthetic halothane and caffeine. Both tests categorize patients as being MHS, MH equivocal (MHE), or MH negative (MHN). These tests are invasive and must be performed using a skeletal muscle biopsy that is < 5 hours old. Sequence variants in the ryanodine receptor 1 (skeletal) (RYR1) gene have been shown to be associated with MH susceptibility (MHS) and are found in up to 80% of patients with confirmed MH, usually with an autosomal dominant pattern of inheritance. Although additional genetic loci have been associated with MH, the contribution of these other loci to MH is low.

Genetic testing for RYR1 sequence variants from commercial providers is performed by polymerase chain reaction (PCR) followed by direct sequencing. Genetic tests for RYR1 sequence variants can be performed to either identify sequence variants in genetic hot spots of the RYR1 gene that cover all exons on which causative MH variants can be found, or for screening of sequence variants across the entire 106 exons of the RYR1 gene.

Examples of commercially available tests are: Malignant Hyperthermia/Central Core Disease (570-572) RYR1 Sequencing (Prevention Genetics); Malignant hyperthermia (RYR1 gene sequenced analysis, partial) (University of Pittsburgh Medical Center, Division of Molecular Diagnostics [UPMC Molecular Diagnostics]).

Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT), also called Osler-Weber-Rendu syndrome, is an autosomal dominant trait disorder that results in the development of multiple abnormalities in the blood vessels. Some arterial vessels flow directly into veins rather than into the capillaries resulting in arteriovenous malformations. When they occur in vessels near the surface of the skin, where they are visible as red markings, they are known as telangiectases (the singular is telangiectasia). Nosebleeds are very common in people with HHT, and more serious problems may arise from hemorrhages in the brain, liver, lungs, or other organs. Forms of HHT include type 1, type 2, type 3, and juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome. People with type 1 tend to develop symptoms earlier than those with type 2, and are more likely to have blood vessel malformations in the lungs and brain. Type 2 and type 3 may be associated with a higher risk of liver involvement. Women are more likely than men to develop blood vessel malformations in the lungs with type 1, and are also at higher risk of liver involvement with both type 1 and type 2. Individuals with any form of hereditary hemorrhagic telangiectasia, however, can have any of these problems.

Genetic testing utilizes a blood test to determine whether or not an at risk individual carries the genes responsible for the development of disease. Mutations in two genes, endoglin and ALK-1, have been shown to be responsible for pure HHT, with the disease subtypes designated HHT1 and HHT2. Mutations in Smad4 result in a juvenile polyposis-HHT overlap syndrome.

In 2010, Shah and group wrote that hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder with age-dependent penetrance characterized by recurrent epistaxis, mucocutaneous telangiectasias, and visceral arteriovenous malformations (AVMs). AVMs can occur in multiple organs, including brain, liver, and lungs, and are associated with a large

portion of disease morbidity. Pulmonary AVMs (PAVMs) can be asymptomatic or manifest as dyspnea and hypoxemia secondary to shunting. The presence of untreated PAVMs can also lead to transient ischemic attacks, stroke, hemothorax, and systemic infection, including cerebral abscesses. Definitive diagnosis is made when three or more clinical findings are present, which include the features mentioned above and a first-degree relative diagnosed with HHT. Diagnosis is suspected when two findings are present. Genetic testing can help confirm diagnosis. Mutations in three genes are known to cause disease: ENG, ACVRL1, and SMAD4. Genetic testing involves sequence and duplication/deletion analysis and identifies a mutation in roughly 80% of patients with clinical disease.

The textbook *Flint: Cummings Otolaryngology: Head & Neck Surgery* (2010) states that genetic testing is available for prenatal diagnosis of hereditary hemorrhagic telangiectasia. This is important, because catastrophic hemorrhage can occur in children with clinically silent disease, thus screening imaging for cerebral and pulmonary arteriomalformations is indicated in children who have a family history.

According to the textbook of *Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease* (2010), genetic testing to detect mutations in the ENG, ALK-1, or MAHD4 genes may be helpful in selected cases. Patients suspected of having HHT should be screened for cerebral and pulmonary arteriovenous malformations (AVMs), and family members of the patient should consider genetic testing.

The textbook *Cassidy: Management of Genetic Syndromes* (2005), reports that, to date, mutation testing has not been widely used in the diagnosis of HHT. However, mutations in either ALK1 or endoglin have been demonstrated in over 70% of unrelated, affected individuals tested using direct gene sequencing of genomic DNA. Genetic testing for HHT will have an important role in both the testing of individuals for whom the diagnosis is uncertain and in presymptomatic testing of young adults at risk of HHT.

In 2006, Bossler and group describe the results of mutation analysis on a consecutive series of 200 individuals undergoing clinical genetic testing for HHT. The observed sensitivity of mutation detection was similar to that in other series with strict ascertainment criteria. A total of 127 probands were found, with sequence changes consisting of 103 unique alterations, 68 of which were novel. In addition, eight intragenic rearrangements in the ENG gene and two in the ACVRL1 gene were identified in a subset of coding sequence mutation-negative individuals. Most individuals tested could be categorized by the number of HHT diagnostic criteria present. Surprisingly, almost 50% of the cases with a single symptom were found to have a significant sequence alteration; three of these reported only nosebleeds. The authors concluded, "genetic testing can confirm the clinical diagnosis in individuals and identify presymptomatic mutation carriers. As many of the complications of HHT disease can be prevented, a confirmed molecular diagnosis provides an opportunity for early detection of AVMs and management of the disease."

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA), which has an estimated prevalence of 1 in 10,000, is characterized by proximal muscle weakness resulting from the degeneration of anterior horn cells in the spinal cord. SMA type I is typically diagnosed at birth or within the first 3 to 6 months of life; affected children are unable to sit unassisted and usually die from respiratory failure within 2 years. Those with SMA type II, which is diagnosed before 18 months of age, are unable to stand or walk unaided, although they may be able to sit and may survive beyond age 4. The clinical features of SMA types III and IV are milder and manifest after 18 months of age or in adulthood, respectively. SMA is inherited in an autosomal recessive manner and is caused by alterations in the survival motor neuron 1 (SMN1) gene located on chromosome 5 at band q12.2 to q13.3. Approximately 95% of SMA patients have the condition as a result of a homozygous deletion involving at least exon 7 of SMN1. Approximately 5% are compound heterozygotes, with a deletion in 1 allele of SMN1 and a subtle intragenic variation in the other. SMN2, a gene nearly identical in sequence to SMN1, is located in the same highly repetitive region on chromosome 5. Although it does not cause SMA, it has been shown to modify the phenotype of the condition; those with the milder SMA types II or III tend to have more copies of SMN2 than those with the severe type I.

SMN1 deletions are detected by polymerase chain reaction (PCR) amplification of exon 7 of the SMN genes, followed by restriction fragment length polymorphism (RFLP) analysis. Following amplification, exon 7 of SMN2 will be cut with the restriction enzyme DraI, while exon 7 of SMN1 will remain intact. SMA patients with homozygous SMN1 deletions will show an absence of the uncut SMN1 exon 7 PCR products. To detect heterozygous SMN1 deletions in SMA carriers or compound heterozygotes, quantitative PCR (qPCR) is performed. To identify subtle intragenic variations in SMA patients found to have only 1 copy of the deletion, the SMN1 gene is typically sequenced. Candidates for diagnostic testing include infants, children, and adults with generalized hypotonia and proximal muscle weakness of unknown etiology. Carrier testing may be offered to couples considering pregnancy, including those with a family history of SMA, and prenatal diagnosis should be made available to all identified carriers.

Genetic Test Panels for Nonsyndromic Hearing Loss

There is limited published evidence for the clinical validity and clinical utility of specific genetic test panels for nonsyndromic hearing loss. A number of test panels are currently available commercially (e.g., OtoScope, OtoGenome, OtoSeq. The genes included in these test panels differ siginficantly, and there is limited published information on their clinical utlity and clinical validity.

Hearing loss may be classified as either syndromic or nonsyndromic. Nonsyndromic hearing loss is defined by the absence of malformations of the external ear or other medical problems in the affected individual. With the syndromic hearing loss, malformations of the external ear and/or other medical problems are present. Approximately 50% of nonsyndromic hearing loss can be attributed to a genetic cause, and may be inherited in an autosomal recessive (70% of patients), autosomal dominant (20%

of patients), with mitochondrial, X-linked and other genetic causes making up the remainder of patients.

Sequence variants in approximately 60 genes and some micro-RNAs have been associated with causing nonsyndromic hearing loss. Micro-RNAs are post-transcriptional regulators that consist of 20-25 nucleotides. Usher and Pendred syndromes are the most common forms of the approximately 400 forms of syndromic hearing loss. Both have autosomal recessive inheritance. Usher syndrome is characterized by sensorineural hearing loss and later development of retinitis pigmentosa. Usher syndrome has three forms that vary by the profundity of hearing loss and whether vestibular dysfunction is present. The three types of Usher syndrome have been associated with sequence variants in 9 different genes. Pendred syndrome is characterized by congenital hearing loss and euthyroid goiter that develops in the second or third decade of life. Pendred syndrome is associated with sequence variants in the SLC26A4 gene. Some of the genes associated with Usher and Pendred syndromes may also be associated with nonsyndromic hearing loss.

The OtoSCOPE test has been developed to make use of next generation sequencing capabilities, to simultaneously test for sequence variants in 66 genes associated with nonsyndromic hearing loss as well as both Usher and Pendred syndromes. The claimed advantage of the OtoScope test is that simultaneous analysis of the 66 genes included in the test may reduce the time and cost compared with genetic testing of individual genes.

the OtoSCOPE genetic testing for hereditary hearing loss is considered investigational/experimental because there is inadequate evidence in the peer-reviewed published clinical literature regarding its effectiveness.

Published evidence for the OtoSeq test panel includes an epidemiological study of the use of a component of the OtoSeq panel in identifying certain hearing loss genes in 34 Pakistani families (Shahzad, et al., 2013). In addition, there is a prelimary study of the performance of the OtoSeq in 8 individuals with hearing loss, comparing the results of Next Generation Sequencing with Sanger Sequencing (Sivakumaran, et al., 2013). There is insufficient published information about the performance and clinical utility of this test.

Heterotaxy

Bedard et al (2011) noted that patients with heterotaxy have characteristic cardiovascular malformations, abnormal arrangement of their visceral organs, and midline patterning defects that result from abnormal left-right patterning during embryogenesis. Loss of function of the transcription factor ZIC3 causes X-linked heterotaxy and isolated congenital heart malformations and represents one of the few known monogenic causes of congenital heart disease. The birth incidence of heterotaxy-spectrum malformations is significantly higher in males, but the authors' previous work indicated that mutations within ZIC3 did not account for the male over-representation. Therefore, cross species comparative sequence alignment was used to identify a putative novel fourth exon, and the existence of a novel alternatively spliced transcript was confirmed by amplification from murine embryonic RNA and subsequent sequencing. This transcript, termed Zic3-B, encompasses exons 1, 2, and 4 whereas Zic3-A encompasses exons 1, 2, and 3. The resulting protein isoforms are 466 and 456 amino acid residues respectively, sharing the first 407 residues. Importantly, the last 2 amino acids in the 5th zinc finger DNA binding domain are altered in the Zic3-B isoform, indicating a potential functional difference that was further evaluated by expression, subcellular localization, and transactivation analyses. The temporo-spatial expression pattern of Zic3-B overlaps with Zic3-A in-vivo, and both isoforms are localized to the nucleus in-vitro. Both isoforms can transcriptionally activate a Gli binding site reporter, but only ZIC3-A synergistically activates upon co-transfection with Gli3, suggesting that the isoforms are functionally distinct. The authors concluded that screening 109 familial and sporadic male heterotaxy cases did not identify pathogenic mutations in the newly identified fourth exon and larger studies are necessary to establish the importance of the novel isoform in human disease.

Tariq et al (2011) heterotaxy-spectrum cardiovascular disorders are challenging for traditional genetic analyses because of clinical and genetic heterogeneity, variable expressivity, and non-penetrance. In this study, high-resolution single nucleotide polymorphisms (SNPs) genotyping and exon-targeted array comparative genomic hybridization (CGH) platforms were coupled to whole-exome sequencing to identify a novel disease candidate gene. SNP genotyping identified absence-of-heterozygosity regions in the heterotaxy proband on chromosomes 1, 4, 7, 13, 15, 18, consistent with parental consanguinity. Subsequently, whole-exome sequencing of the proband identified 26,065 coding variants, including 18 non-synonymous homozygous changes not present in dbSNP132 or 1000 Genomes. Of these 18, only 4 -- 1 each in CXCL2, SHROOM3, CTSO, RXFP1 -- were mapped to the absence-of-heterozygosity regions, each of which was flanked by more than 50 homozygous SNPs, confirming recessive segregation of mutant alleles. Sanger sequencing confirmed the SHROOM3 homozygous missense mutation and it was predicted as pathogenic by 4 bio-informatic tools. SHROOM3 has been identified as a central regulator of morphogenetic cell shape changes necessary for organogenesis and can physically bind ROCK2, a rho kinase protein required for left-right patterning. Screening 96 sporadic heterotaxy patients identified 4 additional patients with rare variants in SHROOM3. The authors concluded that using whole exome sequencing, the authors identify a recessive missense mutation in SHROOM3 associated with heterotaxy syndrome and identify rare variants in subsequent screening of a heterotaxy cohort, suggesting SHROOM3 as a novel target for the control of left-right patterning. This study revealed the value of SNP genotyping coupled with high-throughput sequencing for identification of high yield candidates for rare disorders with genetic and phenotypic heterogeneity.

Also, UpToDate reviews on "Clinical manifestations, pathophysiology, and diagnosis of atrioventricular (AV) canal defects" (Fleishman and Tugertimur, 2013) and "Congenital heart disease (CHD) in the newborn: Presentation and screening for critical CHD" (Altman, 2013) do not mention the use of genetic testing as a management tool.

Mitochondrial Recessive Ataxia Syndrome

Lee et al (2007) stated that spino-cerebellar ataxia (SCA) is a heterogeneous group of neurodegenerative disorders with common features of adult-onset cerebellar ataxia. Many patients with clinically suspected SCA are subsequently diagnosed with common

SCA gene mutations. Previous reports suggested some common mitochondrial DNA (mtDNA) point mutations and mitochondrial DNA polymerase gene (POLG1) mutations might be additional underlying genetic causes of cerebellar ataxia. These researchers tested whether mtDNA point mutations A3243G, A8344G, T8993G, and T8993C, or POLG1 mutations W748S and A467T are found in patients with adult-onset ataxia who did not have common SCA mutations. A total of 476 unrelated patients with suspected SCA underwent genetic testing for SCA 1, 2, 3, 6, 7, 8, 10, 12, 17, and DRPLA gene mutations. After excluding these SCA mutations and patients with paternal transmission history, 265 patients were tested for mtDNA mutations A3243G, A8344G, T8993G, T8993C, and POLG1 W748S and A467T mutations. No mtDNA A3243G, A8344G, T8993G, T8993C, or POLG1 W748S and A467T mutation was detected in any of the 265 ataxia patients, suggesting that the upper limit of the 95 % confidence interval (CI) for the prevalence of these mitochondrial mutations in Chinese patients with adult-onset non-SCA ataxia is no higher than 1.1 %. The authors concluded that the mtDNA mutations A3243G, A8344G, T8993G, T8993C, or POLG1 W748S and A467T are very rare causes of adult-onset ataxia in Taiwan. Routine screening for these mutations in ataxia patients with Chinese origin is of limited clinical value.

Gramstad et al (2009) noted that mutations in the catalytic subunit of polymerase gamma (POLG1) produce a wide variety of neurological disorders including a progressive ataxic syndrome with epilepsy: mitochondrial SCA and epilepsy (MSCAE). The authors' earlier studies of patients with this syndrome raised the possibility of more prominent right than left hemisphere dysfunction. To investigate this in more detail, 8 patients (6 women, 2 men; mean age of 22.3 years) were studied. All completed an intelligence test (Wechsler Adult Intelligence Scale; WAIS), and 4 were also given memory tests and a comprehensive neuropsychological test battery. Patients with MSCAE showed significant cognitive dysfunction. Mean Verbal IQ (84.3) was significantly better than Performance IQ (71.8) ($t = 5.23$, $p = 0.001$), but memory testing and neuropsychological testing failed to detect a consistent unilateral dysfunction. The authors concluded that further studies are needed to define the profile and development of cognitive symptoms in this disorder.

Isohanni et al (2011) stated that mitochondrial DNA polymerase γ (POLG1) mutations in children often manifest as Alpers syndrome, whereas in adults, a common manifestation is mitochondrial recessive ataxia syndrome (MIRAS) with severe epilepsy. Because some patients with MIRAS have presented with ataxia or epilepsy already in childhood, these investigators searched for POLG1 mutations in neurologic manifestations in childhood. They investigated POLG1 in 136 children, all clinically suspected to have mitochondrial disease, with one or more of the following: ataxia, axonal neuropathy, severe epilepsy without known epilepsy syndrome, epileptic encephalopathy, encephalohepatopathy, or neuropathologically verified Alpers syndrome. A total of 7 patients had POLG1 mutations, and all of them had severe encephalopathy with intractable epilepsy. Four patients had died after exposure to sodium valproate. Brain MRI showed parieto-occipital or thalamic hyper-intense lesions, white matter abnormality, and atrophy. Muscle histology and mitochondrial biochemistry results were normal in all. The authors concluded that POLG1 analysis should belong to the first-line DNA diagnostic tests for children with an encephalitis-like presentation evolving into epileptic encephalopathy with liver involvement (Alpers syndrome), even if brain MRI and morphology, respiratory chain activities, and the amount of mitochondrial DNA in the skeletal muscle are normal. POLG1 analysis should precede valproate therapy in pediatric patients with a typical phenotype. However, POLG1 is not a common cause of isolated epilepsy or ataxia in childhood.

Tang et al (2012) determined the prevalence of MNGIE-like phenotype in patients with recessive POLG1 mutations. Mutations in the POLG1 gene, which encodes for the catalytic subunit of the mitochondrial DNA polymerase gamma essential for mitochondrial DNA replication, cause a wide spectrum of mitochondrial disorders. Common phenotypes associated with POLG1 mutations include Alpers syndrome, ataxia-neuropathy syndrome, and progressive external ophthalmoplegia (PEO). Mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder characterized by severe gastrointestinal dysmotility, cachexia, PEO and/or ptosis, peripheral neuropathy, and leukoencephalopathy. MNGIE is caused by TYMP mutations. Rare cases of MNGIE-like phenotype have been linked to RRM2B mutations. Recently, POLG1 mutations were identified in a family with clinical features of MNGIE but no leukoencephalopathy. The coding regions and exon-intron boundaries of POLG1 were sequence analyzed in patients suspected of POLG1-related disorders. Clinical features of 92 unrelated patients with 2 pathogenic POLG1 alleles were carefully reviewed. Three patients, accounting for 3.3 % of all patients with 2 pathogenic POLG1 mutations, were found to have clinical features consistent with MNGIE but no leukoencephalopathy. Patient 1 carries p.W748S and p.R953C; patient 2 is homozygous for p.W748S, and patient 3 is homozygous for p.A467T. In addition, patient 2 has a similarly affected sibling with the same POLG1 genotype. POLG1 mutations may cause MNGIE-like syndrome, but the lack of leukoencephalopathy and the normal plasma thymidine favor POLG1 mutations as responsible molecular defect.

Furthermore, UpToDate reviews on "Overview of the hereditary ataxias" (Opal and Zoghbi, 2013a) and "The spinocerebellar ataxias" (Opal and Zoghbi, 2013b) do not mention the use of POLG1 genetic testing.

Myelodysplastic Syndromes

National Comprehensive Cancer Network's clinical practice guideline on "Myelodysplastic syndromes" (2014) stated that further evaluations are necessary to establish the role of these genetic lesions on risk stratification systems in myelodysplastic syndrome. The guidelines stated that mutations in TET2 are among the most common mutations reported in patients with myelodysplastic syndromes (about 20 % of cases). Mutations in SF3B1 are one of several common molecular abnormalities involving the RNA splicing machinery, occurring in 14.5 to 16.0 % of MDS cases.

Exome Sequencing

A special report on "Exome sequencing for clinical diagnosis of patients with suspected genetic disorders" by the BCBSA's Technology Evaluation Center (2013) stated that "Exome sequencing has the capacity to determine in a single assay an individual's exomic variation profile, limited to most of the protein coding sequence of an individual (approximately 85 %), composed of about 20,000 genes, 180,000 exons (protein-coding segments of a gene), and constituting approximately 1 % of the

whole genome. It is believed that the exome contains about 85 % of heritable disease-causing mutations …. Exome sequencing, relying on next-generation sequencing technologies, is not without challenges and limitations …. Detailed guidance from regulatory or professional organizations is under development, and the variability contributed by the different platforms and procedures used by clinical laboratories offering exome sequencing as a clinical service is unknown …. Currently, the diagnostic yield for single-gene disorders appears to be no greater than 50 % and possibly less, depending on the patient population and provider expertise. Medical management options may be available for only a subset of those diagnosed".

May-Hegglin Anomaly

Strasser et al (2012) stated that Alport syndrome (ATS) is a type-IV collagen inherited disorder, caused by mutations in COL4A3 and COL4A4 (autosomal recessive) or COL4A5 (X-linked). Clinical symptoms include progressive renal disease, eye abnormalities and high-tone sensori-neural deafness. A renal histology very similar to ATS is observed in a subset of patients affected by mutations in MYH9, encoding non-muscle-myosin Type IIa -- a cytoskeletal contractile protein. MYH9-associated disorders (May-Hegglin anomaly, Epstein and Fechtner syndrome, and others) are inherited in an autosomal dominant manner and characterized by defects in different organs (including eyes, ears, kidneys and thrombocytes). These researchers described here a 6-year old girl with hematuria, proteinuria, and early sensori-neural hearing loss. The father of the patient is affected by ATS, the mother by isolated inner ear deafness. Genetic testing revealed a pathogenic mutation in COL4A5 (c.2605G>A) in the girl and her father and a heterozygous mutation in MYH9 (c.4952T>G) in the girl and her mother. The paternal COL4A5 mutation seems to account for the complete phenotype of ATS in the father and the maternal mutation in MYH9 for the inner ear deafness in the mother. It has been discussed that the interaction of both mutations could be responsible for both the unexpected severity of ATS symptoms and the very early onset of inner ear deafness in the girl.

An UpToDate review on "Congenital and acquired disorders of platelet function" (Coutre, 2013) states that "Giant platelet disorders -- Inherited platelet disorders with giant platelets are quite rare (picture 2 and algorithm 1 and table 4). These include platelet glycoprotein abnormalities (e.g., Bernard-Soulier syndrome), deficiency of platelet alpha granules (e.g., gray platelet syndrome), the May-Hegglin anomaly, which also involves the presence of abnormal neutrophil inclusions (i.e., Dohle-like bodies), and some kindreds with type 2B von Willebrand disease (the Montreal platelet syndrome)". This review does not mention the use of genetic testing as a management tool for giant platelet disorders.

UpToDate reviews on "Inborn errors of metabolism: Epidemiology, pathogenesis, and clinical features" (Sutton, 2013a) and "Inborn errors of metabolism: Classification" (Sutton, 2013b) do not mention the use of genetic testing as a management tool.

Very Long Chain AcylCoA Dehydrogenase Deficiency (VLCADD)

An UpToDate review on "Newborn screening" (Sielski, 2013) states that "MS-MS [tandem mass spectrometry] detects more cases of inborn errors of metabolism than clinical diagnosis. In a study from New South Wales and the Australian Capital Territory, Australia, the prevalence of 31 inborn errors of metabolism affecting the urea cycle, amino acids (excluding PKU), organic acids, and fatty acid oxidation detected by MS-MS in 1998 to 2002 was 15.7 per 100,000 births, compared to 8.6 to 9.5 per 100,000 births in the four four-year cohorts preceding expanded screening. The increased rate of diagnosis was most apparent for the medium-chain and short-chain acyl-Co-A dehydrogenase deficiencies. Whether all children with disorders detected by MS-MS would have become symptomatic is uncertain …. The American Academy of Pediatrics has developed newborn screening fact sheets for 12 disorders, biotinidase deficiency, congenital adrenal hyperplasia, congenital hearing loss, congenital hypothyroidism, cystic fibrosis, galactosemia, homocystinuria, maple syrup urine disease, medium-chain acyl-coenzyme A dehydrogenase deficiency, PKU, sickle cell disease and other hemoglobinopathies, and tyrosinemia …. With the use of tandem mass spectrometry (MS-MS), the prevalence of a confirmed metabolic disorder detected by newborn screening is 1:4000 live births (about 12,500 diagnoses each year) in the United States. The most commonly diagnosed conditions are hearing loss, primary congenital hypothyroidism, cystic fibrosis, sickle cell disease, and medium-chain acyl-CoA dehydrogenase deficiency". This review does not mention very long chain acylCoA dehydrogenase deficiency.

Congenital Stationary Night Blindness

According to Orphanet (a portal for rare diseases and orphan drugs), congenital stationary night blindness (CSNB) is an inherited retinal disorder that predominates on rods. It is a rare disease; and 3 types of transmission can be found: (i) autosomal dominant, (ii) recessive, and (iii) X-linked recessive. The affection is heterogeneous. The only symptom is hemeralopia with a moderate loss of visual acuity. Both the funduscopy and visual field are normal. In recessive forms, the "b" wave on the electroretinogram/electroretinography (ERG) is not found in the scotoscopic study, while the "a" wave is normal and increases with light intensity. In dominant forms, the "b" wave is seen. Levels of rhodopsine are normal and regenerate normally. Signal transmission may be the affected function. There is no specific treatment for CSNB. http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=215.

According to Genetic Home Reference, X-linked CSNB is a disorder of the retina. People with this condition typically have difficulty seeing in low light (night blindness). They also have other vision problems, including reduced acuity, high myopia, nystagmus, and strabismus. Color vision is typically not affected by this disorder. The visual problems associated with this condition are congenital. They tend to remain stable (stationary) over time. Researchers have identified 2 major types of X-linked CSNB: (i) the complete form, and (ii) the incomplete form. The types have very similar signs and symptoms. However, everyone with the complete form has night blindness, while not all people with the incomplete form have night blindness. The types are distinguished by their genetic cause and by the results of ERG. http://ghr.nlm.nih.gov/condition/x-linked-congenital-stationary-night-blindness.

In general, the diagnosis of X-linked CSNB can be made by ophthalmologic examination (including ERG) and family history consistent with X-linked inheritance (Boycott et al, 2012) http://www.ncbi.nlm.nih.gov/books/NBK1245/.

According to a Medscape review on "The Genetics of Hereditary Retinopathies and Optic Neuropathies" (Iannaccone, 2005), CSNB can be inherited according to all Mendelian inheritance patterns; 2 X-linked and 2 autosomal dominant genes have been cloned. In all types of CSNB, night vision is congenitally but non-progressively impaired and the retinal examination is normal. Most CSNB patients also have congenital nystagmus as the presenting sign, which can create a differential diagnostic challenge with Leber congenital amaurosis. Typically, patients with complete X-linked CSNB are also moderate-to-high myopes. The X-linked CSNB forms, which are the most common ones, all share an electronegative electroretinogram response similar to that seen in X-linked retinoschisis, and are distinguished in CSNB type 1 (also known as complete CSNB) and CSNB type 2 (incomplete CSNB) based on additional electroretinogram features, a distinction that has been confirmed at the genetic level". http://www.medscape.com/viewarticle/501761_6.

Price et al (1988) reported that 7 of 8 patients presented initially or were followed for decreased acuity and nystagmus without complaints of night blindness. The diagnosis of CSNB was established with ERG and dark adaptation testing. They stated that careful electrodiagnostic testing is needed to provide accurate genetic counseling. Two patients showed pupillary constriction to darkness, which is a sign of retinal disease in young patients.

Lorenz et al (1996) presented the clinical data of 2 families with X-linked incomplete CSNB previously undiagnosed; ERG recordings in both families were suggestive of CSNB. The ERG of the obligate carrier was normal. In an attempt to distinguish between the complete and the incomplete type, and to identify further carrier signs, scotopic perimetry and dark adaptation were performed in both affected males and carriers. Scotopic perimetry tested the rod-mediated visual pathway in its spatial distribution. In affected males with non-recordable ERGs, scotopic perimetry and dark adaptation disclosed residual rod function indicating an incomplete type. In carriers, there was a sensitivity loss at 600 nm, which may be a new carrier sign. The authors concluded that correct diagnosis of the different forms of CSNB together with the identification of carriers is important for (i) genetic counseling, and (ii) linkage studies to identify the gene(s) for CSNB.

Kim et al (2012) evaluated the frequency of negative waveform ERGs in a tertiary referral center. All patients who had an ERG performed at the electrophysiology clinic at Emory University from January 1999 through March 2008 were included in the study. Patients with b-wave amplitude less than or equal to a-wave amplitude during the dark-adapted bright flash recording, in at least 1 eye, were identified as having a "negative ERG". Clinical information, such as age, gender, symptoms, best corrected visual acuity, and diagnoses were recorded for these patients when available. A total of 1,837 patients underwent ERG testing during the study period. Of those, 73 patients had a negative ERG, for a frequency of 4.0 %. Within the adult (greater than or equal to 18 years of age) and pediatric populations, the frequencies of a negative ERG were 2.5 and 7.2 %, respectively. Among the 73 cases, negative ERGs were more common among male than female patients, 6.7 % versus 1.8 % ($p < 0.0001$). Negative ERGs were most common among male children and least common among female adults, 9.6 % versus 1.1 %, respectively, ($p < 0.0001$). Overall in this group of patients, the most common diagnoses associated with a negative ERG were CSNB (n = 29) and X-linked retinoschisis (XLRS, n = 7). The authors concluded that the overall frequency of negative ERGs in this large retrospective review was 4.0 %. Negative ERGs were most common among male children and least common among female adults. Despite the growing number of new diagnoses associated with negative ERGs, CSNB, and XLRS appear to be the most likely diagnoses for a pediatric patient who presents with a negative ERG.

It is also interesting to note that in a recently completed clinical trial (last verified June 2012) of "Treatment of Congenital Stationary Night Blindness with an Alga Containing High Dose of Beta Carotene", the selection criteria for participants of this trial do not include genetic testing. They included the following: http://www.clinicaltrials.gov/ct2/show/NCT00569023.

- Isolated rod response markedly reduced (less than 20 % of normal) after 20 mins dark adaptation and improved by 50 % after 2 hrs
- Negative maximal response ("a" wave to "b" wave ratio less than 2)
- Retinal mid-peripheral white dots (more than 3,000 dots).

Appendix

Amsterdam II criteria:

At least 3 relatives must have an HNPCC-related cancer*, and all of the following criteria must be present:

- At least 1 of the relatives with cancer associated with HNPC should be diagnosed before age 50 years; and
- At least 2 successive generations must be affected; and
- FAP should be excluded in the colorectal cancer cases (if any); and
- One must be a 1st-degree relative of the other two; and
- Tumors should be verified whenerver possible.

Revised Bethesda criteria:

Member must meet 1 or more of the following criteria:

- Colorectal cancer is diagnosed in a member with 1 or more 1st-degree relatives with an HNPCC-related cancer*, with one of the cancers diagnosed under age 50 years; or
- Colorectal cancer is diagnosed in a member with 2 or more 1st- or 2nd-degree relatives with an HNPCC-related cancer*, regardless of age; or
- Member has colorectal cancer diagnosed before age 50 years; or
- Member has colorectal cancer with microsatellite instability-high (MSI-H) histology, where cancer is diagnosed before age 60 years; or

- Member has synchronous or metachronous HNPCC-related cancers*, regardless of age.

* Hereditary nonpolyposis colorectal cancer (HNPCC)-related cancers include colorectal, endometrial, gastric, ovarian, pancreas, ureter and renal pelvis, brain (usually glioblastoma as seen in Turcot syndrome), and small intestinal cancers, as well as sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes covered if selection criteria are met:

81161
81200
81201 - 81203
81205
81209
81220
81221
81240
81242
81243
81250
81251
81252 - 81253
81255
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81257
81260
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81282
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81292
81293
81294
81295
81296
81297
81298
81299
81300
81301
81302
81310
81317
81318
81319
81321 - 81323
81324 - 81326
81330
81331

81332

88245 - 88269

88271 - 88275

88323

88342

88343

88360 - 88361

CPT codes not covered for indications listed in the CPB:

81222

81223

81377

81383

Other CPT codes related to the CPB:

81400 - 81408

96040

HCPCS codes covered if selection criteria are met:

S3840	DNA analysis for germline mutations of the RET proto-oncogene for susceptibility to multiple endocrine neoplasia type 2
S3841	Genetic testing for retinoblastoma
S3842	Genetic testing for von Hippel-Lindau disease
S3844	DNA analysis of the connexin 26 gene (GJB2) for susceptibility to congenital, profound deafness
S3845	Genetic testing for alpha-thalassemia
S3846	Genetic testing for hemoglobin E beta-thalassemia
S3850	Genetic testing for sickle cell anemia
S3852	DNA analysis for APOE epsilon 4 allele for susceptibility to Alzheimer's disease
S3853	Genetic testing for myotonic muscular dystrophy
S3855	Genetic testing for detection of mutations in the presenilin, 1 gene
S3866	Genetic analysis for a specific gene mutation for hypertrophic cardiomyopathy (HCM) in an individual with a known HCM mutation in the family

HCPCS codes not covered for indications listed in the CPB:

S3861	Genetic testing, sodium channel, voltage-gated, type V, alpha subunit (SCN5A) and variants for suspected Brugada syndrome
S3865	Comprehensive gene sequence analysis for hypertrophic cardiomyopathy

Other HCPCS codes related to the CPB:

G0461	Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462	each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)
S0265	Genetic counseling, under physician supervision, each 15 minutes

ICD-9 codes covered if selection criteria are met:

151.0 - 151.9	Malignant neoplasm of stomach [with 2 HNPCC-related cancers]
152.0 - 152.9	Malignant neoplasm of small intestine, including duodenum [with 2 HNPCC-related cancers]
153.0 - 154.9	Malignant neoplasm of colon [hereditary non-polyposis colorectal cancer (HNPCC) (MLH1, MSH2)
155.0 - 155.2	Malignant neoplasm of liver and intrahepatic bile ducts [with 2 HNPCC cancers]
162.2 - 162.9	Malignant neoplasm of bronchus or lung
170.0 - 170.9	Malignant neoplasm of bone and articular cartilage [osteosarcoma]
171.0 - 171.9	Malignant neoplasm of connective and other soft tissue [soft tissue sarcoma]
174.0 - 175.9	Malignant neoplasm of breast

182.0	Malignant neoplasm of body of uterus
183.0	Malignant neoplasm of ovary and other uterine adnexa [with 2 HNPCC cancers]
188.0 - 188.9	Malignant neoplasm of bladder [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPCC]
189.0	Malignant neoplasm of kidney, except pelvis [renal cell cancer syndrome]
189.1	Malignant neoplasm of renal pelvis [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPCC]
189.2	Malignant neoplasm of ureter [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPCC]
190.5	Malignant neoplasm of retina [retinoblastoma]
191.0 - 191.9	Malignant neoplasm of brain [except glioblastoma multiforme]
193	Malignant neoplasm of thyroid gland [medullary thyroid carcinoma]
194.0	Malignant neoplasm of adrenal gland [adrenocortical carcinoma]
204.00 - 208.92	Leukemias
211.3	Benign neoplasm of colon [hereditary polyposis coli (APC)]
227.0	Benign neoplasm of adrenal gland [hereditary paraganglioma (SDHS, SDHB)]
237.71	Neurofibromatosis, type 1 [von Recklinghausen's disease] [neurofibromin] [not covered for Legius syndrome]
237.72	Neurofibromatosis, type 2 [acoustic neurofibromatosis] [Merlin]
238.1	Neoplasm of uncertain behavior of connective and other soft tissue [hereditary leiomyomatosis]
250.0x - 250.9x w/5th digit of 0 or 2	Type 2 Diabetes mellitus [covered for maturity-onset diabetes of the young (MODY]
253.4	Other anterior pituitary disorders [Kallmann syndrome (FGFR1)]
255.2	Adrenogenital disorders [congenital adrenal hyperplasia]
258.01	Multiple endocrine neoplasia [MEN] type I
259.4	Dwarfism, not elsewhere classified [hypochondroplasia (FGFR 3), thanatophoric dysplasia (FGFR3)]
270.1	Phenylketonuria
270.2	Other disturbances of aromatic amino-acid metabolism [albinism]
270.3	Disturbances of branched-chain amino-acid metabolism
271.0	Glycogenosis [McArdle's disease]
271.1	Galactosemia
272.7	Lipidoses [Fabry/Gaucher (acid beta glucosidase)/Niemann-Pick (sphingomyelin phosphodiesterase)]
273.4	Alpha-1-antitrypsin deficiency
275.01	Hereditary hemochromatosis
275.2	Disorders of magnesium metabolism [Gitelman's syndrome]
275.42	Hypercalcemia (familial hypocalciuric)
277.00 - 277.09	Cystic fibrosis [CTFR]
277.39	Other amyloidosis [hereditary amyloidosis (TTR variants)]
277.5	Mucopolysaccharidosis [type 1 (MPS-1)]
277.85	Disorders of fatty acid oxidation [medium chain acyl coA dehydrogenase deficiency ACADM)]
277.87	Disorders of mitochondrial metabolism [MELAS (mitochondrial encephalopathy) (MTTL1, tRNAleu)]
282.3	Other hemolytic anemias due to enzyme deficiency [pyruvate kinase deficiency (PKD)]
282.40 - 282.49	Thalassemias [alpha globin/beta globin/hemoglobin E]
282.5	Sickle-cell trait [hemoglobin S]
282.7	Other hemoglobinopathies [hemoglobin C]
286.0	Congenital factor VIII disorder [hemophilia A/VWF]
286.1	Congenital factor IX disorder [hemophilia B]

286.3	Congenital deficiency of other clotting factors [prothrombin factor II, 20210A mutation]
287.33	Congenital and hereditary thrombocytopenia [amegakaryocytic]
288.01	Congenital neutropenia [cyclic]
288.02	Cyclic neutropenia [congenital]
299.00 - 299.01	Autistic disorder, current or active state or residual state
317 - 319	Mental retardation
327.25	Congenital central alveolar hypoventilation syndrome
330.0	Leukodystrophy [Canavan disease (aspartoacylase A)]
330.1	Cerebral lipidoses [Tay-Sachs disease]
330.8	Other specified cerebral degenerations in childhood [Rett syndrome (MECP2)] [Leigh syndrome]
333.4	Huntington's chorea [Huntington's disease]
333.6	Genetic torsion dystonia [primary TOR1A (DYT1)]
334.0	Friedreich's ataxia [frataxin]
334.1	Hereditary spastic paraplegia [hereditary spastic paraplegia 3 (SPG3A) and 4 (SPG4, SPAST)]
334.3	Other cerebellar ataxia [spinocerebellar ataxia (ataxin, CACNA1A)] [SCA types 8, 10, 17 and DRPLA)]
334.8	Other spinocerebellar diseases [spinocerebellar ataxia (ataxin, CACNA1A)]
335.10 - 335.19	Spinal muscular atrophy [Kennedy disease (SBMA) (SMN)]
345.10 - 345.11	Generalized convulsive epilepsy [myoclonic epilepsy (MERRF) (MTTK) (tRNAlys)]
356.1	Peroneal muscular atrophy [Charcot-Marie-Tooth disease]
356.2	Hereditary sensory neuropathy [with liability to pressure palsies (HNPP)]
359.0 - 359.1	Congenital hereditary or hereditary progressive muscular dystrophy [Duchene (dystrophin)] [Becker's type] [limb girdle muscular dystrophy (LGMD1, LGMD2)] [not covered for oculopharyngeal muscular dystrophy (OPMD)] [dystrophy [type 1C] [Emery-Dreifuss muscular dystrophy] [fascioscapulohumeral muscular dystrophy]
359.21 - 359.29	Myotonic disorders [myotonic dystrophy (CMPK, ZNF-9)]
359.89	Other myopathies [dysferlin]
362.74	Pigmentary retinal dystrophy [retinitis pigmentosa]
377.39	Other optic neuritis [Leber hereditary optic neuropathy (LHON)]
389.00 - 389.9	Hearing loss [hereditary (Connexin-26, GJB2)]
425.11 - 425.18	Hypertrophic cardiomyopathy
425.4	Other primary cardiomyopathies [arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)]
426.82	Long QT syndrome
427.1	Paroxysmal ventricular tachycardia [persons that display exercise- or emotion-induced polymorphic ventricular tachycardia or ventricular fibrillation, occurring in a structurally normal heart]
427.41	Ventricular fibrillation
441.01	Thoracic aneurysm and dissection
448.0	Hereditary hemorrhagic telangiectasia
577.0	Acute pancreatitis [unexplained episode in a child requiring hospitalization with significant concern that hereditary pancreatitis (PRSS1) should be excluded]
577.1	Chronic pancreatitis [unexplained (idiopathic) for hereditary pancreatitis (PRSS1)]
581.0 - 581.9	Nephrotic syndrome [congenital (NPHS1, NPHS2)]
742.2	Reduction deformities of brain [classical lissencephaly]
742.8	Other specified congenital anomalies of nervous system [familial dysautonomia]
755.55	Acrocephalosyndactyly [Pfeiffer syndrome (FGFR1)]
756.0	Anomalies of skull and face bones [Crouzon syndrome (CTFR), Saethre-Chotzen syndrome (TWIST, FGFR2)]
756.4	Chondrodystrophy [achondroplasia]

756.51	Osteogenesis imperfecta
756.83	Ehlers-Danlos syndrome
756.89	Other specified anomalies of muscle, tendon, fascia, and connective tissue [Jackson-Weiss syndrome] [Muencke syndrome (FGFR2)]
757.39	Other specified congenital anomalies of skin [Bloom syndrome]
758.32	Velo-cardio-facial syndrome [22q11 deletion syndrome (CATCH-22)]
759.6	Other hamartoses, not elsewhere classified [Von Hippel Lindau syndrome (VHL)]
759.81	Prader-Willi syndrome [GABRA, SNRPN]
759.82	Marfan syndrome
759.83	Fragile X syndrome
759.89	Other specified anomalies [Angelman syndrome (GABRA, SNRPN) Smith-Lemli-Opitz syndrome]
783.43	Short stature [SHOX-related]
790.5	Other nonspecific abnormal serum enzyme levels [hyper-amylasemia]
995.86	Malignant hyperthermia
V10.05	Personal history of malignant neoplasm of large intestine [with HNPCC related cancers]
V10.06	Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus
V16.0	Family history of malignant neoplasm of gastrointestinal tract
V17.2	Family history of other neurological diseases
V17.41	Family history of sudden cardiac death (SCD)
V17.49	Family history of other cardiovascular diseases [children or young adults (less than 40 years of age) with a first degree relative with a clinical diagnosis of CPVT, or a first or second degree relative with a defined CPVT mutation]
V18.4	Family history of mental retardation
V18.51	Family history, colonic polyps [members with first-degree relatives (i.e., siblings, parents, and offspring) diagnosed with familial adenomatous polyposis (FAP) or with a documented APC mutation]
V18.59	Family history of other digestive disorders [pancreatitis]
V18.9	Family history of genetic disease carrier
V19.5	Family history of congenital anomalies
V26.31	Testing of female for genetic disease carrier status
V26.34	Testing of male for genetic disease carrier status
V77.0	Special screening for thyroid disorders
V77.3	Special screening for phenylketonuria
V77.4	Special screening for galactosemia
V77.6	Special screening for cystic fibrosis
V77.7	Special screening for other inborn errors of metabolism
V80.3	Special screening for ear diseases
V83.01	Asymptomatic hemophilia A carrier
V83.02	Symptomatic hemophilia A carrier
V83.81	Cystic fibrosis gene carrier
V83.89	Other genetic carrier status
V84.01	Genetic susceptibility to malignant neoplasm of breast [covered for Li-Fraumeni syndrome testing other than OncoVue]

ICD-9 codes not covered for indications listed in the CPB:

172.0 - 172.9	Malignant melanoma of skin
185	Malignant neoplasm of prostate
271.3	Intestinal disaccharidase deficiencies and disaccharide malabsorption [lactose intolerance]
272.6	Lipodystrophy [familial partial lipodystrophy]
277.85	Disorders of fatty acid oxidation [very long chain acylCoA dehydrogenase deficiency (VLCADD)]

284.01	Constitutional red blood cell aplasia [Diamond-Blackfan anemia]
286.2	Congenital factor XI deficiency [hemophilia C]
286.4	von Willebrand's disease
288.2	Genetic anomalies of leukocytes [May-Hegglin anomaly]
307.46	Sleep arousal disorder [somnambulation]
331.0	Alzheimer's disease
332.0 - 332.1	Parkinson's disease
333.2	Myoclonus [-dystonia]
335.20	Amyotrophic lateral sclerosis [familial (SOD1 mutation)]
346.30 - 346.33	Hemiplegic migraine
347.00 - 347.11	Cataplexy and narcolepsy
362.16	Retinal neovascularization NOS
362.50	Macular degeneration (senile), unspecified
362.51	Nonexudative senile macular degeneration
362.52	Exudative senile macular degeneration
368.61	Congenital night blindness
389.00 - 389.9	Hearing loss
410.00 - 410.92	Acute myocardial infarction
411.0 - 411.89	Other acute and subacute forms of ischemic heart disease
412	Other acute and subacute forms of ischemic heart disease
413.0 - 413.9	Angina pectoris
414.00 - 414.9	Other forms of chronic ischemic heart disease
746.89	Other congenital anomalies of heart [Brugada syndrome]
753.12 - 753.14	Polycystic kidney
756.16	Klippel-Feil syndrome
756.59	Other congenital osteodystrophies with bracketed info [Mccune-Albright syndrome]
757.1	Ichthyosis congenita [epidemolytic hyperkatosis]
759.9	Congenital anomaly, unspecified [heterotaxy]
780.31 - 780.32	Febrile convulsions
780.4	Dizziness and giddiness [migrainous vertigo]
V16.3	Family history of malignant neoplasm, breast
V81.0	Special screening for ischemic heart disease
V81.2	Special screening for other and unspecified cardiovascular conditions

Other ICD-9 codes related to the CPB:

V13.69	Personal history of other congenital malformations
V26.32	Other genetic testing of female
V26.33	Genetic counseling
V26.39	Other genetic testing of male
V28.0	Screening for chromosomal anomalies by amniocentesis
V29.3	Observation for suspected genetic or metabolic condition
V77.91	Screening for lipoid disorders
V77.99	Special screening for other and unspecified endocrine, nutritional, metabolic, and immunity disorders
V78.1	Special screening for other and unspecified deficiency anemia
V78.2	Special screening for sickle-cell disease or trait
V78.3	Special screening for other hemoglobinopathies
V78.8	Special screening for other disorders of blood and blood-forming organs

V80.09	Special screening for other neurological conditions
V82.4	Maternal postnatal screening for chromosomal anomalies
V82.71 - V82.79	Genetic screening
V84.09	Genetic susceptibility to other malignant neoplasm
V84.81 - V84.89	Genetic susceptibility to other disease

Factor V Leiden:

CPT codes covered if selection criteria are met:

81241	F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant

ICD-9 codes covered if selection criteria are met:

410.00 - 412	Myocardial infarction
415.11 - 415.19	Pulmonary embolism and infarction
671.20 - 671.33	Pregnancy complicated by thrombosis
V12.51 - V12.52, V12.55	Personal history of thrombosis
V17.41 - V17.49	Family history of thrombosis

EpiSEEK:

No specific code

ICD-9 codes not covered for indications listed in the CPB:

345.00 - 345.91	Epilepsy and recurrent seizures

POLG1:

No specific code

ICD-9 codes not covered for indications listed in the CPB:

334.3	Other cerebellar ataxia

SF3B1 and TET2:

No specific code

ICD-9 codes not covered for indications listed in the CPB:

238.72 -238.75	Myelodysplastic syndrome

There are no specific codes for the genetic testing listed:

Exome Sequencing

The above policy is based on the following references:

1. Whittaker L. Clinical applications of genetic testing: Implications for the family physician. Am Fam Physician. 1996;53(6):2077-2084.
2. Paulson GW, Prior TW. Issues related to DNA testing for Huntington's disease in symptomatic patients. Semin Neurol. 1997;17(3):235-238.
3. Sanchez A, Castellvi-Bel S, Mila M, et al. Huntington's disease: Confirmation of diagnosis and presymptomatic testing in Spanish families by genetic analysis. J Neurol Neurosurg Psychiatry. 1996;61(6):625-627.
4. Wilmott RW. Making the diagnosis of cystic fibrosis. J Pediatr. 1998;132(4):563-565.
5. Doherty RA. National Institutes of Health consensus development conference statement on genetic testing for cystic fibrosis. J Med Screen. 1997;4(4):179-180.
6. Chotai KA, Payne SJ. A rapid, PCR based test for differential molecular diagnosis of Prader-Willi and Angelman syndromes. J Med Genet. 1998;35(6):472-475.
7. Kosaki K, McGinniss MJ, Veraksa AN, et al. Prader-Willi and Angelman syndromes: Diagnosis with a bisulfite-treated methylation-specific PCR method. Am J Med Genet. 1997;73(3):308-313.
8. Chakrabarti L, Davies KE. Fragile X syndrome. Curr Opin Neurol. 1997;10(2):142-147.
9. American College of Obstetricians and Gynecologists (ACOG) Committee on Genetics. Fragile X syndrome. ACOG committee opinion. Int J Gynaecol Obstet. 1996;52(2):209-210.
10. Warren ST, Nelson DL. Advances in molecular analysis of fragile X syndrome. JAMA. 1994;271(7):536-542.
11. Kaul R, Gao GP, Balamurugan K, et al. Canavan disease: Molecular basis of aspartoacylase deficiency. J Inherit Metab Dis. 1994;17(3):295-297.
12. Matalon R, Kaul R, Michals K. Canavan disease: Biochemical and molecular studies. J Inherit Metab Dis. 1993;16(4):744-752.
13. Ikeuchi T, Koide R, Onodera O, et al. Dentatorubral-pallidoluysian atrophy (DRPLA). Molecular basis for wide clinical features of DRPLA. Clin Neurosci. 1995;3(1):23-27.

14. Becher MW, Rubinsztein DC, Leggo J, et al. Dentatorubral and pallidoluysian atrophy (DRPLA). Clinical and neuropathological findings in genetically confirmed North American and European pedigrees. Mov Disord. 1997;12(4):519-530.
15. Wood NW. Diagnosing Friedreich's ataxia. Arch Dis Child. 1998;78(3):204-207.
16. Madhani HD. Genetic abnormalities in Friedreich's ataxia. N Engl J Med. 1997;336(14):1022.
17. Durr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med. 1996;335(16):1169-1175.
18. Brissot P, Moirand R, Guyader D, et al. Hemochromatosis after the gene discovery: Revisiting the diagnostic strategy. J Hepatol. 1998;28 Suppl 1:14-18.
19. Bothwell TH, MacPhail AP. Hereditary hemochromatosis: Etiologic, pathologic, and clinical aspects. Semin Hematol. 1998;35(1):55-71.
20. Beutler E. The significance of the 187G (H63D) mutation in hemochromatosis. Am J Hum Genet. 1997;61(3):762-764.
21. American College of Medical Genetics/American Society of Human Genetics Working Group on ApoE and Alzheimer disease. Statement on use of apolipoprotein E testing for Alzheimer disease. JAMA. 1995;274(20):1627-1629.
22. Mayeux R, Saunders AM, Shea S, et al. Utility of the Apolipoprotein E genotype in the diagnosis of Alzheimer's disease. N Engl J Med. 1998;338:506-511.
23. Post SG, Whitehouse PJ, Binstock, RH, et al. The clinical introduction of genetic testing for Alzheimer disease. JAMA. 1997;277(10):832-836.
24. Ionasescu VV. Charcot-Marie-Tooth Neuropathies: From clinical description to molecular genetics. Muscle Nerve. 1995;18:267-275.
25. Saito M, Hayashi Y, Suzuki T, et al. Linkage mapping of the gene for Charcot-Marie-Tooth disease type 2 to chromosome 1p (CMT 2A) and the clinical features of CMT 2A. Neurology. 1997;49:1630-1635.
26. Chance PF, Alderson MK, Leppig KA, et al. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell. 1993;72:143-151.
27. Curry CJ, Stevenson RE, Aughton D, et al. Evaluation of mental retardation: Recommendations of a Consensus Conference: American College of Medical Genetics. Am J Med Genet. 1997;72:468-477.
28. de Vries BBA, van den Ouweland AMW, Mohkamsing S, et al. Screening and diagnosis for the fragile X syndrome among the mentally retarded: An epidemiological and psychological survey. Am J Hum Genet. 1997;61:660-667.
29. Gonnaud PM, Sturtz F, Bonnebouche C, et al. DNA analysis as a tool to confirm the diagnosis of asymptomatic hereditary neuropathy with liability to pressure palsies (HNPP) with further evidence for the occurrence of de novo mutations. Acta Neurol Scand. 1995;92:313-318.
30. Lupski JR, Chance PF, Garcia CA. Inherited primary peripheral neuropathies. Molecular genetics and clinical implications of CMT1A and HNPP. JAMA. 1993;270:2326-2330.
31. Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet. 1996;33:2-17.
32. American Society of Human Genetics (ASHG) and American College of Medical Genetics (ACMG). Diagnostic testing for Prader-Willi and Angelman syndromes: Report of the ASHG/ACMG Test and Technology Transfer Committee. Am J Hum Genet. 1996;58(5):1085-1088.
33. Cassidy SB. Prader-Willi syndrome. J Med Genet. 1997;34:917-923.
34. Heshmati HM, Gharib H, van Heerden JA, et al. Advances and controversies in the diagnosis and management of medullary thyroid carcinoma. Am J Med. 1997;103(1):60-69.
35. Heshmati HM, Gharib H, Khosla S, et al. Genetic testing in medullary thyroid carcinoma syndromes: Mutation types and clinical significance. Mayo Clin Proc. 1997;72(5):430-436.
36. Tarin JJ, Handyside AH. Embryo biopsy strategies for preimplantation diagnosis. Fertil Steril. 1993;59(5):943-952.
37. Cui KH, Matthews CD. Preimplantation diagnosis. Lancet. 1994;343(8903):972-973.
38. Delhanty JDA, Handyside AH, Winston RML. Preimplantation diagnosis. Lancet. 1994;343:1569-1570.
39. Munne S, Sultan KM, Weier HU, et al. Assessment of numeric abnormalities of X, Y, 18, and 16 chromosomes in preimplantation human embryos before transfer. Am J Obstet Gynecol. 1995;172:1191-1201.
40. Santalo J, Veiga A, Calafell JM, et al. Evaluation of cytogenetic analysis for clinical preimplantation diagnosis. Fertil Steril. 1995;64:44-50.
41. Carson SA, Buster JE. Diagnosis and treatment before implantation: The ultimate prenatal medicine. Contemp Obstet Gynecol. 1995;Dec:71-85.
42. American Society for Reproductive Medicine (ASRM). Preimplantation genetic diagnosis. Fact Sheet. Birmingham, AL: ASRM; December 1996. Available at: http://www.asrm.com/Patients/FactSheets/PGD-Fact.pdf. Accessed July 12, 2001.
43. Munne S, Scott R, Sable D, Cohen J. First pregnancies after preconception diagnosis of translocations of maternal origin. Fertil Steril. 1998;69:675-681.
44. National Center for Biotechnology Information (NCBI). Online Mendelian Inheritance in Man. Bethesda, MD: NCBI; 2001. Available at: http://www3.ncbi/nlm.nih.gov/omim/. Accessed July 12, 2001.
45. Carden SM, Boissy RE, Schoettker PJ, et al. Albinism: Modern molecular diagnosis. Br J Ophthalmol. 1998;82(2):189-195.
46. American College of Medical Genetics (ACMG) and American Society of Human Genetics (ASHG). Genetic testing for colon cancer: Joint statement of the American College of Medical Genetics and the American Society of Human Genetics. Genetics Med. 2000;2(6):362-366.
47. Creinin MD, Lisman R, Strickler RC. Screening for factor V Leiden mutation before prescribing combination oral contraceptives. Fertil Steril. 1999;72(4):646-651.
48. McIntyre KM. Medicolegal implications of the consensus conference, with special attention to the Fifth Antithrombotic Therapy Consensus Conference. Chest. 1998;114(5 Suppl):742S-747S.
49. Bick RL, Haas SK. International consensus recommendations. Summary statement and additional suggested guidelines. European Consensus Conference, November 1991. American College of Chest Physicians consensus statement of 1995. International Consensus Statement, 1997. Med Clin North Am. 1998;82(3):613-633.
50. Thrombophilia Awareness Project (TAP). Factor V Leiden [web site]. FVL Thrombophilia Support Page. Blue Lake, CA:

TAP; 2005. Available at: http://www.fvleiden.org/. Accessed February 11, 2005.
51. Grody WW, Griffin JH, Taylor AK, et al. American College of Medical Genetics consensus statement on factor V Leiden genetic testing. Genet Med. 2001;3(2):139-148.
52. Waldemar G, Dubois B, Emre M, et al. Diagnosis and management of Alzheimer's disease and other disorders associated with dementia. The role of neurologists in Europe. European Federation of Neurological Societies. Eur J Neurol. 2000;7(2):133-144.
53. Gasser T, Dichgans M, Finsterer J, et al. EFNS Task Force on Molecular Diagnosis of Neurologic Disorders. Guidelines for the molecular diagnosis of inherited neurologic disorders. First of two parts. Eur J Neurol. 2001;8(4):299-314.
54. Oberstein L, Breuning MH, Haan J, et al. CADASIL. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; 2002. Available at: http://www.geneclinics.org/profiles/cadasil/details.html. Accessed January 9, 2002.
55. Athena Diagnostics, Inc. CADASIL. NeuroCAST Sessions. Worcester, MA: Athena; 2002. available at: http://www.neurocast.com/site/content/sessions_12_2000.asp. Accessed January 9, 2002.
56. American Society of Human Genetics. Statement of the American Society of Human Genetics on Cystic Fibrosis Carrier Screening. Am J Hum Genet. 1992;51(6):1443-1444.
57. Wallach J. Cystic fibrosis of pancreas (mucoviscidosis). In: Interpretation of Diagnostic Tests: A Synopsis of Laboratory Medicine. 5th ed. Boston, MA: Little, Brown & Co.; 1991:214-215.
58. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: Cloning and characterization of complimentary DNA. Science. 1989;245(4922):1066-1073.
59. Cutting GR, Curristin SM, Nash E, et al. Analysis of four diverse population groups indicates that a subset of cystic fibrosis mutations occur in common among Caucasians. Am J Hum Genet. 1992; 50(6):1185-1194.
60. Elias S, Annas GJ, Simpson JL. Carrier screening for cystic fibrosis: Implications for obstetric and gynecologic practice. Am J Obstet Gynecol. 1991;164(4):1077-1083.
61. Lemna WK, Feldman GL, Kerem B, et al. Mutation analysis for heterozygote detection and the prenatal diagnosis of cystic fibrosis. N Engl J Med. 1990;322(5):291-296.
62. No authors listed. Statement from the National Institutes of Health workshop on population screening for the cystic fibrosis gene. N Engl J Med. 1990;323(1):70-71.
63. Mennie ME, Gilfillan A, Compton M, et al. Prenatal screening for cystic fibrosis. Lancet. 1992;340(8813):214-216.
64. Watson EK, Mayall ES, Lamb J, et al. Psychological and social consequences of community carrier screening programme for cystic fibrosis. Lancet. 1992;340(8813):217-220.
65. Nishimi RY. From the Office of Technology Assessment. Cystic fibrosis and DNA tests -- The implications of carrier screening. JAMA. 1993;269(15):1921.
66. Asch DA, Patton JP, Hershey JC, Mennuti MT. Reporting the results of cystic fibrosis carrier screening. Am J Obstet Gynecol. 1993;168(1 Pt 1):1-6.
67. Bekker H, Modell M, Denniss G, et al. Uptake of cystic fibrosis testing in primary care: Supply push or demand pull ? Br Med J. 1993;306(6892):1584-1586.
68. National Institutes of Health. Genetic testing for cystic fibrosis. NIH Consens Statement. Bethesda, MD: NIH, April 14-16, 1997;15(4):1-37.
69. Mennuti MT, Thomson E, Press N. Screening for cystic fibrosis carrier state. Obstet Gynecol. 1999;93:456-461.
70. American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG). Preconception and Prenatal Carrier Screening for Cystic Fibrosis: Clinical and Laboratory Provider Guidelines. ACOG/ACMG Position Statement. Washington, DC: ACOG; 2001.
71. Grody WW, Cutting GR, Klinger KW, et al. and the American College of Medical Genetics Accreditation of Genetic Services Committee, Subcommittee on Cystic Fibrosis Screening. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. American College of Medical Genetics Policy Statements. Genetics Med. 2001;3(2):149-154.
72. American College of Obstetricians and Gynecologists (ACOG), Screening Subcommittee of the Clinical Practice Committee. Fragile X syndrome. ACOG Committee Opinion No. 161. Washington, DC: ACOG; October 1995.
73. American College of Medical Genetics. Fragile X syndrome: Diagnosis and carrier testing. Working Group of the Genetic Screening Subcommittee of the Clinical Practice Committee. American College of Medical Genetics. Am J Med Genet. 1994;53(4):380-381.
74. Maddalena A, Richards CS, GmGinniss MJ, et al., and the Quality Assurance Subcommittee of the Laboratory Practice Committee, American College of Medical Genetics. Technical guidelines and standards for fragile X: The first of a series of disease-specific supplements to the standards and guidelines for clinical genetics laboratories of the American College of Medical Genetics. ACMG Statement. Genet Med. 2001;3(3):200-205.
75. McIntosh N, Gane LW, McConkie-Rosell A, Bennett RL. Genetic counseling for fragile X syndrome: Recommendations of the National Society of Genetic Counselors. J Genet Counsel. 2000;9(4):303-325.
76. Bergqvist D, Blomqvist P, Eliasson M, et al. Prevention, diagnosis, and treatment of venous thromboembolism. Report No. 158. Stockholm, Sweden: SBU; 2002.
77. Swedish Council on Technology Assessment in Health Care (SBU). Genetic test in screening for hereditary hemochromatosis - early assessment briefs (Alert). Stockholm, Sweden: SBU; 2001.
78. Murray J, Cuckle H, Taylor G, et al. Screening for cystic fibrosis. Health Technol Assess. 1999;3(8).
79. Pembrey ME, Barnicoat AJ, Carmichael B, et al. An assessment of screening strategies for fragile X syndrome in the UK. Health Technol Assess. 2001;5(7).
80. Medical Services Advisory Committee (MSAC). Antenatal screening for heritable thrombophilia. MSAC reference 9b. Canberra, ACT; MSAC; 2002.
81. Song FJ, Barton P, Sleightholme V, et al. Screening for fragile X syndrome: A literature review and modelling study. Health Technol Assess. 2003;7(16).
82. Mundy L, Merlin T. Population genetic screening for haemochromatosis: Identifying asymptomatic 'at risk' homozygous individuals. Horizon Scanning Prioritising Summary - Volume 1. Adelaide, SA: Adelaide Health Technology Assessment

(AHTA) on behalf of National Horizon Scanning Unit (HealthPACT and MSAC); 2003.
83. Medical Services Advisory Committee (MSAC). Genetic test for fragile X syndrome. MSAC Application 1035. Canberra, ACT: MSAC; 2002.
84. Institute for Clinical Systems Improvement (ICSI). Genetic testing for hereditary nonpolyposis colorectal cancer (HNPCC). ICSI Healthcare Guidelines. Bloomington, MN: ICSI; 2002.
85. Institute for Clinical Systems Improvement (ICSI). Genetic carrier testing for cystic fibrosis. ICSI Healthcare Guidelines. Bloomington, MN: ICSI; 2003.
86. BlueCross BlueShield Association, Technology Evaluation Center. Genetic testing for HFE gene mutations related to hereditary hemachromatosis. TEC Assessment Program. Chicago, IL: BCBSA; April 2002;16(22).
87. Symanski JD, Gettes LS. Drug effects on the electrocardiogram: A review of their clinical importance. Drugs. 1993;46(2):219-248.
88. McLeod R and the Canadian Task Force on Preventive Health Care. Screening strategies for colorectal cancer: Systematic review and recommendations. London, ON: Canadian Task Force on Preventive Health Care; 2001:1-35.
89. Blancquaert I, Caron L. Fragile X syndrome: The role of molecular diagnosis and screening in an integrated approach to services. AETMIS 01-1 RE. Montreal, QC: Agence d'Evaluation des Technologies et des Modes d'Intervention en Sante (AETMIS); 2002:1-176.
90. Ho C, Banerjee S, Mensinkai S. Molecular diagnosis for hereditary cancer predisposing syndromes: Genetic testing and clinical impact. Technology Report Issue 41. Ottawa, ON: Canadian Coordinating Office for Health Technology Assessment (CCOHTA); 2003:1-82.
91. Moric E, Herbert E, Trusz-Gluza M, et al. The implications of genetic mutations in the sodium channel gene (SCN5A). Europace. 2003;5(4):325-334.
92. Chen S, Zhang L, Bryant RM, et al. KCNQ1 mutations in patients with a family history of lethal cardiac arrhythmias and sudden death. Clin Genet. 2003;63(4):273-282.
93. Viswanathan PC, Balser JR. Inherited sodium channelopathies: A continuum of channel dysfunction. Trends Cardiovasc Med. 2004;14(1):28-35.
94. Priori SG, Aliot E, Blomstrom-Lundqvist C, et al. Task Force on Sudden Cardiac Death of the European Society of Cardiology. Eur Heart J. 2001;22(16):1374-1450.
95. Maron BJ, Moller JH, Seidman CE, et al. Impact of laboratory molecular diagnosis on contemporary diagnostic criteria for genetically transmitted cardiovascular diseases: Hypertrophic cardiomyopathy, long-QT syndrome, and Marfan syndrome. A statement for healthcare professionals from the Councils on Clinical Cardiology, Cardiovascular Disease in the Young, and Basic Science, American Heart Association. Circulation. 1998;98:1460-1471.
96. Hadley DW, Jenkins JF, Dimond E, et al. Colon cancer screening practices after genetic counseling and testing for hereditary nonpolyposis colorectal cancer. J Clin Oncol. 2004;22(1):39-44.
97. Kmet L, Lee RC, Cook LS, et al. Systematic review of the social, ethical, and legal dimensions of genetic cancer risk assessment technologies. Edmonton, AB: Alberta Heritage Foundation for Medical Research (AHFMR); 2004:1-85.
98. American Gastroenterological Association medical position statement: Hereditary colorectal cancer and genetic testing. Gastroenterology. 2001;121(1):195-197.
99. Church J, Simmang C. Practice parameters for the treatment of patients with dominantly inherited colorectal cancer (familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer). Dis Colon Rectum. 2003;46(8):1001-1012.
100. March of Dimes Birth Defects Foundation. Marfan syndrome. Quick Reference and Fact Sheets. White Plains, NY; March of Dimes; 2006. Available at: http://www.marchofdimes.com/professionals/681_1216.asp. Accessed January 26, 2006.
101. Chen H. Marfan syndrome. eMedicine Pediatrics Topic 1372. Omaha, NE: eMedicine.com; updated May 14, 2003. Available at: http://www.emedicine.com/ped/topic1372.htm. Accessed August 24, 2004.
102. Montgomery RK, Buller HA, Rings EH, Grand RJ. Lactose intolerance and the genetic regulation of intestinal lactase-phlorizin hydrolase. FASEB J. 1991;5(13):2824-2832.
103. UK National Health Service (NHS). What is the most practical way to test patients for lactose intolerance? National Library for Health (NLH) Question Answering Service. London, UK: NHS; March 24, 2005.
104. National Public Health Service for Wales. What tests are available for lactose intolerance? ATTRACT Database. Gwent, Wales, UK: National Health Service; May 17, 2005.
105. Jo WS, Bandipalliam P, Shannon KM, et al. Correlation of polyp number and family history of colon cancer with germline MYH mutations. Clin Gastroenterol Hepatol. 2005;3(10):1022-1028.
106. National Comprehensive Cancer Network (NCCN). Colorectal cancer screening. Clinical Practice Guidelines in Oncology -- v.1.2006. Jenkintown, PA: NCCN; 2006. Available at: www.nccn.org/professionals/physician_gls/PDF/colorectal_screening.pdf. Accessed February 20, 2006.
107. Marquez Calderon S, Briones Perez de la Blanca E. Genetic testing assessment framework in the Andalusian Public Health System - guidelines [summary]. Report 2/2006. Seville, Spain: Agencia e Evaluacion de Tecnologias Sanitarias de Andalucia (AETSA); 2005.
108. Adelaide Health Technology Assessment (AHTA). Genetic testing for long QT syndrome; Horizon scanning prioritising summary - volume 13. Adelaide, SA: Adelaide Health Technology Assessment (AHTA) on behalf of National Horizon Scanning Unit (HealthPACT and MSAC); June 2006. Available at: http://www.horizonscanning.gov.au/internet/horizon/publishing.nsf/Content/prioritising-summaries-2006-2. Accessed January 31, 2007.
109. Chin KM, Wessler B, Chew P, Lau J. Genetic tests for cancer. Technology Assessment. Prepared for the Agency for Healthcare Research and Quality (AHRQ) for the Tufts-New England Medical Center Evidence-based Practice Center. Rockville, MD: AHRQ; January 9, 2006. Available at: http://www.ahrq.gov/clinic/ta/gentests/gentests.pdf. Accessed January 31, 2007.
110. Whitlock P, Garlitz BA, Harris EL, et al. Screening for hereditary hemochromatosis: A systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2006;145(3):209-223.
111. Schmitt B, Golub RM, Green R. Screening primary care patients for hereditary hemochromatosis with transferrin

saturation and serum ferritin level: Systematic review for the American College of Physicians. Ann Intern Med. 2005;143(7):522-536.
112. Burke W. Genetic testing. N Engl J Med. 2002;347(23):1867-1875.
113. Limdi JK, Crampton JR. Hereditary haemochromatosis. Q J Med, 2004;97(6):315-324.
114. Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: A HuGE review. Am J Epidemiol. 2001;154(3):193-206.
115. U.S. Preventive Services Task Force. Screening for hemochromatosis: Recommendation statement. Ann Intern Med. 2006 Aug 1;145(3):204-208.
116. Jankovic J. Hyperkinetic movement disorders in children. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; updated April 2007.
117. Weber S, Tonshoff B. Recurrence of focal-segmental glomerulosclerosis in children after renal transplantation: Clinical and genetic aspects. Transplantation. 2005;80(1S) Supplement:S128-S134.
118. Franceschini N, North KE, Kopp JB, et al. NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: A HuGE review. Genet Med. 2006;8(2):63-75.
119. Niaudet P. Treatment of idiopathic nephrotic syndrome in children. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; updated April 2007.
120. Niendorf KB, Tsao H. Cutaneous melanoma: Family screening and genetic testing. Dermatol Ther. 2006;19(1):1-8.
121. Goldstein AM, Chan M, Harland M, et al; Melanoma Genetics Consortium (GenoMEL). High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. Cancer Res. 2006;66(20):9818-9828.
122. Stark M, Puig-Butille JA, Walker G, et al. Mutation of the tumour suppressor p33ING1b is rare in melanoma. Br J Dermatol. 2006 l;155(1):94-99.
123. Tsao H, Haluska F. Inherited susceptibility to melanoma. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; April 2007.
124. American Society of Clinical Oncology (ASCO). Statement of the American Society of Clinical Oncology: Genetic testing for cancer susceptibility, Adopted on February 20, 1996. J Clin Oncol. 1996;14(5):1730-1740.
125. American Society of Clinical Oncology (ASCO). American Society of Clinical Oncology policy statement update: Genetic testing for cancer susceptibility. J Clin Oncol. 2003;21(12):2397-2406.
126. Kefford RF, Newton Bishop JA, Bergman W, Tucker MA. Counseling and DNA testing for individuals perceived to be genetically predisposed to melanoma: A consensus statement of the Melanoma Genetics Consortium. J Clin Oncol. 1999;17(10):3245-3251.
127. Kefford R, Bishop JN, Tucker M, et al.; Melanoma Genetics Consortium. Genetic testing for melanoma. Lancet Oncol. 2002;3(11):653-654.
128. Scottish Intercollegiate Guidelines Network (SIGN). Cutaneous melanoma. A National Clinical Guideline. SIGN Guideline No. 72. Edinburgh, Scotland: SIGN; July 2003.
129. British Committee for Standards in Haematology. Guidelines on the diagnosis and management of AL amyloidosis. London, UK: British Society for Haematology; February 2003. Available at: http://www.bcshguidelines.com/pdf/UKMFAL070703.pdf. Accessed May 7, 2007.
130. Kujovich JL. Factor V leiden thrombophilia. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; February 12, 2007. Available at: http://www.geneclinics.org/. Accessed January 31, 2008.
131. Bernardi F, Faioni EM, Castoldi E, et al. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. Blood. 1997;90(4):1552-1557.
132. de Visser MC, Guasch JF, Kamphuisen PW, et al. The HR2 haplotype of factor V: Effects on factor V levels, normalized activated protein C sensitivity ratios and the risk of venous thrombosis. Thromb Haemost. 2000;83(4):577-582.
133. Mingozzi F, Legnani C, Lunghi B, et al. A FV multiallelic marker detects genetic components of APC resistance contributing to venous thromboembolism in FV Leiden carriers. Thromb Haemost. 2003;89(6):983-989.
134. Faioni EM, Franchi F, Bucciarelli P, et al. Coinheritance of the HR2 haplotype in the factor V gene confers an increased risk of venous thromboembolism to carriers of factor V R506Q (factor V Leiden). Blood. 1999;94(9):3062-3066.
135. Zammiti W, Mtiraoui N, Mercier E, et al. Association of factor V gene polymorphisms (Leiden; Cambridge; Hong Kong and HR2
haplotype) with recurrent idiopathic pregnancy loss in Tunisia. A case-control study. Thromb Haemost. 2006;95(4):612-617.
136. Alhenc-Gelas M, Arnaud E, Nicaud V, et al. Venous thromboembolic disease and the prothrombin, methylene tetrahydrofolate reductase and factor V genes. Thromb Haemost. 1999;81(4):506-510.
137. Jadaon MM, Dashti AA. HR2 haplotype in Arab population and patients with venous thrombosis in Kuwait. J Thromb Haemost. 2005;3(7):1467-1471.
138. de Visser MC, Guasch JF, Kamphuisen PW, et al.The HR2 haplotype of factor V: Effects on factor V levels, normalized activated protein C sensitivity ratios and the risk of venous thrombosis. Thromb Haemost. 2000;83(4):577-582.
139. Luddington R, Jackson A, Pannerselvam S, et al. The factor V R2 allele: Risk of venous thromboembolism, factor V levels and resistance to activated protein C. Thromb Haemost. 2000;83(2):204-208.
140. Dindagur N, Kruthika-Vinod TP, Christopher R.Factor V gene A4070G mutation and the risk of cerebral veno-sinus thrombosis occurring during puerperium. Thromb Res. 2007;119(4):497-500.
141. McCluskey L. Familial amyotrophic lateral sclerosis. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; updated May 2007.
142. Battistini S, Giannini F, Greco G, et al. SOD1 mutations in amyotrophic lateral sclerosis. Results from a multicenter Italian study. J Neurol. 2005;252(7):782-788.
143. Dellefave L, Siddique T. Amyotrophic lateral sclerosis overview. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; November 21, 2007. Available at: http://www.genetests.org/query?dz=als-overview. Accessed January 31, 2008.

144. Smith CO, Michelson S, Bennett R, et al.; U.S. Department of Education, National Institute on Disability and Rehabilitation Research. Spinocerebellar ataxia: Making an informed choice about genetic testing. Seattle, WA: University of Washington Medical Center, Medical Genetics and Neurology; 2004. Available at: http://depts.washington.edu/neurogen/documents/43134SpinoAtaxia.pdf. Accessed January 31, 2008.
145. National Institutes of Health (NIH), National Library of Medicine (NLM). Leber hereditary optic neuropathy. Genetics Home Reference. Bethesda, MD; NIH; August 2006. Available at: http://ghr.nlm.nih.gov/ghr/. Accessed January 31, 2008.
146. Man PY, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. J Med Genet. 2002;39(3):162-169.
147. Mroczek-Tonska K, Kisiel B, Piechota J, et al. Leber hereditary optic neuropathy--a disease with a known molecular basis but a mysterious mechanism of pathology. J Appl Genet. 2003;44(4):529-538.
148. Thajeb P, Dai D, Chiang MF, et al. Genotype-phenotype correlation of maternally inherited disorders due to mutations in mitochondrial DNA. Taiwan J Obstet Gynecol. 2006;45(3):201-207.
149. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. NEJM. 2003;348(26):2656-2668.
150. Thorburn DR, Rahman S. Mitochondrial DNA-associated Leigh Syndrome and NARP. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; September 22, 2006. Available at: http://www.geneclinics.org/profiles/narp/index.html. Accessed January 31, 2008.
151. Maniura-Weber K, Helm M, Engemann K, et al. Molecular dysfunction associated with the human mitochondrial 3302A>G mutation in the MTTL1 (mt-tRNALeu(UUR)) gene. Nucleic Acids Res. 2006; 34(22): 6404–6415.
152. Rahman S, Poulton J, Marchington D, et al. Decrease of 3243 A-G mtDNA mutation from blood in MELAS syndrome: A longitudinal study. AJHG. 2001; 68(1):238–240.
153. Brais B, Rouleau G. Oculopharyngeal muscular dystrophy. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; June 26, 2006. Available at: http://www.geneclinics.org/profiles/opmd/index.html. Accessed January 31, 2008.
154. Fink J. Hereditary spastic paraplegia. Neurol Clin. 2002;20(3):711-726.
155. Fink J. Hereditary spastic paraplegia overview. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; October 4, 2007. Available at: http://www.geneclinics.org/. Accessed January 31, 2008.
156. Eisen A. Disorders affecting the spinal cord. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; updated September 2007.
157. Raman G, Chew P, Trikalimos T, et al. Agency for Healthcare Research and Quality (AHRQ). Genetic tests for non-cancer diseases/conditions: A horizon scan. Technology Assessment. Prepared by the Tufts-New England Medical Center Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ). Rockville, MD: AHRQ; 2007. Available at: http://www.cms.hhs.gov/determinationprocess/downloads/id49TA.pdf. Accessed January 31, 2008.
158. Hall J. Clinical indicators for SHOX gene testing in children with short stature. Journal Watch Pediatrics and Adolescent Medicine. October 17, 2007.
159. Munns, C and Glass I. SHOX-related haploinsufficiency disorders. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; October 4, 2007. Available at: http://www.geneclinics.org/. Accessed January 31, 2008.
160. Frye R. Pyruvate kinase deficiency. eMedicine Metabolic Disorders, Topic 1980. Omaha, NE: eMedicine.com; updated February 4, 2005.
161. Yaish H. Pyruvate kinase deficiency. eMedicine Hematology, Topic 1971. Omaha, NE: eMedicine.com; updated July 31, 2007.
162. Black D. Migrainous vertigo. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; updated October 2006.
163. von Brevern M, Ta N, Shankar A, et al. Migrainous vertigo: Mutation analysis of the candidate genes CACNA1A, ATP1A2, SCN1A, and CACNB4. Headache. 2006;46(7):1136-1141.
164. Schmidt U, Fuessel S, Koch R, et al. Quantitative multi-gene expression profiling of primary prostate cancer. Prostate. 2006;66(14):1521-1534.
165. U.S. National Institutes of Health, National Cancer Institute (NCI). Genetics of prostate cancer (PDQ). Prostate cancer risk assessment. Bethesda, MD; NIH; December 19, 2007. Available at: http://www.cancer.gov/cancertopics/pdq/genetics/prostate/HealthProfessional/page6. Accessed January 31, 2008.
166. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special report: Recent developments in prostate cancer genetics and genetic testing. TEC Assessments in Press. Chicago, IL: BCBSA; November 2008.
167. Falchetti A, Marini F, Brandi ML. Multiple endocrine neoplasia type 1. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; August 31, 2005. Available at: http://www.geneclinics.org/. Accessed January 31, 2008.
168. Arnold A. Clinical manifestations and diagnosis of multiple endocrine neoplasia type 1. In: UpToDate Online Journal [serial online]. Waltham, MA: UpToDate; updated January 2007.
169. Roden DM. Long-QT syndrome. New Engl J Med. 2008;358(2):169-176.
170. Heart Rhythm UK (HRUK) Familial Sudden Death Syndromes Statement Development Group. Clinical indications for genetic testing in familial sudden cardiac death syndromes: An HRUK position statement. Heart. 2008;94(4):502-507.
171. Napolitano C, Priori SG, Schwartz PJ, et al. Genetic testing in the long QT syndrome: Development and validation of an efficient approach to genotyping in clinical practice. JAMA. 2005;294(23):2975-2980.
172. Brugada P, Brugada R, Antzelevitch C, et al. The Brugada syndrome. Arch Mal Coeur Vaiss. 2005; 98(2):115-122.
173. Brugada R, Brugada P, Brugada J, Hong K. Brugada syndrome. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: University of Washington; December 7, 2007. Available at: http://www.geneclinics.org/. Accessed September 11, 2008.
174. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Genetic testing for long QT syndrome. TEC Assessment Program. Chicago, IL: BCBSA; February 2008;22(9). Available at: http://www.bcbs.com/blueresources/tec/vols/22/22_09.pdf. Accessed September 11, 2008.

175. Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008;359(21):2208-2219.
176. Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med. 2008;359(21):2220-2232.
177. Zipes,DP, Camm AJ, Borggrefe M, et al. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: A report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death). J Am Coll Cardiol. 2006;48:e247. Available at: http://circ.ahajournals.org/cgi/reprint/114/10/e385. Accessed on May 18, 2009.
178. Calkins H. Arrhythmogenic right-ventricular dysplasia/cardiomyopathy. Curr Opin Cardiol. 2006;21(1):55-63.
179. Corrado D, Basso C, Thiene G. Arrhythmogenic right ventricular cardiomyopathy: Diagnosis, prognosis, and treatment. Heart. 2000;83(5):588-595.
180. Colombo MG, Botto N, Vittorini S, et al. Clinical utility of genetic tests for inherited hypertrophic and dilated cardiomyopathies. Cardiovasc Ultrasound. 2008;6:62.
181. McNally E, MacLeod H, Dellefave L. Arrhythmogenic right ventricular dysplasia/cardiomyopathy, autosomal dominant. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Seattle, WA: Universtiy of Washington; 2008. Available at: http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=arvd. Accessed May 18, 2009.
182. Johns Hopkins Medicine [website]. Arrhythmogenic right ventricular dysplasia. Baltimore, MD: The Johns Hopkins Hospital; 2008. Available at: http://www.arvd.com/registry.html. Accessed May 18, 2009.
183. Hershberger RE, Lindenfeld J, Mestroni L, et al. Genetic evaluation of cardiomyopathy -- a Heart Failure Society of America Practice Guideline. J Card Fail. 2009;15(2):83-97.
184. American College of Obstetricians and Gynecologists (ACOG). Update on carrier screening for cystic fibrosis. ACOG Committee Opinion No. 325. Washington, DC: ACOG; December 2005.
185. Gail MH. Value of adding single-nucleotide polymorphism genotypes to a breast cancer risk model. J Natl Cancer Inst. 2009;101(13):959-963.
186. Intergenetics, Inc [website]. What is OncoVue? Intergenetics, Inc. Oklahoma City, OK; 2009. Available at: http://www.intergenetics.com/intergenetics/oncovue.html. Accessed on December 15, 2009.
187. American College of Medical Genetics (ACMG). ACMG statement on direct-to-consumer genetic testing. Bethesda, MD; ACMG; 2008. Available at: http://www.acmg.net/StaticContent/StaticPages/DTC_Statement.pdf. Accessed February 2, 2010.
188. U.S. Food and Drug Administration (FDA). Guidance on pharmacogenetic tests and genetic tests for heritable markers. Rockville, MD: FDA; 2010. Available at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm077862.htm. Accessed February 2, 2010.
189. Counsyl. The universal genetic test [website]. Redwood City, CA: Counsyl; 2010 Available at: https://www.counsyl.com/learn/universal-genetic-test/. Accessed February 2, 2010.
190. American College of Obstetricians and Gynecologists (ACOG), Committee on Genetics. Preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. ACOG Commitee Opinion No. 442. Washington, DC: ACOG; October 2009.
191. Bos JM, Towbin JA, Ackerman MJ. Diagnostic, prognostic, and therapeutic implications of genetic testing for hypertrophic cardiomyopathy. J Am Coll Cardiol. 2009;54(3):201-211.
192. Hudecova K, Simkova I, Gardlik R, Bernadic M. Genetic screening of patients with hypertrophic cardiomyopathy -- a new diagnostic strategy for risk stratification? Bratisl Lek Listy. 2009;110(2):85-92.
193. Shephard R, Semsarian C. Advances in the prevention of sudden cardiac death in the young. Ther Adv Cardiovasc Dis. 2009;3(2):145-155.
194. Hershberger RE, Lindenfeld J, Mestroni L, et al; Heart Failure Society of America. Genetic evaluation of cardiomyopathy -- a Heart Failure Society of America practice guideline. J Card Fail. 2009;15(2):83-97.
195. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Genetic testing for predisposition to inherited hypertrophic cardiomyopathy. TEC Assessment Program. Chicago, IL: BCBSA; August 2010. .
196. Rose BD. Pathogenesis and diagnosis of focal segmental glomerulosclerosis. UpToDate [online serial]. Waltham, MA: UpToDate; 2010.
197. Rao TKS. Focal segmental glomerulosclerosis. eMedicine. Nephrology. February 17, 2009. Available at: http://emedicine.medscape.com/article/245915-overview. Accessed January 31, 2011.
198. Frassetto LA, Lo L. Bartter syndrome. eMedicine. Nephrology. September 18, 2009. Available at: http://emedicine.medscape.com/article/238670-overview. Accessed January 31, 2011.
199. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol. 2009;27(35):5924-5930.
200. Siena S, Sartore-Bianchi A, Di Nicolantonio F, et al. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst. 2009;101(19):1308-1324.
201. Walker EP. Home genetic tests get 'F' from GAO. July 22, 2010. Medpage today. Available at: http://www.medpagetoday.com/Genetics/GeneticTesting/21326utm_content=&utm_medium=email&impressionId=&utm_campaign=DailyHeadlines&utm_source=&userid. Accessed January 31, 2011.
202. Monaghan KG, Feldman GL, Palomaki GE, et al.; Molecular Subcommittee of the ACMG Laboratory Quality Assurance Committee. Technical standards and guidelines for reproductive screening in the Ashkenazi Jewish population. Genet Med. 2008;10(1):57-72.
203. Maurer M. Cold urticaria. Last reviewed September 2011. UpToDate, In. Waltham, MA.
204. Nigrovic PA. Cryopyrin-associated periodic syndromes and related disorders. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed September 2011.

205. Urwyler A, Deufel T, McCarthy T, et al. Guidelines for molecular genetic detection of susceptibility to malignant hyperthermia. Br J Anaesth. 2001;86(2):283-287.
206. Santome Collazo JL, Cirujano Segura A, Ferreiro Fernandez B, et al. Simple virilizing forms of congenital adrenal hyperplasia: Adaptation and prospective validation of the molecular screening. Med Clin (Barc). 2010;135(5):195-201.
207. Speiser PW, Azziz R, Baskin LS, et al; Endocrine Society. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2010;95(9):4133-4160.
208. Wappler F. Anesthesia for patients with a history of malignant hyperthermia. Curr Opin Anaesthesiol. 2010;23(3):417-422.
209. Chudova D, Wilde JI, Wang ET, et al. Molecular classification of thyroid nodules using high-dimensionality genomic data. J Clin Endocrinol Metab. 2010;95(12):5296-5304.
210. Merke DP. Diagnosis of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed September 2011.
211. Litman RS. Susceptibility to malignant hyperthermia. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed September 2011.
212. Licis AK, Desruisseau DM, Yamada KA, et al. Novel genetic findings in an extended family pedigree with sleepwalking. Neurology. 2011;76(1):49-52.
213. Dogu O, Pressman MR. Identification of sleepwalking gene(s). Not yet, but soon? Neurology. 2011;76(1):12-13.
214. American College of Obstetricians and Gynecologists. Update on carrier screening for cystic fibrosis. Committee Opinion No. 486. Obstet Gynecol. 2011;117(4):1028-1031.
215. National Comprehensive Cancer Network (NCCN). Thyroid Carcinoma. NCCN Clinical Practice Guidelines in Oncology.v.2.2012. Fort Washington, PA: NCCN; 2012.
216. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases. Report of the American Academy of Ophthalmology Task Force on Genetic Testing. Ophthalmology. 2012; 119:2408–2410.
217. Bayrak-Toydemir P; Mao R; Lewin S; McDonald J. Hereditary hemorrhagic telangiectasia: An overview of diagnosis and management in the molecular era for clinicians. Genet Med, 6(4): 175-91 Jul 1 2004.
218. Bossler AD et al. Novel mutations in ENG and ACVRL1 identified in a series of 200 individuals undergoing clinical genetic testing for hereditary hemorrhagic telangiectasia (HHT): correlation of genotype with phenotype. Hum Mutat, 27(7): 667-75 2006.
219. Cassidy: Management of Genetic Syndromes. Second Edition. 2005.
220. Flint: Cummings Otolaryngology: Head & Neck Surgery. Fifth Edition. 2010.
221. Mei-Zahav M et al.. Symptomatic children with hereditary hemorrhagic telangiectasia: a pediatric center experience. Arch Pediatr Adolesc Med, 160(6): 596-601 2006.
222. Shah P et al. Diagnosis of adult hereditary pulmonary disease and the role of genetic testing. Chest, Volume 137, Issue 4 2010.
223. Shahzad M, Sivakumaran TA, Qaiser TA, et al. Genetic analysis through OtoSeq of Pakistani families segregating prelingual hearing loss. Otolaryngol Head Neck Surg. 2013 Jun 14. [Epub ahead of print]
224. Sivakumaran TA, Husami A, Kissell D, et al. Performance evaluation of the next-generation sequencing approach for molecular diagnosis of hereditary hearing loss. Otolaryngol Head Neck Surg. 2013;148(6):1007-1016.
225. Eppsteiner RW, Shearer AE, Hildebrand MS, et al. Prediction of cochlear implant performance by genetic mutation: the spiral ganglion hypothesis. Hear Res. 2012;292(1-2):51-58.
226. Bedard JE, Haaning AM, Ware SM. Identification of a novel ZIC3 isoform and mutation screening in patients with heterotaxy and congenital heart disease. PLoS One. 2011;6(8):e23755.
227. Tariq M, Belmont JW, Lalani S, et al. SHROOM3 is a novel candidate for heterotaxy identified by whole exome sequencing. Genome Biol. 2011;12(9):R91.
228. Strasser K, Hoefele J, Bergmann C, et al. COL4A5-associated X-linked Alport syndrome in a female patient with early inner ear deafness due to a mutation in MYH9. Nephrol Dial Transplant. 2012;27(11):4236-4240.
229. Fleishman CE, Tugertimur A. Clinical manifestations, pathophysiology, and diagnosis of atrioventricular (AV) canal defects. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013.
230. Altman CA. Congenital heart disease (CHD) in the newborn: Presentation and screening for critical CHD. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013.
231. Coutre S. Congenital and acquired disorders of platelet function. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013.
232. Sutton VR. Inborn errors of metabolism: Epidemiology, pathogenesis, and clinical features. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013a.
233. Sutton VR. Inborn errors of metabolism: Classification. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013b.
234. Sielski LA. Newborn screening. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed February 2013.
235. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology: Myelodysplastic syndromes. Version 2.2014. Fort Washington, PA: NCCN; 2014. Available at: . Accessed January 21, 2014.
236. Perlee LT, Bansal AT, Gehrs K, et al. Inclusion of genotype with fundus phenotype improves accuracy of predicting choroidal neovascularization and geographic atrophy. Ophthalmology. 2013;120(9):1880-1892.
237. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special report: Exome sequencing for clinical diagnosis of patients with suspected genetic disorders. Chicago, IL: BCBSA; August 2013. Available at: http://www.bcbs.com/blueresources/tec/vols/28/28_03.pdf. Accessed January 21, 2014.
238. Lee YC, Lu YC, Chang MH, Soong BW. Common mitochondrial DNA and POLG1 mutations are rare in the Chinese patients with adult-onset ataxia on Taiwan. J Neurol Sci. 2007;254(1-2):65-68.
239. Gramstad A, Bindoff LA, Lillebo A, et al. Neuropsychological performance in patients with POLG1 mutations and the syndrome of mitochondrial spinocerebellar ataxia and epilepsy. Epilepsy Behav. 2009;16(1):172-174.
240. Isohanni P, Hakonen AH, Euro L, et al. POLG1 manifestations in childhood. Neurology. 2011;76(9):811-815.
241. Tang S, Dimberg EL, Milone M, Wong LJ. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)-like phenotype: An expanded clinical spectrum of POLG1 mutations. J Neurol. 2012;259(5):862-868.

242. Opal P, Zoghbi HY. Overview of the hereditary ataxias. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013a.
243. Opal P, Zoghbi HY. The spinocerebellar ataxias. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2013b.
244. Price MJ, Judisch GF, Thompson HS. X-linked congenital stationary night blindness with myopia and nystagmus without clinical complaints of nyctalopia. J Pediatr Ophthalmol Strabismus. 1988;25(1):33-36.
245. Lorenz B, Andrassi M, Miliczek KD. Incomplete congenital stationary night blindness (CSNB). An important differential diagnosis of congenital nystagmus. Klin Monbl Augenheilkd. 1996;208(1):48-55.
246. Kim JM, Payne JF, Yan J, Barnes CS. Negative electroretinograms in the pediatric and adult population. Doc Ophthalmol. 2012;124(1):41-48.

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Medical Policy

Subject:	Diagnostic Genetic Testing of a Potentially Affected Individual (Adult or Child)		
Policy #:	GENE.00013	**Current Effective Date:**	04/15/2014
Status:	Revised	**Last Review Date:**	02/13/2014

Description/Scope

This document addresses diagnostic genetic testing of a potentially affected individual (adult or child).

Note(s):

- This document is limited to the use molecular genetic testing and does not provide criteria for karyotype analysis or biochemical testing.
- Please refer to the following documents for additional information on genetic testing:

  GENE.00001 - Genetic Testing for Cancer Susceptibility;
  GENE.00003 - Genetic Testing and Biochemical Markers for the Diagnosis of Alzheimer's Disease;
  GENE.00007 - Cardiac Ion Channel Genetic Testing;
  GENE.00012 - Preconceptional or Prenatal Genetic Testing of a Parent or Prospective Parent;
  GENE.00015 - Predictive Genetic Testing for Non-Malignant Diseases;
  GENE.000021 - Comparative Genomic Hybridization (CGH) Microarray Testing for Developmental Delay, Autism Spectrum Disorder and Mental Retardation

Position Statement

Medically Necessary:

Genetic testing is considered **medically necessary** for diagnostic purposes in individuals with symptoms of a genetic disorder or individuals who are at risk for a late onset genetic disorder or slowly evolving genetic disorder who meet ALL of the following criteria:

- A specific mutation, set of mutations or gene expression profile has been established in the scientific literature to be reliably associated with the disease; **and**
- A biochemical or other test is identified but the results are indeterminate, or the genetic disorder cannot be identified through biochemical or other testing; **and**
- The results of the genetic test could impact the medical management of the individual with a resulting improvement in health outcomes; **and**
- Testing is accompanied by genetic counseling.

Investigational and Not Medically Necessary:

Genetic testing for diagnostic purposes in individuals not meeting the above criteria is considered **investigational and not medically necessary,** including, but not limited to, melanoma (hereditary), amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease) or ataxia telangiectasia.

Diagnostic genetic testing using panels of genes (with or without next generation sequencing), including but not limited to whole genome and whole exome sequencing, is considered **investigational and not medically necessary** unless all components of the panel have been determined to be medically necessary based on the criteria above. However, individual components of a panel may be considered medically necessary when criteria above are met.

Rationale

Diagnosis of a genetic disorder in individuals with suggestive symptoms can typically be made using biochemical testing. However, in limited situations, genetic testing may be used to provide a definitive diagnosis in conjunction with clinical signs and symptoms. Because of the rapidly evolving field of genetic testing, each genetic test must be carefully evaluated to determine whether or not the identified genetic mutations reliably identify a genetic disorder, resulting in the initiation of effective therapy.

There has been a surge of interest in the genetic mutation responsible for ALS. ALS is a progressive neurodegenerative disease which involves both upper and lower motor neurons. The mean age of onset is 56 years in individuals with no known family history of the disease and 46 years in individuals with more than one affected family member (familial ALS or FALS). The average duration of the disease is about three years, but it can vary significantly. Death usually occurs as the result of respiratory failure.

ALS can be inherited in an autosomal recessive, autosomal dominant or X-linked manner. The diagnosis of ALS is typically based on clinical features, electrodiagnostic testing, and exclusion of other health conditions with related symptoms. Researchers have identified several possible causes of ALS including, but not limited to mutations in the gene that produces the superoxide dismutase (SOD1) enzyme. While genetic tests are available to identify mutations in the SOD1 gene, it is not clear how the results of diagnostic genetic testing would improve the care or outcome of the affected individual.

Gene Expression Profiling

Researchers continue to explore the role of genetic function in the development diseases. Gene expression profile (gene expression signature, gene expression pattern [GEP] or RNA expression profiling) assays are being investigated as a tool to assist in the risk stratification and clinical management of individuals with various diseases. GEP assays measure the activity (expression) of RNA in a given tissue or bodily fluid at a given point in time. By measuring the levels of RNA of thousands of genes simultaneously, RNA expression profiling captures the rate at which those genes are expressed in a tissue at a particular moment in time. Because the rate of gene expression changes under pathological conditions, RNA expression profiling can identify genes that may be involved in disease pathogenesis resulting from both environmental and genetic factors, as well as any associated interactions. Dynamic changes in these processes can be studied over time. GEP is being investigated as a means to identify panels of genes for disease risk prediction, to diagnose a disease or condition or for clinical management (Arnett, 2007).

Techniques to identify the GEP of a particular tissue or cell type include, but are not necessarily limited to, northern blotting, cDNA/oligonucleotide microarrays, real-time quantitative polymerase chain reaction (RT-qPCR) and serial analysis of gene expression (SAGE, SuperSAGE). Each of these techniques has intrinsic advantages and limitations (Arcellana-Panlilio, 2002; Arnett, 2007; Kawasaki, 2006).

Whole Genome Sequencing

Whole genome sequencing (WGS), also known as full genome sequencing (FGS), complete genome sequencing, or entire genome sequencing, is a laboratory procedure which seeks to determine an individual's entire DNA sequence, specifying the order of every base pair within the

genome at a single time. WGS allows researchers to study not just the 98 percent of the human genome made up of genes that are in the non-coding and structural regions, but to examine 100 percent of the genome. In humans, this process involves obtaining a DNA sample from the individual (typically from saliva, bone marrow, epithelial cells, or hair) and sequencing an individual's entire chromosomal and mitochondrial DNA. Because of the large volume of genomic data involved in this process, the genomic information is processed by and stored on microprocessors and computers.

Researchers continue to explore the relationship between mutations in the genomic material and the development or presence of disease. The role of WGS in the clinical setting has yet to be established. Research is still being done to determine if WGS can be used to accurately identify the presence of a disease, predict the development of a particular disease in asymptomatic individuals as well as how an individual might respond to pharmacological therapy. It has been theorized that WGS might eventually improve clinical outcomes by preventing the development of disease.

Whole Exome Sequencing

It is estimated that most disease-causing mutations (around 85 percent) of clinically important sequence variants occur within the regions of the genome that encode proteins. While similar to WGS, whole-exome sequencing (WES) reads only the parts of the human genome that encode proteins, leaving the other regions of the genome unread (Choi, 2009). Since most of the errors that occur in DNA sequences that then lead to genetic disorders are located in the exons, sequencing of the exome is being explored as a more efficient method of analyzing an individual's DNA to discover the genetic cause of diseases or disabilities. It has been theorized that sequencing of the human exome can be used to identify genetic variants in individuals to diagnose diseases and to predict the development of a particular disease in asymptomatic individuals without the high cost associated with WGS.

The American College of Medical Genetics and Genomics (ACMG, 2012) published a position statement addressing points to consider in the clinical application of genomic sequencing. The policy statement:

> Was developed primarily as an educational resource for clinical and laboratory geneticists to help them provide quality clinical and laboratory genetic services. Adherence to these Points to Consider is voluntary and, in determining the relevance of and weight to be given to any specific point, the clinical and laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen.

The document contains indications for whole genome and WES as both screening and diagnostic tools. The ACMG states that clinical diagnostic testing using whole genome or WES is indicated for the following phenotypically affected individuals:

a. The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.
b. A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.
c. A patient presents with a likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
d. A fetus with a likely genetic disorder in which specific genetic tests , including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.
    i. Prenatal diagnosis by genomic (i.e., next-generation whole exome- or whole genome-) sequencing has significant limitations. The current technology does not support short

turn-around times which are often expected in the prenatal setting. There are high false positive, false negative, and variants of unknown clinical significance rates. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis (2012).

The ACMG document does not include references to peer reviewed literature in support of the recommendations made, or describe the process by which the recommendations were developed.

WES and WGS present ethical questions about informing individuals about incidental findings that have clinical significance. Ongoing discussions continue to explore whether or not, and how to inform individuals about medically relevant mutations in genes unrelated to the diagnostic question (i.e., mutations of unknown significance, non-paternity and sex chromosome abnormalities). This type of information may not only affect the individual being tested, but may also implicate family members. Also, in light of the small sample sizes and the limited number of studies exploring treatment outcomes, there may be safety considerations if a treatment decision is based on WES or WGS findings.

In 2013, the ACMG charged a Working Group with evaluating the need for and principles that would govern recommendations for analyzing and reporting incidental findings from genome and exome sequencing in the clinical context. The Working Group acknowledged that there was insufficient evidence about benefits, risks, and costs of reporting incidental findings to make evidence-based recommendations. However, based on available evidence and clinical consensus among its members, the Working Group determined that reporting some incidental findings might provide medical benefit for the individuals and families of individuals undergoing clinical sequencing. The Working Group recommended that when a report is issued for clinically indicated exome and genome sequencing, a minimum list of conditions, genes and variants should be routinely evaluated and reported to the ordering clinician. The conditions included for reporting were those that the Working Group and external reviewers considered most likely to be verifiable by other diagnostic methods and amenable to medical intervention. A complete list of these specified conditions can be found in the ACMG document. It should be noted that these recommendations address incidental findings sought and reported during clinical sequencing for a specific clinical indication. Conditions that were part of routine newborn screening were not addressed because they have their own assessment criteria and are applied in a specific public health framework. The Working Group recommendations also do not address preconception sequencing, prenatal sequencing, newborn sequencing, or sequencing of healthy children and adults (Green, 2013).

A potential major indication for use is molecular diagnosis of individuals with a phenotype that is suspicious for a genetic disorder or for individuals with known genetic disorders that have a large degree of genetic heterogeneity involving substantial gene complexity. Such individuals may be left without a clinical diagnosis of their disorder, despite a lengthy diagnostic work-up involving a variety of traditional molecular and other types of conventional diagnostic tests. For some of these individuals, WES, after initial conventional testing has failed to make the diagnosis, may return a likely pathogenic variant.

While some of the potential advantages of WES include the fact that it can be carried out more quickly than traditional genetic testing and it may be less expensive than some other tests (for example, WGS), it is not without limitations. WES typically covers only 85–90% of the exome and has no, or limited coverage of other areas of the genome. Areas of concern with this technology include: (1) gaps in the identification of exons prior to sequencing; (2) the need to narrow the large initial number of variants to manageable numbers without losing the likely candidate mutation; (3) difficulty identifying the potential causative variant when large numbers of variants of unknown significance are generated for each individual; (4) whole exome sequencing will not identify chromosomal changes, duplications, large deletions, rearrangements within genes,

epigenetic changes or nucleotide repeats (ACMG, 2012; National Cancer Institute, 2012; Teer, 2010[a]; Teer, 2010[b]).

At this time, there are limitations to WES that prohibit its use in routine clinical care. The limited experience with WES on a population level leads to gaps in understanding and interpreting ancillary information and variants of uncertain significance. As a result, the risk/benefit ratio of WES testing is poorly defined. Because the peer-reviewed literature on WES for clinical purposes consists primarily of case reports and small case series, the clinical applications of WES has yet to be established (Bilgüvar, 2010; Choi, 2009; Clayton-Smith, 2011, Saitsu, 2011; Vissers, 2011).

Background/Overview

Biochemical tests or other common laboratory tests or imaging procedures are typically adequate to diagnose a genetic disorder in symptomatic individuals; therefore, genetic testing plays only a small role in this setting. Additionally, there may be doubt in many cases that identification of genetic mutations identified with a specific disease will result in improved care. While a wide array of genetic medical conditions may be effectively treated or managed, many conditions have no known effective medical recourse. In these instances, there is very little evidence to demonstrate that the added information provided by genetic testing has any significant impact on individual care or outcomes. Conditions that fit into this category include ataxia telangiectasia, ALS, and Alzheimer's disease, among others. In the case of Alzheimer's disease specifically, several genetic alleles have been identified and have commercially available tests to identify them. Unfortunately, identification of these genes in a person's body does not allow improved prevention, care or management of their condition.

GEPs measure the degree of "expression" of multiple genes using a single sample. GEPs provide information about a cell's type, its current activity status and its local environment. GEPs are being investigated as a means to identify panels of genes for disease risk prediction, to diagnose a disease or condition or for clinical management.

Definitions

Amyotrophic lateral sclerosis (ALS,also known asLou Gehrig's disease):A progressive neurodegenerative disorder that affects nerve cells in the spinal cord and brain which eventually results in paralysis and death.

Ataxia telangiectasia: A rare, progressive, neurodegenerative childhood disease that affects the brain and other body systems.

DNA (deoxyribonucleic acid): A type of molecule that contains the code for genetic information.

Exome: All the exons in a genome.

Exon: The portion of the genome that predominantly encodes protein.

First-degree relative: Any relative who is a parent, sibling, or offspring to another.

Gene expression: The process by which a gene is turned on in a cell to make RNA and proteins. Gene expression may be measured by identifying the RNA, or the protein made from the RNA, or what the protein does in a cell.

Gene expression profiling: Any genomic technique that measures the degree of expression of multiple genes from an individual's tissue or cell sample.

Genetic counseling: A process involving the guidance of a specially trained professional in the

evaluation of family history, medical records, and genetic test results, in assessing the risk of genetic diseases, understanding the ramifications of diagnosis, and explanation of available treatment options available.

Genetic molecular testing: A type of test that is used to determine the presence or absence of a specific gene or set of genes to help diagnose a disease, screen for specific health conditions, and for other purposes.

Genome: An organism's entire set of DNA.

Mutation: A change in DNA sequence.

Phenotype: The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences.

Second-degree relative: Any relative who is a grandparent, grandchild, uncle, aunt, niece, nephew, or half-sibling to another.

Whole exome sequencing: A testing method which analyzes the exons (coding regions) of thousands of genes simultaneously using next-generation sequencing techniques.

Whole genome sequencing: A testing method which seeks to determine an individual's entire DNA sequence, specifying the order of every base pair within the genome.

Coding

The following codes for treatments and procedures applicable to this document are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement policy. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

When services are Medically Necessary:

CPT

81228	Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (eg, Bacterial Artificial Chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)
81229	Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities
81599	Unlisted multianalyte assay with algorithmic analysis [when specified as gene expression profiling reliably associated with a specific disease]

ICD-9 Diagnosis ***[For dates of service prior to 10/01/2014]***
All other diagnoses not listed below as investigational and not medically necessary

ICD-10 Diagnosis ***[For dates of service on or after 10/01/2014]***
All other diagnoses not listed below as investigational and not medically necessary

When services may be Medically Necessary when criteria are met:

CPT

81479	Unlisted molecular pathology procedure [for specific gene testing]

81599 Unlisted multianalyte assay with algorithmic analysis [when specified as gene expression profiling]

ICD-9 Diagnosis ***[For dates of service prior to 10/01/2014]***
All other diagnoses not listed as investigational and not medically necessary

ICD-10 Diagnosis ***[For dates of service on or after 10/01/2014]***
All other diagnoses not listed as investigational and not medically necessary

When services are Investigational and Not Medically Necessary:

CPT

81403

Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons) [when specified as the following]:

- *ANG (angiogenin, ribonuclease, RNase A family, 5)* (eg, amyotrophic lateral sclerosis), full gene sequence

81404

Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis) [when specified as the following]:

- *CDKN2A (cyclin-dependent kinase inhibitor 2A)* (eg, CDKN2A-related cutaneous malignant melanoma, familial atypical mole-malignant melanoma syndrome), full gene sequence
- *SOD1 (superoxide dismutase 1, soluble) (eg, amyotrophic lateral sclerosis), full gene sequence*

81405

Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis) [when specified as the following]:

- *TARDBP (TAR DNA binding protein) (eg, amyotrophic lateral sclerosis), full gene sequence*

81406

Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia) [when specified as the following]:

- *FUS (fused in sarcoma)* (eg, amyotrophic lateral sclerosis), full gene sequence;
- *OPTN (optineurin)* (eg, amyotrophic lateral sclerosis), full gene sequence

81408

Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis) [when specified as the following]:

- *ATM (ataxia telangiectasia mutated)* (eg, ataxia telangiectasia), full gene sequence

ICD-9 Diagnosis ***[For dates of service prior to 10/01/2014]***

172.0-172.9 Malignant melanoma of skin [when test is specified for hereditary melanoma]

Note: BRAF molecular diagnostic testing for melanoma is addressed in a separate document.

334.8 Other spinocerebellar diseases (ataxia telangiectasia)
335.20 Amyotrophic lateral sclerosis

ICD-10 Diagnosis ***[For dates of service on or after 10/01/2014]***

C43.0-C43.9 Malignant melanoma of skin [when test is specified for hereditary melanoma]

Note: BRAF molecular diagnostic testing for melanoma is addressed in a separate document.

G11.3 Cerebellar ataxia with defective DNA repair (ataxia telangiectasia)
G12.21 Amyotrophic lateral sclerosis

When services are also Investigational and Not Medically Necessary:

CPT

81479 Unlisted molecular pathology procedure [when specified as diagnostic genetic testing using panels of genes (with or without next generation sequencing), whole genome sequencing or whole exome sequencing]

HCPCS

S3800 Genetic testing for amyotrophic lateral sclerosis (ALS)

ICD-9 Diagnosis ***[For dates of service prior to 10/01/2014]***
All diagnoses

ICD-10 Diagnosis ***[For dates of service on or after 10/01/2014]***
All diagnoses

References

Peer Reviewed Publications:

1. Arcellana-Panlilio M, Robbins SM. Cutting-edge technology. I. Global gene expression profiling using DNA microarrays. Am J Physiol Gastrointest Liver Physiol. 2002; 282(3):G397-G402.
2. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet. 2011; 12(11):745-755.
3. Biesecker LG. Opportunities and challenges for the integration of massively parallel genomic sequencing into clinical practice: lessons from the ClinSeq project. Genet Med. 2012; 14(4):393-398.
4. Kawasaki ES. The end of the microarray Tower of Babel: will universal standards lead the way? J Biomol Tech. 2006; 17(3):200-206.

5. Teer JK, Bonnycastle LL, Chines PS, et al. Systematic comparison of three genomic enrichment methods for massively parallel DNA sequencing. Genome Res. 2010(a) 20(10):1420–1431.
6. Teer JK, Mullikin JC. Exome sequencing: the sweet spot before whole genomes. Hum Mol Genet. 2010(b) 19(R2):R145–151.
7. Weinstein LB. Selected genetic disorders affecting Ashkenazi Jewish families. Fam Community Health. 2007; 30(1):50-62.

Government Agency, Medical Society, and Other Authoritative Publications:

1. American College of Medical Genetics and Genomics. Position Statement. Points to consider in the clinical application of genomic sequencing. 2012. Approved May 15, 2012. Available at: http://www.acmg.net/StaticContent/PPG/Clinical_Application_of_Genomic_Sequencing.pdf. Accessed on December 19, 2013.
2. Arnett DK, Baird AE, Barkley RA, et al. American Heart Association Council on Epidemiology and Prevention; American Heart Association Stroke Council; Functional Genomics and Translational Biology Interdisciplinary Working Group. Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group. Circulation. 2007; 115(22):2878-2901.
3. Blue Cross and Blue Shield Association. Sequencing for Clinical Diagnosis of Patients with Suspected Genetic Disorders. TEC Assessment, 2013; 28(3).
4. Centers for Disease Control and Prevention (CDC). Genomic Testing. Available at: http://www.cdc.gov/genomics/gtesting/ACCE/. Accessed on December 19, 2013.
5. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013; 15(7):565-574.
6. Holtzman NA, Watson MS. Promoting Safe and Effective Genetic Tests in the United States: Work of the Task Force on Genetic Testing Clin. Chem.1999; 45:732-738.
7. National Cancer Institute. Backgrounder. Sequencing techniques uncover mutations in genes that can increase cancer risk. Posted August 15, 2012. Available at: http://www.cancer.gov/newscenter/featurednews/2012/SequencingTechniquesBackgrounder. Accessed on December 19, 2013.
8. National Library of Medicine (NLM). Genetic Conditions: Amyotrophic lateral sclerosis. Reviewed August 2012. Published December 16, 2013. Available at: http://ghr.nlm.nih.gov/condition=amyotrophiclateralsclerosis. Accessed on December 19, 2013.
9. National Library of Medicine (NLM). Genetic Conditions: Ataxia-telangiectasia. Reviewed January 2013. Published December 16, 2013 Available at: http://ghr.nlm.nih.gov/condition=ataxiatelangiectasia. Accessed on December 19, 2013.
10. National Library of Medicine (NLM). Genetics Home Reference Glossary: Published January 17, 2013. Available at: http://ghr.nlm.nih.gov/ghr/glossary. Accessed on December 19, 2013.

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Amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease)
Ataxia telangiectasia
Melanoma (hereditary)
XomeDx

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete

listing of all products available.

Document History

Status	Date	Action
Revised	02/13/2014	Medical Policy & Technology Assessment Committee (MPTAC) review. Medically necessary position statement expanded to address gene expression profiling. Additional investigational and not medically necessary position statement added to address diagnostic genetic testing using panels of genes (with or without next generation sequencing), including but not limited to whole genome and whole exome sequencing. Updated Rationale, Background/Overview, Coding, References and History sections.
	01/01/2014	Updated Coding section with 01/01/2014 CPT descriptor changes.
	07/01/2013	Updated Coding section with 07/01/2013 CPT changes.
Reviewed	02/14/2013	MPTAC review. Updated review date, References and History sections.
	01/01/2013	Updated Coding section to include 01/01/2013 CPT changes; removed 83890-83914, 88384-88386 deleted 12/31/2012.
Reviewed	02/16/2012	MPTAC review. Updated review date, Reference and History sections.
	01/01/2012	Updated Coding section to include 01/01/2012 CPT changes.
	07/13/2011	Updated Coding section; removed S3870 which is now addressed in GENE.00021.
Reviewed	02/17/2011	MPTAC review. Updated review date, Description/Scope, Rationale, Background/Overview, Reference and History sections.
	01/12/2011	Updated Coding section; removed S3865, S3866 which are now addressed in GENE.00017.
Reviewed	02/25/2010	MPTAC review. Updated review date, Reference and History sections. Removed the word "patient" from the title and description and replaced it with the word "individual."
Review	02/26/2009	MPTAC review. Updated review date, Reference, Coding and History sections.
New	02/21/2008	MPTAC initial document development. Document created to addresses diagnostic genetic testing, which was formerly addressed in GENE.00001.

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American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss

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Disclaimer: This guideline is designed primarily as an educational resource for clinicians to help them provide quality medical services. Adherence to this guideline is completely voluntary and does not necessarily assure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinician should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. Clinicians are encouraged to document the reasons for the use of a particular procedure or test, whether or not it is in conformance with this guideline. Clinicians also are advised to take notice of the date this guideline was adopted, and to consider other medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Hearing loss is a common and complex condition that can occur at any age, can be inherited or acquired, and is associated with a remarkably wide array of etiologies. The diverse causes of hearing loss, combined with the highly variable and often overlapping presentations of different forms of hearing loss, challenge the ability of traditional clinical evaluations to arrive at an etiologic diagnosis for many deaf and hard-of-hearing individuals. However, identifying the etiology of a hearing loss may affect clinical management, improve prognostic accuracy, and refine genetic counseling and assessment of the likelihood of recurrence for relatives of deaf and hard-of-hearing individuals. Linguistic and cultural identities associated with being deaf or hard of hearing can complicate access to and the effectiveness of clinical care. These concerns can be minimized when genetic and other health-care services are provided in a linguistically and culturally sensitive manner. This guideline offers information about the frequency, causes, and presentations of hearing loss and suggests approaches to the clinical evaluation of deaf and hard-of-hearing individuals aimed at identifying an etiologic diagnosis and providing informative and effective patient education and genetic counseling.

Genet Med advance online publication 20 March 2014

Key Words: genetics evaluation; deaf; Deaf; genetic counseling; genetic testing; guideline; hard of hearing; hearing loss

DEFINITIONS

Deaf: a community with a distinct culture and language shaped by the experience of being deaf or hard of hearing, which may include deaf, hard-of-hearing, and hearing individuals

deaf: an auditory phenotype characterized by a total or near-total loss of the ability to hear

hard of hearing: an auditory phenotype characterized by a partial loss of the ability to hear

hearing loss: an auditory phenotype characterized by any degree of loss of the ability to hear; depending on cause, hearing loss can be temporary or permanent—this guideline focuses on permanent hearing loss

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INTRODUCTION

Two to three of every 1,000 children born in the United States are deaf or have a hearing loss significant enough to affect speech and language development.[1] Early intervention has been shown to be effective in facilitating speech and language development in deaf and hard-of-hearing children.[2] As a result, newborn hearing screening, which began in 2001, is now mandated throughout the United States. Not all childhood hearing loss is present at birth, however, and hearing screening is recommended throughout childhood and adolescence to identify children with later-onset hearing loss and to permit early intervention.[3,4]

Ninety-five percent of newborns with hearing loss identified by newborn hearing screening programs are born to hearing parents, obscuring the fact that the majority of newborns have a hereditary cause for their hearing loss.[5,6] Analysis of family history data from school-aged children in the United States estimated that up to 60% of educationally significant congenital and early-onset hearing loss is caused by genetic factors.[5,6] The majority of genetic hearing loss is inherited in an autosomal recessive pattern and often presents in the absence of a positive family history for hearing loss. One gene, *GJB2*, which encodes the gap junction protein connexin 26, accounts for the largest proportion of autosomal recessive early childhood hearing loss in many populations.[7]

The prevalence of hearing loss increases with age, with 40–50% of the population experiencing hearing loss by age 75.[8] The contribution of genetic causes to cases of adult-onset hearing loss is less clear. However, it is evident that a significant proportion of adult-onset hearing loss is likely to be caused, or strongly influenced, by genetic factors.[9–14]

The goal of a genetics evaluation for deaf and hard-of-hearing individuals of any age is to identify an etiologic diagnosis and, in doing so, enable implementation of an individualized health-maintenance strategy.[15–17] Identification of a previously unrecognized syndromic form of hearing loss can be particularly important because it may allow early management of associated medical concerns. Obtaining an etiologic diagnosis also provides the basis for precise genetic counseling that includes an accurate estimation of the chances for recurrence of hearing loss within families.

AUDIOMETRIC AND CLINICAL ASPECTS OF HEARING LOSS

Hearing loss is typically described in terms related to its clinical presentation. In general, hearing loss is categorized as either syndromic or nonsyndromic, depending on the presence or absence of associated defects in other organ systems. Hearing loss is also typically described by the following:

- The age of onset—congenital, prelingual (before the acquisition of speech), postlingual (after the acquisition of speech), adult-onset, or presbycusis (age-related late-onset hearing loss);
- The type of hearing loss—sensorineural, conductive, mixed, or auditory neuropathy;
- The laterality and symmetry of the hearing loss—unilateral or bilateral, symmetric or asymmetric;
- The stability of the hearing loss—progressive, nonprogressive, or fluctuating;
- The degree of hearing loss—slight (16–25 decibels (dB)), mild (26–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB), or profound (91 dB or greater)[18]; and
- The configuration of the hearing loss as seen on audiometric analysis—sloping, flat, rising, or midfrequency (cookie-bite) loss.

Hearing loss may also be described according to an apparent pattern of inheritance—autosomal recessive, autosomal dominant, X-linked, or matrilineal (mitochondrial). If a specific etiology is known, descriptions of hearing loss may also include the etiologic diagnosis, such as Usher syndrome type 1–related hearing loss or *GJB2*-related hearing loss.[15,16,19,20]

GENETIC AND NONGENETIC ETIOLOGIES OF HEARING LOSS

Hearing loss is among the most etiologically heterogeneous disorders, with more than 400 genetic syndromes that include hearing loss as a feature, more than 100 genes associated with nonsyndromic genetic hearing loss, and a number of nongenetic causes.[20,21] Genes associated with syndromic and nonsyndromic genetic hearing loss encode a variety of proteins involved in the development and function of the auditory system, including transcription factors, structural proteins, gap junction proteins, and ion channels, to name just a few.

An estimated 30% of genetic hearing loss is syndromic. A few syndromes, such as Pendred (enlarged vestibular aqueduct, thyroid problems), Usher (retinitis pigmentosa), Waardenburg (pigmentary anomalies), and branchio-oto-renal (branchial arch and renal anomalies) syndromes, account for substantial percentages of hearing loss in some populations.[20,22–25] Syndromic hearing loss may be transmitted as an autosomal recessive, autosomal dominant, X-linked, or matrilineal trait. A review of individual conditions can be found in *Hereditary Hearing Loss and Its Syndromes* by Toriello and Smith[20] and the online database GeneReviews.[19]

For some syndromic forms of hearing loss, such as Usher syndrome or Pendred syndrome, the nonauditory features can be subtle, especially in early childhood. For others, hearing loss is not the presenting finding or the most pressing concern. For many syndromic forms of hearing loss, there is marked variability in the phenotypic presentation and in the age of onset of syndromic features. This variability can exist both between and within families. For example, hearing loss is observed in only 20–50% of individuals with Waardenburg syndrome. As a result, this diagnosis can be easily missed if specific information about pigmentary changes or gastrointestinal disturbances is not elicited.[26] Furthermore, some hereditary forms of hearing loss, such as neurofibromatosis type 2, enlarged vestibular aqueduct syndrome, and Pendred syndrome, may present

initially as unilateral hearing loss.[19,20,27–29] Given the challenges that can exist in distinguishing between syndromic and nonsyndromic forms of hearing loss, all children and adolescents showing hearing loss without a known etiology, e.g., confirmed *GJB2* mutations or documented congenital cytomegalovirus (CMV) infection, should be evaluated for syndromic conditions by a clinical geneticist.[15,16]

An estimated 70% of genetic hearing loss is nonsyndromic. Nonsyndromic hearing loss may be transmitted as an autosomal recessive (~80%), autosomal dominant (~15%), or X-linked trait (~1%).[20] In addition, matrilineal (mitochondrial) transmission of nonsyndromic hearing loss occurs with a frequency of ~1% in Western nations but has a slightly higher incidence in Spain and East Asian countries including China, Mongolia, Korea, and Japan.[30,31]

Of particular note, the DFNB1 locus, which includes the *GJB2* gene encoding the gap junction protein connexin 26 and the *GJB6* gene encoding the gap junction protein connexin 30, accounts for an estimated 50% of all autosomal recessive nonsyndromic hearing loss and 15–40% of all deaf individuals in a variety of populations.[7,32–38] More than 150 deafness-causing variants have been identified in *GJB2,* but a few common mutations account for a large percentage of alleles in several populations.[7,34–36] *GJB2*-related hearing loss is sensorineural, usually present at birth, typically bilateral and nonprogressive, and can range from mild to profound in severity. However, progressive or later-onset hearing loss—with infants passing their newborn hearing screen—have also been described, particularly in association with nontruncating mutations.[39–42] Nonsyndromic hearing loss due to mutations at the DFNB1 locus may also be caused by (i) interaction of a *GJB2* mutation on one allele and a deletion involving *GJB6* on the other allele or (ii) biallelic deletions involving *GJB6*.[43–45] *GJB6* deletions have been observed in multiple populations, although they appear to be a relatively uncommon explanation for hearing loss in the United States.[46–48] Notably, hearing loss caused by certain dominant mutations in *GJB2*, although uncommon, may present as a syndromic hearing loss, with associated skin findings.[49–51]

Nonsyndromic mitochondrial hearing loss is characterized by audiograms that fall into the moderate-to-profound range and is associated with variants in either the *MT-RNR1* gene encoding the mitochondrial 12S ribosomal RNA or the *MT-TS1* gene encoding the mitochondrial transfer RNA Ser(UCN).[30,31,52] Of particular note, mutations in *MT-RNR1* are associated with predisposition to aminoglycoside ototoxicity.[53] Hearing loss in individuals exposed to aminoglycoside antibiotics who carry susceptibility mutations in *MT-RNR1* is bilateral, severe to profound, and typically develops within a few days to weeks after administration of any amount, including just a single dose, of an aminoglycoside antibiotic.[54] Studies offer conflicting findings with regard to the likelihood of hearing loss in individuals carrying a deafness-causing variant in *MT-RNR1* who are not exposed to aminoglycosides.[53–55]

Age-related hearing loss, or presbycusis, is a common neurosensory deficit. In the United States, presbycusis is present in 40–50% of individuals aged 75 and older. Presbycusis generally affects higher frequencies of sound disproportionately, making it difficult for those with presbycusis to understand speech.[8] Men have presbycusis more frequently than women.[56] Presbycusis is a complex condition influenced by genetic and environmental factors.[13] Much of the literature about age-related hearing loss has focused on environmental factors such as noise exposure.[9,57,58] More recently, however, several susceptibility loci for age-related hearing loss have been identified. Genes implicated in this process using linkage and genome-wide association studies include genes previously implicated in other forms of hearing loss (such as *KCNQ4* and *ACTG1*), and genes involved in oxidative stress (such as *GRM7*, *GRHL2*, mitochondrial oxidative genes, and *N*-acetyltransferase).[9,10,12–14, 20]

Certain environmental (nongenetic) factors play a major etiologic role in hearing loss.[59] In the United States, congenital CMV infection is the most common nongenetic cause of hearing loss among children. Of the 20,000-40,000 infants born with congenital CMV infection each year, 90% have no detectable clinical abnormalities at birth, yet 10–15% of these asymptomatic infants will develop sensorineural hearing loss which can present in early childhood, can be unilateral or bilateral, and is often progressive.[60–62] As a result, congenital CMV infection may go undetected even in children who undergo newborn hearing screening and receive a thorough physical examination in the neonatal period.[16,20,62]

Congenital rubella, which was a common cause of hearing loss in the mid-1960s, occurs less frequently in Western populations today as a result of successful immunization programs.[63,64] According to the World Health Organization, no cases of endemic rubella infection are known to have occurred in the Americas between 2009 and 2012.[65] Similarly, the occurrence of postmeningitic hearing loss in children has been substantially reduced in developed countries as a result of vaccination against *Haemophilus influenzae*.[66] However, other environmental causes for hearing loss—including prematurity and exposure to noise or ototoxic drugs such as aminoglycosides and cyclophosphamides (which may have a genetically determined predisposition in some cases)—persist in the United States today.[20, 67–69]

THE IMPORTANCE OF GENETIC EVALUATION AND GENETIC COUNSELING FOR DEAF AND HARD-OF-HEARING INDIVIDUALS

When a genetic etiology is possible, a clinical genetics evaluation, including genetic counseling, offers a number of potential benefits for children and adults with hearing loss and their families. Benefits can include providing etiologic information, identifying (or allaying concerns about) comorbidities that may need referral for specialty care, planning for future medical and educational needs, facilitating estimations of the likelihood of recurrence, allowing families to better plan for the birth of a deaf or hard-of-hearing child, relieving the guilt that some parents may feel about having a child with hearing loss, enhancing

psychological well-being, dispelling misinformation, and facilitating referral for unrelated hereditary conditions such as familial cancer.[48,70–79] Furthermore, if mitochondrial DNA mutations associated with genetic susceptibility to aminoglycoside ototoxicity are discovered, it may be possible for relatives to avoid precipitating medications.[53–55]

As with any genetics evaluation, clear communication between the genetics professionals and their patients is important for the provision of effective genetics services. Deaf and hard-of-hearing individuals use a variety of communication methods, including spoken and signed language, lip reading, and written notes. Special training may be needed to optimize communication between individuals with hearing loss and genetics professionals. Such training may include (i) training sign language interpreters in medical and genetics terminology and (ii) training genetics professionals to work effectively with sign language interpreters and use a variety of communication aids, including videophones, video relay services, instant messaging, and visual aids.[80]

In addition, deafness is considered by some to be a nonmedical trait. Many deaf individuals consider themselves to be part of a linguistic and cultural minority group, viewing their deafness as a neutral or positive trait.[81,82] By contrast, the medical perspective—which views deafness as a pathology—is pervasive among most hearing individuals and some deaf individuals. This difference in perspective may affect the willingness of some individuals to obtain genetic services and genetic counseling.[83,84] However, when given accurate information about the nature of genetic counseling and how to obtain a referral, Deaf adults are often interested in receiving genetic services in order to learn more about themselves and why they are deaf or hard of hearing. In addition, many Deaf and hard-of-hearing individuals report an enhanced sense of self-understanding and self-identity, as well as an enhanced cultural and group identity, as a result of genetic testing.[72,85] Providing genetic services in a linguistically and culturally sensitive manner has been shown to improve outcomes such as genetics knowledge and understanding.[86,87] Furthermore, using neutral or balanced terminology, such as "chance" instead of "risk," "deaf" or "hearing" instead of "affected" or "unaffected," and exercising caution in the use of terms such as "handicapped," "pathology," and "impairment" can enhance the provision of genetic services to deaf and hard-of-hearing individuals and their families.[86,88,89]

GENETIC TESTING FOR THE ETIOLOGIC DIAGNOSIS OF HEREDITARY HEARING LOSS

Historically, molecular diagnostic tests for hearing loss have used genotyping or DNA sequencing to identify specific hearing loss variants or to screen individual genes, or small collections of genes, for changes associated with hearing loss. This approach has proven to be effective in cases in which there is a single gene, or limited number of genes, responsible for a subtype of hearing loss. Examples include *SLC26A4* gene sequencing in individuals suspected of having Pendred syndrome, *PAX3* gene sequencing in individuals with features of Waardenburg syndrome type I, *MITF* and *SOX10* gene sequencing in individuals with features of Waardenburg syndrome type II, or sequencing of *MYO7A* or *USH2A*, the most common genes involved in Usher syndrome types I and II, respectively.[90,91] Such screening can also be cost effective in individuals with genetically heterogeneous hearing loss phenotypes when a single gene is responsible for a significant percentage of cases. For example, *GJB2* gene sequencing can identify the underlying etiology for many individuals whose clinical presentation is consistent with autosomal recessive nonsyndromic hearing loss.

Today, tests based on next-generation sequencing (NGS) technologies are rapidly replacing many single gene–sequencing tests for hearing loss (**Figure 1**). These tests use disease-targeted exon capture, whole-exome sequencing (WES), or whole-genome sequencing (WGS) strategies. The main advantage of these tests is their ability to address the problem of genetic heterogeneity, wherein many different genes result in phenotypes that cannot be easily distinguished clinically.[92–96] Several NGS tests are now clinically available and can be found by querying the GeneTests and Genetic Testing Registry websites.[97,98]

NGS tests that use disease-targeted exon-capture approaches restrict sequencing to specific genes, such as genes known to be associated with hearing loss. Such tests can provide excellent coverage of the genes selected for study but are limited by our present knowledge of which genes are involved in hearing loss. Furthermore, some tests may sequence only a subset of the genes known to be associated with hearing loss. WES is also based on exon capture but does not rely on a list of genes involved in a particular disease process. Instead, WES seeks to evaluate all exons in the genome for variations. This approach can identify variants in known hearing loss–related genes and genes that have yet to be associated with hearing loss. WGS is not limited to screening exons and therefore has the potential to identify changes outside of exons that may be related to hearing loss.

The ability of WES and WGS approaches to detect a larger subset of all hearing loss–related changes needs to be balanced with the difficulties in interpretation that come from identifying an ever-increasing number of variants, the challenge of causally linking variants in new genes to hearing loss, and the likelihood of identifying genetic susceptibilities unrelated to hearing loss (i.e., incidental findings).[99] In 2013, the ACMG published recommendations for reporting incidental findings from genomic sequencing.[100]

Furthermore, not all regions of the genome are efficiently captured and analyzed by current exon-capture or WGS approaches, and large deletions and duplications, in addition to copy-number and structural variations, may not be efficiently detected.[99] These limitations of NGS technologies may necessitate use of alternative or complementary genetic testing strategies in some cases.

NGS technologies are expected to continue to improve over time, but it will always be important to pay close attention to the performance characteristics of tests, including coverage, analytic sensitivity, the genes that are and are not analyzed, and the types of mutations that are and are not detected. In some cases, it may be helpful to have tests performed in laboratories that

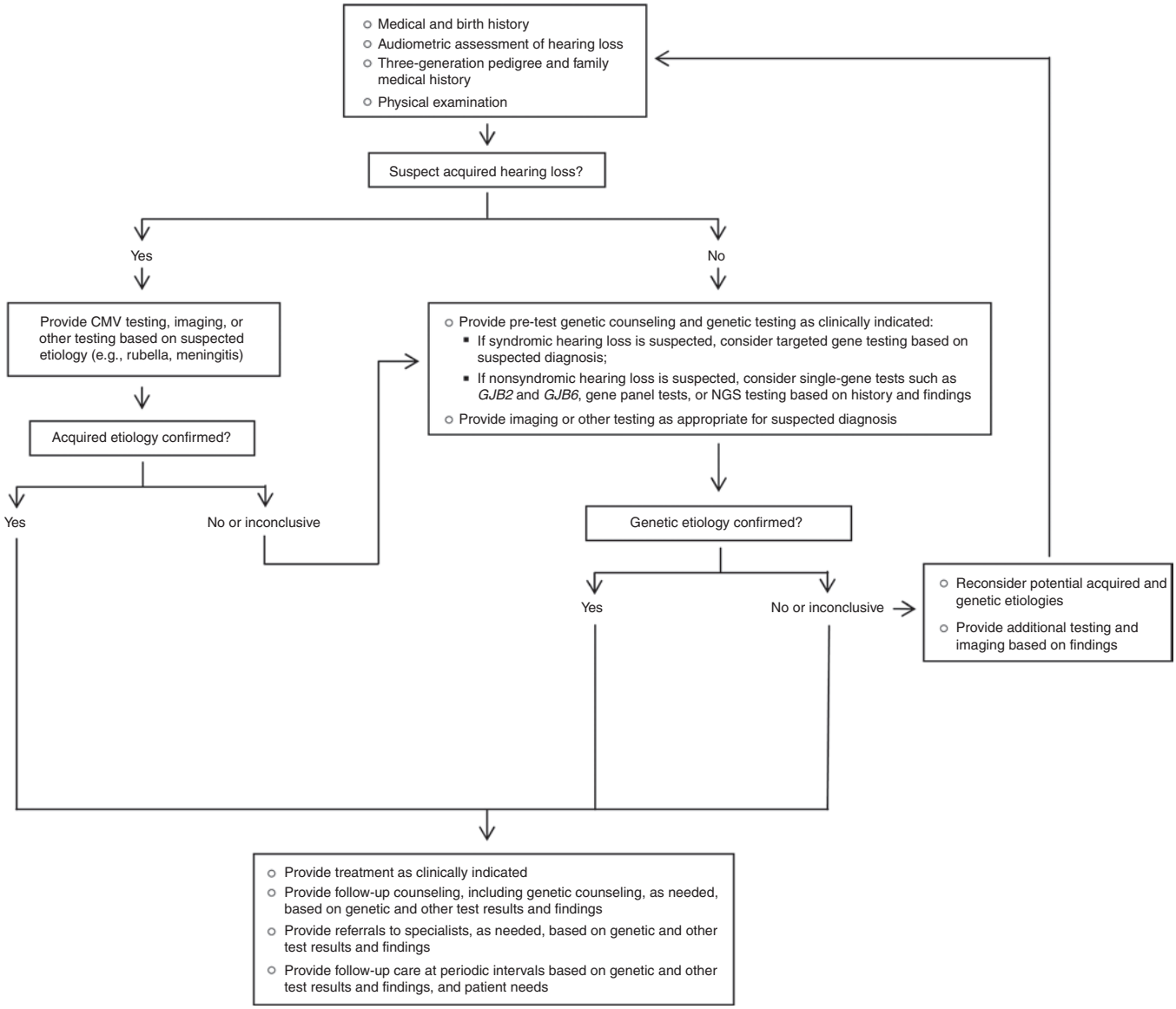

Figure 1 Graphic overview of approaches to the clinical evaluation and etiologic diagnosis of hearing loss. CMV, cytomegalovirus; NGS, next-generation sequencing.

focus on genetic causes of hearing loss because these laboratories may be more likely to report test performance with respect to hearing-related genes and to have developed approaches to specifically analyze relevant regions of the genome that may be refractory to more general NGS approaches.[92–96,99]

OTHER TESTING IMPORTANT TO THE ETIOLOGIC DIAGNOSIS OF HEARING LOSS

Because CMV remains a common cause of pediatric hearing loss, testing for congenital CMV infection by rapid culture or polymerase chain reaction of saliva or urine samples from newborns is recommended as an initial test once a newborn hearing loss is confirmed (**Figure 1**). However, testing for CMV is most diagnostic when done before ~6 weeks of age.[101–105] A negative result most likely excludes CMV as the cause of the hearing loss, but a positive result may not necessarily indicate that the hearing loss is due to CMV infection, especially if obtained in older children who may have been exposed to CMV after birth.

Recent algorithms for the evaluation of hearing loss suggest that other nongenetic tests, such as computed tomography, magnetic resonance imaging, renal ultrasonography, electrocardiography, and ophthalmologic consultation, have an important role because their results can guide genetic testing or interpretation of DNA sequence variants.[106] For example, temporal bone imaging is commonly recommended to look for an enlarged vestibular aqueduct, which would prompt genetic testing for Pendred syndrome.[27,107,108] However, many nongenetic tests have low diagnostic yield in patients with hearing loss.[109] Furthermore, recent advances in genetic testing technologies that permit the analysis of many genes simultaneously at rapidly decreasing cost may soon prompt reassessment of the clinical utility of certain nongenetic tests as part of the initial

workup for the etiologic diagnosis of hearing loss. Such reassessments will need to consider the clinical utility of various nongenetic tests versus the risks associated with those tests, such as the clinical utility of computed tomography and magnetic resonance imaging versus the risks associated with radiation exposure and sedation.[17,109] As evidence for the clinical utility of NGS tests for the etiologic diagnosis of hearing loss is accumulated and evaluated, physicians should continue to rely on their best clinical judgment and consider the use of nongenetic tests for the evaluation of hearing loss on a case-by-case basis. For example, unless cochlear implantation is being considered, auditory neuropathy is detected, progressive hearing loss is identified, or other specific clinical concerns exist, it could be argued that temporal bone imaging might, in some cases, be better used as a complement or follow-up to genetic testing rather than as a part of the initial diagnostic evaluation.[109,110] In addition, in the absence of specific clinical concerns or family history, tests such as electrocardiographic studies, thyroid function testing, urinalysis, and renal ultrasonography might also be postponed until results of genetic testing are obtained, and then ordered as clinically indicated.[109,111,112]

GUIDELINE

1. All newborns and infants with confirmed hearing loss should undergo a comprehensive evaluation in which patient-focused medical and birth histories and a three-generation pedigree and family medical history are obtained, and a physical examination that focuses on dysmorphic physical findings is performed. Evaluation of children and young adults with hearing loss should follow a similar approach. Evaluation of deaf or hard-of-hearing adults should be customized based on the age of onset and other characteristics of the hearing loss (**Figure 1**).
   - The medical and birth histories may be helpful in differentiating between acquired versus inherited causes of hearing loss. Elements of medical and birth histories focused on hearing loss include the following:
     - Prenatal history, including maternal infections (e.g., CMV, rubella) and illnesses (e.g., syphilis), or medication or drug exposures (e.g., thalidomide, retinoic acid)[113,114];
     - Neonatal history, including premature birth, low birth weight, birth hypoxia, hyperbilirubinemia, sepsis, and exposure to ototoxic medications;
     - Postnatal history, including viral illnesses, bacterial meningitis, head trauma, noise exposure, and exposure to ototoxic medications; and
     - Audiometric assessment of the hearing loss, including sensorineural versus conductive or mixed hearing loss; age of onset; progressive, nonprogressive, or fluctuating nature of the hearing loss; laterality, symmetry, severity, and configuration of the hearing loss; and the presence or absence of vestibular dysfunction or auditory neuropathy.
   - The pedigree and family medical history should focus on identifying the following:
     - First- and second-degree relatives with hearing loss or with features commonly associated with hearing loss (such as pigmentary, branchial, or renal anomalies) or sudden cardiac death;
     - A pattern of inheritance;
     - Ethnicity and country of origin;
     - A common origin from ethnically or geographically isolated areas; and
     - Consanguinity.
   - The physical examination should focus on dysmorphic and other physical findings such as the following:
     - Unusual facial appearance, with attention to asymmetry;
     - Pigmentary anomalies;
     - Neck, skin, facial, or ear anomalies;
     - Neurological abnormalities;
     - Balance disturbances;
     - Skeletal abnormalities; and
     - Other unusual physical findings.
2. For individuals with findings that suggest a syndromic genetic etiology for their hearing loss,
   - Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing, if available, should be ordered to confirm the diagnosis—this testing may include single-gene tests, hearing loss sequencing panels, WES, WGS, chromosome analysis, or microarray-based copy-number analysis, depending on clinical findings;
   - Appropriate studies should be undertaken to determine whether other organs are involved; and
   - Appropriate near-term and long-term screening and management should be arranged, including referrals to specialists, as indicated by the associated manifestations of the particular syndrome.
3. For individuals lacking physical findings suggestive of a known syndrome and having medical and birth histories that do not suggest an environmental cause of hearing loss, a tiered diagnostic approach should be implemented.
   - Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing should be ordered.
     - Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics.
     - In the absence of any specific clinical indications and for singleton cases and cases with apparent autosomal recessive inheritance, the next step should be testing for DFNB1-related hearing loss (due to mutations in *GJB2* and adjacent deletions in *GJB6*).

  - If initial genetic testing is negative, genetic testing using gene panel tests, NGS technologies such as large sequencing panels targeted toward hearing loss–related genes, WES, or WGS may be considered. Because several tests are clinically available, the clinician must be aware of the genes included in the test (panel) chosen and the performance characteristics of the platform chosen, including coverage, analytic sensitivity, and what types of mutations will be detected. It should be noted that the cost of these new genetic sequencing technologies is decreasing so rapidly that a tiered approach to testing may soon no longer be cost effective. In particular, for large sequencing panels targeted toward hearing loss–related genes, it may, in some cases, already be more cost effective to use NGS technologies as the initial test in the evaluation of hearing loss. However, issues related to genomic testing, such as the likelihood of incidental findings, will have to be addressed.
  - If genetic testing reveals mutation(s) in a hearing loss–related gene, mutation-specific genetic counseling should be provided, followed by appropriate medical evaluations and referrals.
  - If genetic testing fails to identify an etiology for a patient's hearing loss, the possibility of a genetic or acquired etiology remains. This point must be emphasized because it can be misunderstood by clinicians and by patients and their families. For interested patients and families, further genetic testing may be pursued on a research basis.
- Temporal bone imaging by computed tomography or magnetic resonance imaging should be considered as a complement to genetic testing, particularly if the diagnosis remains unclear, if cochlear implantation is being considered, if auditory neuropathy is noted, in cases of progressive hearing loss, or if other clinical concerns exist. The anticipated clinical utility of imaging studies should be balanced against the risks associated with radiation exposure and sedation.
- CMV testing should be done at the same time as genetic testing for infants with congenital hearing loss. For later-onset or progressive hearing loss, CMV testing can be obtained, but the likelihood that a positive test is due to postnatal exposure increases with age.

4. Referral to a multidisciplinary care center, when available, is recommended.
   - A team approach that includes otolaryngologists, clinical geneticists, genetic counselors, audiologists, speech and language specialists, early hearing intervention and family support specialists (which may include other individuals who are deaf or hard of hearing or other parents of deaf or hard-of-hearing children), and other appropriate specialists offers optimal opportunity to provide ongoing management and support of deaf and hard-of-hearing individuals and their families as their needs change over time.
   - For cases in which the genetic evaluation failed to identify an underlying cause, periodic follow-up care every 3 years with a geneticist may be appropriate for several reasons. First, subtle features of syndromic forms of hearing loss may not be apparent at birth or early in childhood but may appear as deaf or hard-of-hearing individuals grow into adulthood. These may prompt additional medical tests or referrals for specialty care. Second, follow-up visits offer the opportunity to inform individuals about new genetic tests that may have become available or changes in the interpretation of previous test results as medical knowledge advances. Finally, follow-up visits may also help identify clinical concerns unrelated to hearing loss, for which referral for specialty care may be appropriate (**Figure 1**).
5. Regardless of whether genetic test results are positive, negative, or inconclusive, results should be communicated through the process of genetic counseling.

DISCLOSURE

C.G.P. has received grant support to develop educational materials on cancer for the Deaf community. H.L.R. is employed by a fee-for-service laboratory that offers diagnostic testing for hearing loss. The other authors declare no conflict of interest.

REFERENCES

1. Finitzo T, Albright K, O'Neal J. The newborn with hearing loss: detection in the nursery. *Pediatrics* 1998;102:1452–1460.
2. Yoshinaga-Itano C, Coulter D, Thomson V. Developmental outcomes of children with hearing loss born in Colorado hospitals with and without universal newborn hearing screening programs. *Semin Neonatol* 2001;6:521–529.
3. American Academy of Audiology Childhood Hearing Screening Guidelines. *American Academy of Audiology* [serial online] 2012; American Academy of Audiology. http://www.cdc.gov/ncbddd/hearingloss/documents/AAA_Childhood%20Hearing%20Guidelines_2011.pdf. Accessed 12 June 2012.
4. Harlor AD Jr, Bower C; Committee on Practice and Ambulatory Medicine; Section on Otolaryngology-Head and Neck Surgery. Hearing assessment in infants and children: recommendations beyond neonatal screening. *Pediatrics* 2009;124:1252–1263.
5. Marazita ML, Ploughman LM, Rawlings B, Remington E, Arnos KS, Nance WE. Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. *Am J Med Genet* 1993;46:486–491.
6. Mitchell RE, Karchmer MA. Chasing the mythical ten percent: parental hearing status of deaf and hard of hearing students in the United States. *Sign Lang Studies* 2004;4:138–163.
7. Pandya A, Arnos KS, Xia XJ, et al. Frequency and distribution of GJB2 (connexin 26) and GJB6 (connexin 30) mutations in a large North American repository of deaf probands. *Genet Med* 2003;5:295–303.
8. National Institute on Deafness and Other Communication Disorders (NIDCD): Presbycusis Health Statistics. *National Institute on Deafness and Other Communication Disorders* [serial online] 2011; http://www.nidcd.nih.gov/health/hearing/presbycusis.htm. Accessed 12 June 2012.
9. Carlsson PI, Van Laer L, Borg E, et al. The influence of genetic variation in oxidative stress genes on human noise susceptibility. *Hear Res* 2005;202:87–96.
10. Friedman RA, Van Laer L, Huentelman MJ, et al. GRM7 variants confer susceptibility to age-related hearing impairment. *Hum Mol Genet* 2009;18:785–796.
11. Op de Beeck K, Schacht J, Van CG. Apoptosis in acquired and genetic hearing impairment: the programmed death of the hair cell. *Hear Res* 2011;281:18–27.

12. Unal M, Tamer L, Dogruer ZN, Yildirim H, Vayisoglu Y, Camdeviren H. N-acetyltransferase 2 gene polymorphism and presbycusis. *Laryngoscope* 2005;115:2238–2241.
13. Van Eyken E, Van Camp G, Van Laer L. The complexity of age-related hearing impairment: contributing environmental and genetic factors. *Audiol Neurootol* 2007;12:345–358.
14. Van Laer L, Van Eyken E, Fransen E, et al. The grainyhead like 2 gene (GRHL2), alias TFCP2L3, is associated with age-related hearing impairment. *Hum Mol Genet* 2008;17:159–169.
15. Kochhar A, Hildebrand MS, Smith RJ. Clinical aspects of hereditary hearing loss. *Genet Med* 2007;9:393–408.
16. Pandya A, Arnos KS. Genetic evaluation and counseling in the context of early hearing detection and intervention. *Semin Hear* 2006;27:205–212.
17. Robin NH, Prucka SK, Woolley AL, Smith RJ. The use of genetic testing in the evaluation of hearing impairment in a child. *Curr Opin Pediatr* 2005;17: 709–712.
18. American Speech-Language-Hearing Assocation: Degree of Hearing Loss. *American Speech-Language-Hearing Association (AHSA)* [serial online] 2012; American Speech-Language-Hearing Association (AHSA). http://www.asha.org/public/hearing/Degree-of-Hearing-Loss/. Accessed 12 June 2012.
19. Pagon RA, Adam MP, Bird TD, et al., (eds).GeneReviews [Internet] University of Washington: Seattle, WA, 1993–2011. http://www.ncbi.nlm.nih.gov/books/NBK1116. Accessed 7 February 2014.
20. *Hereditary Hearing Loss and Its Syndromes, 3rd edn*. Toriello HV, Smith S (eds). Oxford University Press: New York, NY, 2013.
21. Van Camp G, Smith RJ. Hereditary Hearing Loss Homepage. *Hereditary Hearing Loss Homepage* [serial online] 2011; http://hereditaryhearingloss.org. Accessed 12 June 2012.
22. Kimberling WJ, Hildebrand MS, Shearer AE, et al. Frequency of Usher syndrome in two pediatric populations: implications for genetic screening of deaf and hard of hearing children. *Genet Med* 2010;12:512–516.
23. Park HJ, Shaukat S, Liu XZ, et al. Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness. *J Med Genet* 2003;40:242–248.
24. Smith RJH. Branchiootorenal Spectrum Disorders. *GeneReviews* [serial online] 1993; PMID:20301554. University of Washington, Seattle, WA. http://www.ncbi.nlm.nih.gov/books/NBK1380. Accessed 26 November 2013.
25. Yuan Y, Guo W, Tang J, et al. Molecular epidemiology and functional assessment of novel allelic variants of SLC26A4 in non-syndromic hearing loss patients with enlarged vestibular aqueduct in China. *PLoS ONE* 2012;7:e49984.
26. Schultz JM. Waardenburg syndrome. *Semin Hear* 2006;27:171–181.
27. Madeo AC, Pryor SP, Brewer C et al. Pendred syndrome. *Semin Hear* 2006;27:160–170.
28. Bamiou DE, Savy L, O'Mahoney C, Phelps P, Sirimanna T. Unilateral sensorineural hearing loss and its aetiology in childhood: the contribution of computerised tomography in aetiological diagnosis and management. *Int J Pediatr Otorhinolaryngol* 1999;51:91–99.
29. Dodson KM, Georgolios A, Barr N, et al. Etiology of unilateral hearing loss in a national hereditary deafness repository. *Am J Otolaryngol* 2012;33:590–594.
30. Li Z, Li R, Chen J, et al. Mutational analysis of the mitochondrial 12S rRNA gene in Chinese pediatric subjects with aminoglycoside-induced and non-syndromic hearing loss. *Hum Genet* 2005;117:9–15.
31. Pandya A, Xia X, Radnaabazar J, et al. Mutation in the mitochondrial 12S rRNA gene in two families from Mongolia with matrilineal aminoglycoside ototoxicity. *J Med Genet* 1997;34:169–172.
32. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. *J Med Genet* 2000;37:41–43.
33. Denoyelle F, Marlin S, Weil D, et al. Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counselling. *Lancet* 1999;353:1298–1303.
34. Estivill X, Fortina P, Surrey S, et al. Connexin-26 mutations in sporadic and inherited sensorineural deafness. *Lancet* 1998;351:394–398.
35. Kelsell DP, Dunlop J, Stevens HP, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* 1997;387:80–83.
36. Kenneson A, Van Naarden Braun K, Boyle C. GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: a HuGE review. *Genet Med* 2002;4:258–274.
37. Kudo T, Ikeda K, Kure S, et al. Novel mutations in the connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. *Am J Med Genet* 2000;90:141–145.
38. RamShankar M, Girirajan S, Dagan O, et al. Contribution of connexin26 (GJB2) mutations and founder effect to non-syndromic hearing loss in India. *J Med Genet* 2003;40:e68.
39. Chan DK, Schrijver I, Chang KW. Connexin-26-associated deafness: phenotypic variability and progression of hearing loss. *Genet Med* 2010;12:174–181.
40. Kenna MA, Feldman HA, Neault MW, et al. Audiologic phenotype and progression in GJB2 (Connexin 26) hearing loss. *Arch Otolaryngol Head Neck Surg* 2010;136:81–87.
41. Norris VW, Arnos KS, Hanks WD, Xia X, Nance WE, Pandya A. Does universal newborn hearing screening identify all children with GJB2 (Connexin 26) deafness? Penetrance of GJB2 deafness. *Ear Hear* 2006;27:732–741.
42. Snoeckx RL, Huygen PL, Feldmann D, et al. GJB2 mutations and degree of hearing loss: a multicenter study. *Am J Hum Genet* 2005;77:945–957.
43. del Castillo FJ, Rodríguez-Ballesteros M, Alvarez A, et al. A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. *J Med Genet* 2005;42:588–594.
44. del Castillo I, Villamar M, Moreno-Pelayo MA, et al. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N Engl J Med* 2002;346:243–249.
45. Del Castillo I, Moreno-Pelayo MA, Del Castillo FJ, et al. Prevalence and evolutionary origins of the del(GJB6-D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multicenter study. *Am J Hum Genet* 2003;73:1452–1458.
46. Feldmann D, Denoyelle F, Chauvin P, et al. Large deletion of the GJB6 gene in deaf patients heterozygous for the GJB2 gene mutation: genotypic and phenotypic analysis. *Am J Med Genet A* 2004;127A:263–267.
47. Fitzgerald T, Duva S, Ostrer H, et al. The frequency of GJB2 and GJB6 mutations in the New York State newborn population: feasibility of genetic screening for hearing defects. *Clin Genet* 2004;65:338–342.
48. Palmer CG, Martinez A, Fox M, et al. A prospective, longitudinal study of the impact of GJB2/GJB6 genetic testing on the beliefs and attitudes of parents of deaf and hard-of-hearing infants. *Am J Med Genet A* 2009;149A:1169–1182.
49. de Zwart-Storm EA, van Geel M, Veysey E, et al. A novel missense mutation in GJB2, p.Tyr65His, causes severe Vohwinkel syndrome. *Br J Dermatol* 2011;164:197–199.
50. Iossa S, Marciano E, Franzé A. GJB2 Gene Mutations in Syndromic Skin Diseases with Sensorineural Hearing Loss. *Curr Genomics* 2011;12:475–785.
51. Richard G, Rouan F, Willoughby CE, et al. Missense mutations in GJB2 encoding connexin-26 cause the ectodermal dysplasia keratitis-ichthyosis-deafness syndrome. *Am J Hum Genet* 2002;70:1341–1348.
52. Pandya A. Nonsyndromic Hearing Loss and Deafness, Mitochondrial. 22 October 2004 [updated 21 April 2011]. In: Pagon RA, Adam MP, Bird TD, et al. (eds). GeneReviews [Internet] University of Washington: Seattle, WA, 1993–2014. http://www.ncbi.nlm.nih.gov/books/NBK1422. Accessed 7 February 2014.
53. Estivill X, Govea N, Barceló E, et al. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment of aminoglycosides. *Am J Hum Genet* 1998;62:27–35.
54. Prezant TR, Agapian JV, Bohlman MC, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet* 1993;4:289–294.
55. Rahman S, Ecob R, Costello H, et al. Hearing in 44-45 year olds with m.1555A>G, a genetic mutation predisposing to aminoglycoside-induced deafness: a population based cohort study. *BMJ Open* 2012;2:e000411.
56. Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci* 1991;630:16–31.
57. Davis RR, Kozel P, Erway LC. Genetic influences in individual susceptibility to noise: a review. *Noise Health* 2003;5:19–28.
58. Tak S, Davis RR, Calvert GM. Exposure to hazardous workplace noise and use of hearing protection devices among US workers–NHANES, 1999-2004. *Am J Ind Med* 2009;52:358–371.
59. Grosse SD, Ross DS, Dollard SC. Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment. *J Clin Virol* 2008;41:57–62.
60. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol* 2007;17:355–363.
61. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. *Pediatr Infect Dis J* 2011;30:1043–1046.
62. Morton CC, Nance WE. Newborn hearing screening–a silent revolution. *N Engl J Med* 2006;354:2151–2164.
63. Reef SE, Redd SB, Abernathy E, Kutty P, Icenogle JP. Evidence used to support the achievement and maintenance of elimination of rubella and congenital rubella syndrome in the United States. *J Infect Dis* 2011;204(suppl 2):S593–S597.

64. Plotkin SA. The history of rubella and rubella vaccination leading to elimination. *Clin Infect Dis* 2006;43(suppl 3):S164–S168.
65. Rubella: Fact sheet No. 367. *World Health Organization* [serial online] 2012;http://www.who.int/mediacentre/factsheets/fs367/en/. Accessed 29 November 2013.
66. Stein LK, Boyer KM. Progress in the prevention of hearing loss in infants. *Ear Hear* 1994;15:116–125.
67. Fligor BJ, Neault MW, Mullen CH, Feldman HA, Jones DT. Factors associated with sensorineural hearing loss among survivors of extracorporeal membrane oxygenation therapy. *Pediatrics* 2005;115:1519–1528.
68. Marlow ES, Hunt LP, Marlow N. Sensorineural hearing loss and prematurity. *Arch Dis Child Fetal Neonatal Ed* 2000;82:F141–F144.
69. Robertson CM, Howarth TM, Bork DL, Dinu IA. Permanent bilateral sensory and neural hearing loss of children after neonatal intensive care because of extreme prematurity: a thirty-year study. *Pediatrics* 2009;123:e797–e807.
70. Boudreault P, Baldwin EE, Fox M, et al. Deaf adults' reasons for genetic testing depend on cultural affiliation: results from a prospective, longitudinal genetic counseling and testing study. *J Deaf Stud Deaf Educ* 2010;15:209–227.
71. Brunger JW, Murray GS, O'Riordan M, Matthews AL, Smith RJ, Robin NH. Parental attitudes toward genetic testing for pediatric deafness. *Am J Hum Genet* 2000;67:1621–1625.
72. Burton SK, Withrow K, Arnos KS, Kalfoglou AL, Pandya A. A focus group study of consumer attitudes toward genetic testing and newborn screening for deafness. *Genet Med* 2006;8:779–783.
73. Palmer CG, Martinez A, Fox M, Sininger Y, Grody WW, Schimmenti LA. Ethnic differences in parental perceptions of genetic testing for deaf infants. *J Genet Couns* 2008;17:129–138.
74. Palmer CG, Boudreault P, Baldwin EE, et al. Deaf genetic testing and psychological well-being in deaf adults. *J Genet Couns* 2013;22:492–507.
75. Robin NH. *Medical Genetics: Its Application to Speech, Hearing, and Craniofacial Disorders*. Plural Publishing: San Diego, California, 2008.
76. Robin NH. It does matter: the importance of making the diagnosis of a genetic syndrome. *Curr Opin Pediatr* 2006;18:595–597.
77. Withrow KA, Burton S, Arnos KS, Kalfoglou A, Pandya A. Consumer motivations for pursuing genetic testing and their preferences for the provision of genetic services for hearing loss. *J Genet Couns* 2008;17:252–260.
78. Withrow KA, Tracy KA, Burton SK, et al. Impact of genetic advances and testing for hearing loss: results from a national consumer survey. *Am J Med Genet A* 2009;149A:1159–1168.
79. Withrow KA, Tracy KA, Burton SK, et al. Provision of genetic services for hearing loss: results from a national survey and comparison to insights obtained from previous focus group discussions. *J Genet Couns* 2009;18:618–621.
80. Middleton A, Palmer CGS. Clients who are D/deaf or hard of hearing. In: Wiggins J, Middleton A (eds). *Getting the Message Across: Communication with Diverse Populations in Clinical Genetics.* Oxford University Press: Oxford, UK, 2012.
81. Lane H. Ethnicity, ethics, and the deaf-world. *J Deaf Stud Deaf Educ* 2005;10:291–310.
82. Padden CA, Humphries TL. *Deaf in America: Voices from a Culture*. Harvard University Press: Cambridge, MA, 1988.
83. Martinez A, Linden J, Schimmenti LA, Palmer CG. Attitudes of the broader hearing, deaf, and hard-of-hearing community toward genetic testing for deafness. *Genet Med* 2003;5:106–112.
84. Middleton A, Hewison J, Mueller RF. Attitudes of deaf adults toward genetic testing for hereditary deafness. *Am J Hum Genet* 1998;63:1175–1180.
85. Sankar P, Cho MK, Wolpe PR, Schairer C. What is in a cause? Exploring the relationship between genetic cause and felt stigma. *Genet Med* 2006; 8:33–42.
86. Baldwin EE, Boudreault P, Fox M, Sinsheimer JS, Palmer CG. Effect of pre-test genetic counseling for deaf adults on knowledge of genetic testing. *J Genet Couns* 2012;21:256–272.
87. Middleton A, Emery SD, Turner GH. Views, knowledge, and beliefs about genetics and genetic counseling among deaf people. *Sign Lang Studies* 2010;10:170–196.
88. Arnos KS, Cunningham M, Israel J, Marazita ML. Innovative approach to genetic counseling services for the deaf population. *Am J Med Genet* 1992;44: 345–351.
89. Israel J, Cunningham M, Thumann H, Arnos KS. Genetic counseling for deaf adults: Communication/language and cultural considerations. *J Genet Couns* 1992;1:135–153.
90. Bonnet C, Grati M, Marlin S, et al. Complete exon sequencing of all known Usher syndrome genes greatly improves molecular diagnosis. *Orphanet J Rare Dis* 2011;6:21.
91. Usami S, Nishio SY, Nagano M, Abe S, Yamaguchi T; Deafness Gene Study Consortium. Simultaneous screening of multiple mutations by invader assay improves molecular diagnosis of hereditary hearing loss: a multicenter study. *PLoS ONE* 2012;7:e31276.
92. Brown KK, Rehm HL. Molecular diagnosis of hearing loss. *Curr Protoc Hum Genet* 2012;72:9.16 .1-9.16.16.
93. Brownstein Z, Friedman LM, Shahin H, et al. Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. *Genome Biol* 2011;12:R89.
94. Shearer AE, DeLuca AP, Hildebrand MS, et al. Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. *Proc Natl Acad Sci USA* 2010;107:21104–21109.
95. Shearer AE, Hildebrand MS, Sloan CM, Smith RJ. Deafness in the genomics era. *Hear Res* 2011;282:1–9.
96. Sirmaci A, Edwards YJ, Akay H, Tekin M. Challenges in whole exome sequencing: an example from hereditary deafness. *PLoS ONE* 2012; 7:e32000.
97. GeneTests: Medical Genetics Information Resource (database online). University of Washington, Seattle, WA, 1993-2011 [serial online] 2012. http://www.genetests.org. Accessed 30 June 2011.
98. GTR: Genetic Testing Registry. National Center for Biotechnology Information, *US National Library of Medicine* [serial online] 2012;http://www.ncbi.nlm.nih.gov/gtr/. Accessed 25 September 2012.
99. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al.; Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med* 2013;15:733–747.
100. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med* 2013;15:565–574.
101. Boppana SB, Ross SA, Shimamura M, et al.; National Institute on Deafness and Other Communication Disorders CHIMES Study. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. *N Engl J Med* 2011;364:2111–2118.
102. Lombardi G, Garofoli F, Stronati M. Congenital cytomegalovirus infection: treatment, sequelae and follow-up. *J Matern Fetal Neonatal Med* 2010;23(suppl 3):45–48.
103. Rosenthal LS, Fowler KB, Boppana SB, et al. Cytomegalovirus shedding and delayed sensorineural hearing loss: results from longitudinal follow-up of children with congenital infection. *Pediatr Infect Dis J* 2009;28: 515–520.
104. Shin JJ, Keamy DG Jr, Steinberg EA. Medical and surgical interventions for hearing loss associated with congenital cytomegalovirus: a systematic review. *Otolaryngol Head Neck Surg* 2011;144:662–675.
105. Yamamoto AY, Mussi-Pinhata MM, Marin LJ, Brito RM, Oliveira PF, Coelho TB. Is saliva as reliable as urine for detection of cytomegalovirus DNA for neonatal screening of congenital CMV infection? *J Clin Virol* 2006; 36:228–230.
106. Cohen M, Phillips JA 3rd. Genetic approach to evaluation of hearing loss. *Otolaryngol Clin North Am* 2012;45:25–39.
107. Reardon W, OMahoney CF, Trembath R, Jan H, Phelps PD. Enlarged vestibular aqueduct: a radiological marker of pendred syndrome, and mutation of the PDS gene. *QJM* 2000;93:99–104.
108. Lai R, Hu P, Zhu F, et al. Genetic diagnosis and cochlear implantation for patients with nonsyndromic hearing loss and enlarged vestibular aqueduct. *J Laryngol Otol* 2012;126:349–355.
109. Lin JW, Chowdhury N, Mody A, et al. Comprehensive diagnostic battery for evaluating sensorineural hearing loss in children. *Otol Neurotol* 2011;32:259–264.
110. Madden C, Halsted M, Meinzen-Derr J, et al. The influence of mutations in the SLC26A4 gene on the temporal bone in a population with enlarged vestibular aqueduct. *Arch Otolaryngol Head Neck Surg* 2007;133:162–168.
111. Chiang CE. Congenital and acquired long QT syndrome. Current concepts and management. *Cardiol Rev* 2004;12:222–234.
112. Tranebjaerg L, Samson RA, Green GE. Jervell and Lange-Nielsen Syndrome. GeneReviews [serial online] 2010; PMID:20301579. University of Washington, Seattle, WA. http://www.ncbi.nlm.nih.gov/books/NBK1405. Accessed 15 June 2012.
113. Dyer JJ, Strasnick B, Jacobson JT. Teratogenic hearing loss: a clinical perspective. *Am J Otol* 1998;19:671–678.
114. Takemori S, Tanaka Y, Suzuki JI. Thalidomide anomalies of the ear. *Arch Otolaryngol* 1976;102:425–427.

Neurodevelopmental Manifestations of Mitochondrial Disease

Marni J. Falk, MD

ABSTRACT: Mitochondrial disease is an increasingly recognized but widely heterogeneous group of multisystemic disorders that commonly involve severe neurodevelopmental manifestations in childhood. This review explores the presentation, genetic basis, and diagnostic evaluation of primary mitochondrial disease. Emphasis is placed on neurodevelopmental findings that may be encountered by a Developmental Pediatrician that should provoke consideration of a mitochondrial disorder. The inheritance patterns and mechanisms by which mutations in genes located in either the nuclear or mitochondrial genomes can cause mitochondrial diseases are discussed. A general overview of the current diagnostic evaluation that can be readily initiated by the Developmental Pediatrician is provided, along with a summary of currently available treatment options.

(J Dev Behav Pediatr 31:610–621, 2010) **Index terms:** OXPHOS, mfDNA, nDNA, mitochondrial disease diagnosis.

OVERVIEW OF HUMAN MITOCHONDRIAL DISEASE

A recent development in the world of inherited disease has been the realization that dysfunction of the mitochondrial respiratory chain constitutes a frequent group of disorders that afflict all ages and ethnicities.[1,2] Indeed, mutations in hundreds of discrete genes—originating in either nuclear (nDNA) or mitochondrial (mtDNA) genomes—culminate in energy deficiency and organ failure. The five-complex respiratory chain (RC), also known as the electron transport chain, is the site of oxidative phosphorylation (OXPHOS) within the inner mitochondrial membrane in which the end products of intermediary metabolism are oxidized to generate energy in the form of adenosine triphosphate. *Primary* mitochondrial disease refers to disorders whose underlying genetic cause directly impairs RC composition or function. *Secondary* OXPHOS dysfunction, by contrast, has been described in a host of other genetic or environmental disorders, including other genetic disorders (i.e., Rett syndrome,[3] other metabolic defects,[4–7] chromosomal aneuploidies,[8,9]) or toxicities from drugs (i.e., valproate,[10] statins,[11] pesticides[12,13]). The minimal prevalence of primary mitochondrial disease is one in 5000,[1,2] although pathogenic mutations in mitochondrial DNA (mtDNA) may occur as frequently as one in 200 births.[14]

Primary RC (OXPHOS) dysfunction is commonly characterized by progressive, multisystemic involvement of high-energy demand tissues.[15] Indeed, clinical problems involving three or more systems should invoke consideration of mitochondrial disease into the differential diagnosis. Typical sequelae of RC dysfunction may involve almost any system: neurologic (central, peripheral, or autonomic nervous systems), heart (arrhythmias, cardiomyopathy), skeletal muscle (myopathy), eyes (eye movement abnormalities, vision loss due to retinal or optic nerve disease, ptosis), endocrine organs (diabetes mellitus, hypothyroidism, hypoparathyroidism, growth hormone deficiency, adrenal insufficiency), kidneys (renal tubular acidosis, aminoaciduria, nephropathy), gastrointestinal tract (liver failure, dysmotility), hearing (sensorineural hearing loss, vestibular dysfunction), and/or may cause global metabolic instability with susceptibility to infections and other catabolic stressors.[15] Each gene, and even mutation, implies a different disorder, hence the extensive variability in manifestations, prognosis, and inheritance characteristic of "mitochondrial disease."[16,17]

NEURODEVELOPMENTAL MANIFESTATIONS OF MITOCHONDRIAL DISEASE

Neurologic "Red-Flag" and "Nonspecific" Findings in Mitochondrial Disease

Neurodevelopmental abnormalities are common in primary mitochondrial disease and may present at any age. Table 1 lists both red flag neurologic findings that may present in infants and children with primary mitochondrial disease[15] and a wide range of nonspecific neurologic findings that often occur in combination in mitochondrial disease. By definition, nonspecific presentations also lead to a wide range of other primary diagnoses. Axonal neuropathy and/or autonomic nervous system involvement may occur in mitochondrial disease, although these presentations are more commonly diagnosed in older children and adults. Sensorineural hearing loss is a common finding that occurs in a wide range of

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To the Committee Members of the Health Evidence Review Commission:

My name is Devki Saraiya, and I am a licensed, certified genetic counselor at Myriad Genetic Laboratories.

In 2014, the National Comprehensive Cancer Network (NCCN) significantly updated their hereditary cancer guidelines: 1) Created the Genetic/Familial High-Risk Assessment: Colorectal guideline[1] and 2) updated the Genetic/Familial High-Risk Assessment: Breast and Ovarian guideline[2]. Given these updates, I am writing to respectfully comment on the Genetics Advisory Council's review of new genetics CPT codes for 2015.

Comment #1: Update Diagnostic Guideline D1, Non-Prenatal Genetic Testing Guideline Section A)1)a) as follows:

Lynch syndrome (hereditary colorectal and endometrial cancer) services (CPT 81288, 81292-81300, 81317-81319, 81435, 81436) and familial adenomatous polyposis (FAP) services (CPT 81201-81203) should be provided as defined by the NCCN Clinical Practice Guidelines in Oncology. ~~Colorectal Cancer Screening~~ Genetic/Familial High-Risk Assessment: Colorectal. V.2.2104. ***www.nccn.org***

Rationale: The current wording references the NCCN Colorectal Screening guidelines, which as of 5/19/2014 no longer incorporate testing criteria for Lynch syndrome/FAP. The NCCN has created a new guideline "Genetic/Familial High-Risk Assessment: Colorectal V.2.2014" which outlines criteria for Lynch syndrome/FAP services.

Comment #2: Update Diagnostic Guideline D1, Non-Prenatal Genetic Testing Guideline Section A)1)c) as follows:

BRCA1/BRCA2 testing services (CPT 81211-81217) for women with a personal history of breast and/or ovarian cancer and for men with breast cancer should be provided according to the NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High- Risk Assessment: Breast and Ovarian. ~~V.1.2011 (4/7/11)~~ V.2.2014. ***www.nccn.org***

Rationale: The current wording references the NCCN Genetic/ Familial High-Risk Assessment: Breast and Ovarian guideline from 2011. NCCN has made important updates to BRCA1/2 testing criteria including criteria clarification and incorporation of prostate cancer (Gleason score $\geq$7) in their Genetic/Familial High-Risk Assessment: Breast and Ovarian V.2.2014 guidelines. Given these updates and keeping in line with the GAC's recommendation to update to the NCCN's current hereditary colon guidelines, it is respectfully requested that the GAC utilize the most current version of the breast/ovarian NCCN guidelines.

Comment #3: Update Diagnostic Guideline D1, Non-Prenatal Genetic Testing Guideline Section A)1)b) as follows:

BRCA1/BRCA2 testing services (CPT 81211-81217) for women without a personal history of breast and/or ovarian cancer should be provided to high risk women as defined in Guideline Note 3 or as otherwise defined ~~by the US Preventive Services Task Force~~ by the NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast and Ovarian. V2.2014.

Rationale: Given proven clinical utility of BRCA1/2 gene testing in both affected and unaffected at-risk individuals, it would be beneficial to provide coverage for members based on current peer-reviewed evidence and expert opinion that is found in the NCCN practice guidelines since there are

significant clinical shortcomings when comparing USPSTF recommendations[3] (currently referenced in the recommendation) to NCCN guidelines:

a) Limiting assessment to first-degree relatives (FDR), as the USPSTF does, will miss a significant number of BRCA mutation carriers inheriting the mutation from their fathers since BRCA1/2 mutations are inherited in an autosomal dominant pattern with sex-limited expression[4]. With USPSTF, close paternal female relatives (paternal aunts, paternal grandmothers, paternal nieces) are not FDRs and their cancer history is not considered, while it is in the NCCN criteria.

b) Requiring ≥2 first-/second-degree relatives affected with ovarian cancer in order to be considered at increased risk, as the USPSTF does, will miss a significant number of BRCA mutations carriers. Numerous studies have been published proving the high prevalence of BRCA1/2 mutations among unselected ovarian cancer patients[5-7]. This issue is addressed in the NCCN guidelines.

c) Failing to include triple negative breast cancer (TNBC) as a risk factor for BRCA mutations, as the USPSTF does, will result in missed BRCA mutation carriers since BRCA1/2 mutation carriers have an increased incidence of TNBC[8-16]. This issue is addressed in the NCCN criteria.

Comment #4: Update Diagnostic Guideline D1, Non-Prenatal Genetic Testing Guideline Section A)2) as follows:

Genetic counseling should precede genetic testing for hereditary cancer. Very rarely, it may be appropriate for a genetic test to be performed prior to genetic counseling for a patient with cancer. If this is done, genetic counseling should be provided as soon as practical. a) Pre and post-test genetic counseling ~~by the following providers~~ should be covered when provided by suitably trained health professional with expertise and experience in cancer genetics.

~~i) Medical Geneticist (M.D.) - Board Certified or Active Candidate Status from the American Board of Medical Genetics~~

~~ii) Clinical Geneticist (Ph.D.) - Board Certified or Active Candidate Status from the American Board of Medical Genetics.~~

~~iii) Genetic Counselor - Board Certified or Active Candidate Status from the American Board of Genetic Counseling, or Board Certified by the American Board of Medical Genetics.~~

~~iv) Advance Practice Nurse in Genetics - Credential from the Genetic Nursing Credentialing Commission.~~

Rationale: The NCCN, multiple published guidelines[1-3, 17-23] and government health plans, including CMS, advocate for a variety of licensed healthcare provider (HCP) types to be able to identify/counsel/test patients at risk of hereditary cancers since several studies have demonstrated that <½ of patients referred for genetic counseling attend a session[24-29]. **The main clinical benefit of testing is the opportunity to prevent/reduce the risk of cancer, a disease with significant financial and economic burden to Oregon Medicaid, families, and society**. While genetics professionals are extremely important, and access to such providers should remain open, limiting access to genetic testing for high risk patients to ONLY those that have met with the above limited provider types will result in fewer mutation carriers identified.

Comment #5: Consider addition of hereditary cancer multi-gene panels to coverage policy

Rationale: The NCCN Genetic/Familial High-Risk Assessment: Breast and Ovarian practice guidelines now provide the option of either a targeted (single syndrome) approach, or a multi-gene (panel) as a first-line test, in evaluation of a high risk patient. According to NCCN, individuals who meet testing criteria for BRCA1/2 may consider either comprehensive BRCA1/2 testing or a multi-gene (panel) test given that multiple genes may contribute to breast/ovarian cancer, and there is

often overlap in the cancers associated with different genes. An advantage of the multi-gene panel test is the ability of a "broad and unbiased" testing approach to detect mutations that might otherwise have gone undetected. This reduction in uninformative negative results increases the number of patients who can be provided with tailored medical management recommendations.

Thank you in advance for your consideration of these comments. Please feel free to contact me if I can provide any additional information or answer any questions.

Warm regards,
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References:

1. National Comprehensive Cancer Network Practice Guidelines in Oncology Genetics/Familial High Risk Assessment: Colorectal, v.2.2014. Available at www.nccn.org.
2. National Comprehensive Cancer Network Practice Guidelines in Oncology Genetics/Familial High Risk Assessment: Breast and Ovarian, v.2.2014. Available at www.nccn.org.
3. Nelson HD, et al. Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-related Cancer in Women: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med*. 2014;160(4):255-66.
4. Weitzel JN, et al. Limited family structure and BRCA gene mutation status in single cases of breast cancer. *JAMA*. 2007 Jun 20;297(23):2587-95.
5. Pal T, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases.*Cancer*. 2005 Dec 15;104(12):2807-16.
6. Risch HA, McLaughlin JR, Cole DEC, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *American Journal of Human Genetics.* 2001;68:700-710.
7. Zhang S. et al. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. *Gynecol Oncol.* 2011 May 1;121(2):352-7.
8. Atchley DP et al. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J Clin Oncol 2008;26:4282-4288.
9. Chacon RD and Costanzo MV. Triple negative breast cancer. Breast Cancer Research. 2010;12 (Suppl 2):53-62.
10. Gonzalez-Angulo AM, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin Cancer Res 2011;17(5):1082-1089.
11. Hartman AR, et al. Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. Cancer Online 5 Oct, 2011.
12. Young SR, et al. The prevalence of BRCA1 mutations among young women with triple negative breast cancer. *BMC Cancer*. 2009 Mar 19;9:86.
13. Evans DG, et al. Prevalence of BRCA1 and BRCA2 mutations in triple negative breast cancer. J Med Genet 2011;48:520-522.
14. Fostira F, et al. Prevalence of BRCA1 mutations among 403 women with triple-negative breast cancer: implications for genetic screening selection criteria: a Hellenic Cooperative Oncology Group Study. Breast Cancer Res Treat 2012;134:352-362.

15. Comen E, et al. Relative contributions of BRCA1 and BRCA2 mutations to “triple-negative” breast cancer in Ashkenazi Women. Breast Cancer Res and Treat 2011;129:185-190.
16. Meyer P, et al. BRCA2 mutations and triple-negative breast cancer. PLoS One. 2012; 7(5): e38361.
17. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2009 Apr;113(4):957-66. ACOG Practice Bulletin No. 103: Hereditary breast and ovarian cancer syndrome.
18. Lancaster JM, Powell CD, Kauff ND et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol* 2007 Nov;107(2):159-62
19. Robson, M. et al. American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility. JCO February 10, 2010 vol. 28 no. 5 893-901.
20. National Accreditation Program for Breast Centers (NAPBC) 2011 Breast Center Standards Manual. http://accreditedbreastcenters.org/standards/2011standardsmanual.pdf
21. American Society of Breast Surgeons. BRCA Testing for Patients With and Without Breast Cancer. Revised Sept 30, 2012. https://www.breastsurgeons.org/statements/PDF_Statements/BRCA_Testing.pdf
22. American Academy of Physician Assistants (AAPA). Guidelines for Ethical Conduct for the Physician Assistant Profession. Revised 2008. http://www.aapa.org/images/stories/documents/about_aapa/policymanual/19-EthicalConduct.pdf
23. Oncology Nursing: The Application of Cancer Genetics and Genomics Throughout the Oncology Care Continuum http://www.ons.org/Publications/Positions/HealthCarePolicy
24. Pruski-Clark J et al. *National Interdisciplinary Breast Center Conference*. March 10-14, 2012.
25. Buchanan AH, et al. Cancer genetic counseling in rural North Carolina oncology clinics: program establishment and patient characteristics. *Community Oncology.2*009;6:70-77.
26. Cohen SA, et al. A Collaborative Approach to Genetic Testing: A Community Hospital’s Experience. *J Genet Couns.* 2009 Dec;18(6):530-3.
27. South CD, et al. Immunohistochemistry staining for the mismatch repair proteins in the clinical care of patients with colorectal cancer. *Genet Med.* 2009;11(11):812-7.
28. O'Neill SM, et al. Referral to cancer genetic counseling: are there stages of readiness? *Am J Med Genet C Semin Med Genet*. 2006 Nov 15;142(4):221-31.
29. Fowler ES, et al. Cancer genetic counseling: trends in patient referrals and genetic testing in a community-based program. *Community Oncology*. 2005;2: 253-260.

Definition of High Risk for Breast Cancer

Question: How should high risk of breast cancer be defined?

Question source: Genetics Advisory Panel

Issue: GN3 defines which patients are at high risk for breast cancer, and therefore eligible for prophylactic procedures such as mastectomy or oophorectomy. The specifications in GN3, and referred to in the non-prenatal genetic testing guideline, are based on USPSTF criteria in place at the time the guideline was created. In 2013, the USPSTF changed their criteria for defining high risk to a recommendation for using one of several screening tools (see below). The NCCN published their own criteria for defining high risk in their 2014 guideline Genetic/Familial High Risk Assessment: Breast and Ovarian. Both the revised USPSTF and NCCN risk groups vary from the current GN3 definition. Additionally, NCCN has published an extensive guideline regarding what procedures should be offered to women based on an algorithm of their risk, which includes genetics as well as multiple other factors.

As staff reviewed this topic, it was identified that the current guideline inappropriately allows prophylactic mastectomy and other treatments based on criteria for further genetic testing, not treatment.

The NCCN 2014 guideline regarding treatment of breast cancer contains this statement:

> Except as outlined in the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian and the NCCN Guidelines for Breast Cancer Risk Reduction, prophylactic mastectomy of a breast contralateral to a known unilateral breast cancer is discouraged.

Contralateral mastectomy is still recommended for consideration in cases of invasive lobular carcinoma.

From the USPSTF 2014 recommendations for genetic testing for breast cancer:

> For women who have at least 1 family member with breast, ovarian, or other types of BRCA-related cancer, primary care providers may use 1 of several brief familial risk stratification tools to determine the need for in-depth genetic counseling.
>
> Although several risk tools are available, the tools evaluated by the USPSTF include the Ontario Family History Assessment Tool, Manchester Scoring System, Referral Screening Tool, Pedigree Assessment Tool, and FHS-7. The Referral Screening Tool and FHS-7 are the simplest and quickest to administer. All of these tools seem to be clinically useful predictors of which women should be referred for genetic counseling due to increased risk for potentially harmful BRCA mutations (most sensitivity estimates were >85%), although some models have been evaluated in only 1 study. To determine which patients would benefit from BRCA risk assessment, primary care providers should not use general breast cancer risk assessment models (for example, the National Cancer Institute Breast Cancer

Risk Assessment Tool, which is based on the Gail model) because they are not designed to determine which women should receive genetic counseling or BRCA testing.

In general, these tools elicit information about factors that are associated with increased likelihood of BRCA mutations. Family history factors associated with increased likelihood of potentially harmful BRCA mutations include breast cancer diagnosis before age 50 years, bilateral breast cancer, presence of breast and ovarian cancer, presence of breast cancer in 1 or more male family members, multiple cases of breast cancer in the family, 1 or more family members with 2 primary types of BRCA-related cancer, and Ashkenazi Jewish ethnicity. The USPSTF recognizes that each risk assessment tool has limitations and found insufficient comparative evidence to recommend one tool over another. The USPSTF also found insufficient evidence to support a specific risk threshold for referral for testing.

Definition of who should be offered genetic testing for breast cancer syndrome rom the 2014 NCCN guideline for genetic testing for breast cancer:

An unaffected individual with a family history of one or more of the following:
-A known mutation in a breast cancer susceptibility gene within the family
-≥2 breast primaries in single individual
-≥2 individuals with breast primaries on the same side of family
-≥1 ovarian cancer primary from the same side of family
-First or second degree relative with breast cancer ≤45 y
-≥1 family member on same side of family with a combination of breast cancer and ≥1 of the following: pancreatic cancer, prostate cancer, sarcoma, adrenocortical carcinoma, brain tumors, endometrial cancer, leukemia/lymphoma; thyroid cancer, dermatologic manifestations and/or macrocephaly, hamartomatous polyps of GI tract; diffuse gastric cancer
-male breast cancer

Current guideline

GUIDELINE NOTE 3, PROPHYLACTIC TREATMENT FOR PREVENTION OF BREAST CANCER IN HIGH RISK WOMEN

Line 195

Bilateral prophylactic breast removal is included on Line 195 for women without a personal history of invasive breast cancer who are at high risk for breast cancer. Prior to surgery, women without a personal history of breast cancer must have a genetics consultation. High risk is defined as:

A) Having a BRCA1/BRCA2 mutation;
B) Having a strong family history of breast cancer, defined as one of the following:
    1) 2 first-degree or second degree relatives diagnosed with breast cancer at younger than an average age of 50 years (at least one must be a first-degree relative);
    2) 3 first-degree or second-degree relatives diagnosed with breast cancer at younger than an average age of 60 years (at least one must be a first-degree relative);

3) 4 relatives diagnosed with breast cancer at any age (at least one must be a first-degree relative);
4) 1 relative with ovarian cancer at any age and, on the same side of the family, either 1 first-degree relative (including the relative with ovarian cancer) or second-degree relative diagnosed with breast cancer at younger than age 50 years, or 2 first-degree or second-degree relatives diagnosed with breast cancer at younger than an average age of 60 years, or another ovarian cancer at any age;
5) 1 first-degree relative with cancer diagnosed in both breasts at younger than an average age of 50 years;
6) 1 first-degree or second-degree relative diagnosed with bilateral breast cancer and one first-degree or second-degree relative diagnosed with breast cancer at younger than an average age of 60 years; or,
7) a male relative with breast cancer at any age and on the same side of the family at least 1 first-degree or second-degree relative diagnosed with breast cancer at younger than age 50 years, or 2 first-degree or second-degree relatives diagnosed with breast cancer at younger than an average age of 60 years.

C) A history of LCIS with a family history of breast cancer; or,
D) A history of treatment with thoracic radiation between ages 10 and 30.

Contralateral prophylactic mastectomy is included on Line 195 for women with a personal history of breast cancer and any of the high risk categories listed above. In addition, contralateral prophylactic mastectomy of the unaffected breast is indicated for women with invasive lobular carcinoma.

Prophylactic oophorectomy is included on Line 195 for women who have the BRCA1/BRCA2 mutation.

Expert input:

Dr. Andy Higgens, breast surgeon, Bend

Growing trend nationwide to have contralateral mastectomy for decreasing patient anxiety and decreasing surveillance, despite lack of evidence of improved survival. Commercial insurers are not regulating contralateral mastectomy. The main benefit is peace of mind and symmetry, not medical survival or disease reduction. OHP will be an outlier if contralateral mastectomy is not covered by patient preference. Using risk groups to determine who should have contralateral mastectomy covered is problematic, as this is a rapidly developing area. Someone without a genetic abnormality or a 20% or greater risk of breast cancer would have contralateral mastectomy denied. This is not logical as this patient has more risk than the woman who has never had cancer but meets the NCCN requirement for bilateral prophylactic mastectomy.

Recommends coverage for contralateral mastectomy unless medically contraindicated. Would remove the second paragraph of the proposed new guideline.

Cost effective to do contralateral mastectomy in younger women given cost savings of avoiding years of mammograms and MRIs.

HERC staff recommendation:

1) Modify GN3 as shown below
   a) Wording in green is proposed for possible deletion/non-inclusion based on expert surgical input

[without track changes]

GUIDELINE NOTE 3, PROPHYLACTIC TREATMENT FOR PREVENTION OF BREAST CANCER IN HIGH RISK WOMEN

Line 195

Bilateral prophylactic breast removal and/or oophorectomy are included on Line 195 for women without a personal history of invasive breast cancer who meet the criteria in the NCCN Clinical Practice Guidelines in Oncology. Breast Cancer Risk Reduction. V.1.2014 (1/20/14). ***www.nccn.org***. Prior to surgery, women without a personal history of breast cancer must have a genetics consultation as defined in section A2 of the DIAGNOSTIC GUIDELINE D1, NON-PRENATAL GENETIC TESTING GUIDELINE.

Contralateral prophylactic mastectomy is included on Line 195 for women with a personal history of breast cancer and 1) a hereditary breast and/or ovarian cancer syndrome or 2) a lifetime risk of breast cancer >20%. In addition, contralateral prophylactic mastectomy of the unaffected breast is indicated for women with invasive lobular carcinoma.

GUIDELINE NOTE 3, PROPHYLACTIC TREATMENT FOR PREVENTION OF BREAST CANCER IN HIGH RISK WOMEN

Line 195

Bilateral prophylactic breast removal and/or oophorectomy ~~is~~ are included on Line 195 for women without a personal history of invasive breast cancer who meet the criteria in the NCCN Clinical Practice Guidelines in Oncology. Breast Cancer Risk Reduction. V.1.2014 (1/20/14). ***www.nccn.org***. ~~are at high risk for breast cancer.~~ Prior to surgery, women without a personal history of breast cancer must have a genetics consultation as defined in section A2 of the DIAGNOSTIC GUIDELINE D1, NON-PRENATAL GENETIC TESTING GUIDELINE. ~~High risk is defined as~~

~~A) Having a BRCA1/BRCA2 mutation;~~
~~B) Having a strong family history of breast cancer, defined as one of the following:~~
   ~~1) 2 first-degree or second degree relatives diagnosed with breast cancer at younger than an average age of 50 years (at least one must be a first-degree relative);~~
   ~~2) 3 first-degree or second-degree relatives diagnosed with breast cancer at younger than an average age of 60 years (at least one must be a first-degree relative);~~
   ~~3) 4 relatives diagnosed with breast cancer at any age (at least one must be a first-degree relative);~~
   ~~4) 1 relative with ovarian cancer at any age and, on the same side of the family, either 1 first-degree relative (including the relative with ovarian cancer) or second-degree relative diagnosed with breast cancer at younger than age 50 years, or 2 first-degree or second-degree relatives diagnosed with breast cancer at younger than an average age of 60 years, or another ovarian cancer at any age;~~

5) ~~1 first-degree relative with cancer diagnosed in both breasts at younger than an average age of 50 years;~~
6) ~~1 first-degree or second-degree relative diagnosed with bilateral breast cancer and one first-degree or second-degree relative diagnosed with breast cancer at younger than an average age of 60 years; or,~~
7) ~~a male relative with breast cancer at any age and on the same side of the family at least 1 first-degree or second-degree relative diagnosed with breast cancer at younger than age 50 years, or 2 first-degree or second-degree relatives diagnosed with breast cancer at younger than an average age of 60 years.~~

C) ~~A history of LCIS with a family history of breast cancer; or,~~
D) ~~A history of treatment with thoracic radiation between ages 10 and 30.~~

Contralateral prophylactic mastectomy is included on Line 195 for women with a personal history of breast cancer and 1) a hereditary breast and/or ovarian cancer syndrome or 2) a lifetime risk of breast cancer >20%. ~~any of the high risk categories listed above~~. In addition, contralateral prophylactic mastectomy of the unaffected breast is indicated for women with invasive lobular carcinoma.

~~Prophylactic oophorectomy is included on Line 195 for women who have the BRCA1/BRCA2 mutation.~~

Final Recommendation Statement

BRCA-related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing

Recommendations made by the USPSTF are independent of the U.S. government. They should not be construed as an official position of the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services.

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Recommendation Summary

Summary of Recommendations and Evidence

Population	Recommendation	Grade (What's This?)
Women who have Family Members with Breast, Ovarian, Tubal, or Peritoneal Cancer	The USPSTF recommends that primary care providers screen women who have family members with breast, ovarian, tubal, or peritoneal cancer with 1 of several screening tools designed to identify a family history that may be associated with an increased risk for potentially harmful mutations in breast cancer susceptibility genes	The USPSTF recommends the service. There is high certainty that the net benefit is moderate or

Population	Recommendation	Grade (What's This?)
	(*BRCA1* or *BRCA2*). Women with positive screening results should receive genetic counseling and, if indicated after counseling, BRCA testing.	there is moderate certainty that the net benefit is moderate to substantial.
Women Whose Family History is not Associated with an Increased Risk	The USPSTF recommends against routine genetic counseling or BRCA testing for women whose family history is not associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes.	The USPSTF recommends against the service. There is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits

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Preface

U.S. Preventive Services Task Force Recommendation Statement

Release Date: December 2013

The U.S. Preventive Services Task Force (USPSTF) makes recommendations about the effectiveness of specific preventive care services for patients without related signs or symptoms.

It bases its recommendations on the evidence of both the benefits and harms of the service and an assessment of the balance. The USPSTF does not consider the costs of providing a service in this assessment.

The USPSTF recognizes that clinical decisions involve more considerations than evidence alone. Clinicians should understand the evidence but individualize decision making to the specific patient or situation. Similarly, the USPSTF notes that policy and coverage decisions involve considerations in addition to the evidence of clinical benefits and harms.

This article was first published in *Annals of Internal Medicine* on 24 December 2013. Select for copyright and source information.

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Rationale

Importance

The cancer types related to potentially harmful mutations of the BRCA genes are predominantly breast, ovarian, and fallopian tube cancer, although other types are also associated[1]. In the general population, 12.3% of women will develop breast cancer during their lifetime and 2.74% will die of the disease, whereas 1.4% of women will develop ovarian cancer and 1.0% will die of the disease[2]. A woman's risk for breast cancer increases to 45% to 65% by age 70 years if there are clinically significant mutations in either BRCA gene[3, 4]. Mutations in the *BRCA1* gene increase ovarian cancer risk to 39% by age 70 years, and *BRCA2* mutations increase ovarian cancer risk to 10% to 17% by age 70 years[3, 4]. In the general population, these mutations occur in an estimated 1 in 300 to 500 women (0.2% to 0.3%)[5-8]. In a meta-analysis conducted for the USPSTF, the combined prevalence of *BRCA1* and *BRCA2* mutations was 2.1% in a general population of Ashkenazi Jewish women[9].

Detection of Potentially Harmful BRCA Mutations

Genetic risk assessment and BRCA mutation testing is generally a multistep process involving identification of individuals who may be at increased risk for potentially harmful mutations, followed by genetic counseling from suitably trained health care providers and genetic testing of selected high-risk individuals when indicated. Several familial risk stratification tools are clinically useful for selecting patients who should be offered genetic counseling to further determine their candidacy for possible BRCA mutation testing.

Benefits of Testing for Potentially Harmful Mutations

For women whose family history is associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, adequate evidence suggests that the benefits of testing for potentially harmful BRCA mutations are moderate.

For women whose family history is not associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, there is adequate evidence that the benefits of testing for potentially harmful BRCA mutations are few to none.

Harms of Detection of Potentially Harmful BRCA Mutations and Early Intervention and Treatment

Adequate evidence suggests that the overall harms of detection of and early intervention for potentially harmful BRCA mutations are small to moderate.

USPSTF Assessment

For women whose family history is associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, there is moderate certainty that the net benefit of testing for potentially harmful BRCA mutations and early intervention is moderate.

For women whose family history is not associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, there is moderate certainty that the net benefit of testing for potentially harmful BRCA mutations and early intervention ranges from minimal to potentially harmful.

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Clinical Considerations

Patient Population Under Consideration

This recommendation applies to asymptomatic women who have not been diagnosed with BRCA-related cancer.

Women who have 1 or more family members with a known potentially harmful mutation in the *BRCA1* or *BRCA2* genes should be offered genetic counseling and testing.

The USPSTF recognizes the potential importance of further evaluating women who have a diagnosis of breast or ovarian cancer. Some women receive genetic testing as part of a cancer evaluation at the time of diagnosis of breast cancer. The USPSTF did not review the appropriate use of BRCA testing in the evaluation of women who are newly diagnosed with breast cancer. That assessment is part of disease management and is beyond the scope of this recommendation. Women who have been diagnosed with breast cancer in the past and who did not receive BRCA testing as part of their cancer care but have a family history of breast or ovarian cancer should be encouraged to discuss further evaluation with their clinician.

These recommendations do not apply to men, although male family members may be identified for testing during evaluation.

Family History Screening and Risk Assessment

Mutations in the BRCA genes cluster in families, exhibiting an autosomal dominant pattern of transmission in maternal or paternal lineage. During standard elicitation of family history information from patients, primary care providers should ask about specific types of cancer, primary cancer sites, which family members were affected, relatives with multiple types of primary cancer, and the age at diagnosis and sex of affected family members.

For women who have at least 1 family member with breast, ovarian, or other types of BRCA-related cancer, primary care providers may use 1 of several brief familial risk stratification tools to determine the need for in-depth genetic counseling.

Although several risk tools are available, the tools evaluated by the USPSTF include the Ontario Family History Assessment Tool (Table 1), Manchester Scoring System (Table 2), Referral Screening Tool (Table 3), Pedigree Assessment Tool (Table 4), and FHS-7 (Table 5)[10-19]. The Referral Screening Tool (an updated version, the B-RST, is available at www.breastcancergenescreen.orgThis link goes offsite. Click to read the external link disclaimer and FHS-7 are the simplest and quickest to administer. All of these tools seem to be clinically useful predictors of which women should be referred for genetic counseling due to increased risk for potentially harmful BRCA mutations (most sensitivity estimates were >85%), although some models have been evaluated in only 1 study[9, 20]. To determine which patients would benefit from BRCA risk assessment, primary care providers should not use general breast cancer risk assessment models (for example, the National Cancer Institute Breast Cancer Risk Assessment Tool, which is based on the Gail model) because they are not designed to determine which women should receive genetic counseling or BRCA testing.

In general, these tools elicit information about factors that are associated with increased likelihood of BRCA mutations. Family history factors associated with increased likelihood of potentially harmful BRCA mutations include breast cancer diagnosis before age 50 years, bilateral breast cancer, presence of breast and ovarian cancer, presence of breast cancer in 1 or more male family members, multiple cases of breast cancer in the family, 1 or more family members with 2 primary types of BRCA-related cancer, and Ashkenazi Jewish ethnicity. The USPSTF recognizes that each risk assessment tool has limitations and found insufficient comparative evidence to recommend one tool over another. The USPSTF also found insufficient evidence to support a specific risk threshold for referral for testing.

Genetic Counseling

Genetic counseling about BRCA mutation testing may be done by trained health professionals, including trained primary care providers. Several professional organizations describe the skills and training necessary to provide comprehensive genetic counseling. The process of genetic counseling includes detailed kindred analysis and risk assessment for potentially harmful BRCA mutations; education about the possible results of testing and their implications; identification of affected family members who may be preferred candidates for testing; outlining options for screening, risk-reducing medications, or surgery for eligible patients; and follow-up counseling for interpretation of test results.

BRCA Mutation Testing

Adequate evidence suggests that current genetic sequencing tests can accurately detect BRCA mutations. Testing for BRCA mutations should be done only when an individual has personal or family history that suggests an inherited cancer susceptibility, when an individual has access to a health professional who is trained to provide genetic counseling and interpret test results, and when test results will aid in decision making. Initial testing of a family member who has breast

or ovarian cancer is the preferred strategy in most cases, but it is reasonable to test if no affected relative is available. It is essential that before testing, the individual is fully informed about the implications of testing and has expressed a desire for it.

The type of mutation analysis required depends on family history. Individuals from families with known mutations or from ethnic groups in which certain mutations are more common (for example, Ashkenazi Jewish women) can be tested for these specific mutations.

Individuals without linkages to families or groups with known mutations receive more comprehensive testing. In these cases, when possible, testing should begin with a relative who has breast or ovarian cancer to determine whether affected family members have a clinically significant mutation.

Tests for BRCA mutations are highly sensitive and specific for known mutations, but interpretation of results is complex and generally requires posttest counseling. Test results for genetic mutations are reported as positive (that is, potentially harmful mutation detected), variants of uncertain clinical significance, uninformative-negative, or true-negative. Women who have relatives with known BRCA mutations can be reassured about their inherited risk for a potentially harmful mutation if the results are negative (that is, a true negative). Some studies suggest increased breast cancer risk in some women with true-negative results[21-24]. However, a comprehensive meta-analysis conducted for the USPSTF that included these studies found that breast cancer risk is generally not increased in women with true-negative results[9]. An uninformative-negative result occurs when a woman's test does not detect a potentially harmful mutation but no relatives have been tested or no mutations have been detected in tested relatives. Available tests may not be able to identify mutations in these families. Risk for breast cancer is increased in women with uninformative-negative results[9].

Timing of Screening

Consideration of screening for potentially harmful BRCA mutations should begin once women have reached the age of consent (18 years). Primary care providers should periodically assess all patients for changes in family history (for example, comprehensive review at least every 5 to 10 years[25]).

Interventions for Women Who Are BRCA Mutation Carriers

Interventions that may reduce risk for cancer or cancer-related death in women who are BRCA mutation carriers include earlier, more frequent, or intensive cancer screening; risk-reducing medications (for example, tamoxifen or raloxifene); and risk-reducing surgery (for example, mastectomy or salpingo-oophorectomy). However, the strength of evidence varies across the types of interventions.

Evidence is lacking on the effect of intensive screening for BRCA-related cancer on clinical outcomes in women who are BRCA mutation carriers. Medications, such as tamoxifen and raloxifene, have been shown to reduce the incidence of invasive breast cancer in high-risk

women in the general population, but they have not been studied specifically in women who are BRCA mutation carriers[9, 20, 26].

In high-risk women and those who are BRCA mutation carriers, cohort studies of risk-reducing surgery (mastectomy and salpingo-oophorectomy) showed substantially reduced risk for breast or ovarian cancer. Breast cancer risk was reduced by 85% to 100% with mastectomy[27-29] and by 37% to 100% with oophorectomy, and ovarian cancer risk was reduced by 69% to 100% with oophorectomy or salpingo-oophorectomy[26]. Salpingo-oophorectomy was also associated with a 55% relative reduction in all-cause mortality (as measured during the course of the study) in women with *BRCA1* or *BRCA2* mutations and without a history of breast cancer[27].

Other Approaches to Prevention

The USPSTF recommendations on medications for breast cancer risk reduction are available on the USPSTF Web site (www.uspreventiveservicestaskforce.org).

The USPSTF recommends against screening for ovarian cancer in women. This recommendation does not apply to women with known genetic mutations that increase their risk for ovarian cancer (for example, BRCA mutations).

Useful Resources

The National Cancer Institute Cancer Genetics Services Directory provides a list of professionals who offer services related to cancer genetics, including cancer risk assessment, genetic counseling, and genetic susceptibility testing (available at http://www.cancer.gov/cancertopics/genetics/directoryThis link goes offsite. Click to read the external link disclaimer).

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Other Considerations

Although some studies have reported that women prefer in-person genetic counseling, telephone- or computer-based counseling may be considered for women who would not otherwise have access to these services.

Research Needs and Gaps

Research on risk assessment and testing for BRCA mutations has focused on short-term outcomes for highly selected women in referral centers. Additional studies are needed, including comparative effectiveness trials of approaches to risk screening and strategies to improve access to genetic counseling and BRCA testing for high-risk individuals.

Another unresolved question is what specific training is needed (for persons other than trained genetic counselors) to provide genetic counseling. It would be helpful to understand which

methods of delivery of genetic counseling are most effective, including those that can increase access to genetic counseling in rural or other settings. Trials comparing types of providers and protocols could address these questions.

What happens after patients are identified as high-risk in clinical settings is unknown. The consequences of genetic testing for individuals and their relatives require more study. Well-designed investigations using standardized measures and diverse study populations are needed.

An expanded database or registry of patients receiving genetic counseling for inherited breast and ovarian cancer susceptibility or who are tested for BRCA mutations would provide useful information about predictors of cancer and response to interventions. Additional data are needed from women of varying socioeconomic, racial, and ethnic groups.

For women who are mutation carriers, studies about the effectiveness of intensive cancer screening and risk-reducing medications and the effects of age at intervention on improving long-term outcomes are needed. This research would increase knowledge of the relative benefits and harms of interventions that are provided on the basis of genetic risk information.

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Discussion

Burden of Disease

Breast cancer is the second most common cancer in women in the United States and is the second leading cause of cancer death[30, 31]. In 2013, an estimated 232,340 women in the United States will be diagnosed with breast cancer and 39,620 women will die of the disease[32]. According to lifetime risk estimates for the general population, 12.3% of women will develop breast cancer during their lives and 2.74% will die of it[2].

Ovarian cancer is the fifth leading cause of cancer death in women in the United States[31], accounting for an estimated 22,240 new cases and 14,030 deaths in 2013[33]. According to lifetime risk estimates for the general population, 1.4% of women will develop ovarian cancer during their lives and 1.0% will die of it[2].

Estimates of the prevalence of potentially harmful BRCA mutations vary by population. The estimated prevalence is 0.2% to 0.3% in the general population of women[5-8], 6.0% in women with cancer onset before age 40 years[8, 34, 35], and 2.1% in the general population of Ashkenazi Jewish women[36-39]. In a meta-analysis of studies in which recruitment was based on family history of breast or ovarian cancer, *BRCA1* mutation prevalence was 13.6%, *BRCA2* mutation prevalence was 7.9%, and prevalence of either mutation was 19.8%[9].

Scope of Review

This recommendation applies to women who have no signs or symptoms of BRCA-related cancer. For its updated evidence review, the USPSTF considered risk assessment, genetic

counseling, and genetic testing for potentially harmful *BRCA1* or *BRCA2* mutations in asymptomatic women with a family history of breast or ovarian cancer but no personal history of cancer or known potentially harmful BRCA mutations in their family. The USPSTF also reviewed interventions aimed at reducing the risk for BRCA-related cancer in women with potentially harmful BRCA mutations, including intensive cancer screening (for example, earlier and more frequent mammography or magnetic resonance imaging of the breast), medications (for example, tamoxifen or raloxifene), and risk-reducing surgery (for example, mastectomy or oophorectomy). Studies about patients with current or past breast or ovarian cancer were excluded unless they were designed to address screening issues in women without cancer (for example, retrospective or case–control studies).

Accuracy of Familial Risk Assessment

The USPSTF reviewed several tools that could be used in primary care settings to predict individual risk for breast cancer and potentially harmful BRCA mutations.

Tools specifically designed to determine risk for BRCA-related cancer are primarily intended for use by nongeneticist health care providers to guide referral to genetic counselors for more definitive evaluation. Models that have been validated in studies include the Ontario Family History Assessment Tool (Table 1), Manchester Scoring System (Table 2), Referral Screening Tool (Table 3), Pedigree Assessment Tool (Table 4), and FHS-7 (Table 5)[10-19]. In general, these tools elicit information about factors associated with increased likelihood of BRCA mutations. They are clinically useful predictors of which women should be referred for genetic counseling because of increased risk for potentially harmful BRCA mutations (most sensitivity estimates were >85%), although some models have been evaluated in only 1 study[9, 20]. The USPSTF recognizes that each risk assessment tool has limitations and found insufficient evidence to recommend one tool over another.

Accuracy of BRCA Mutation Testing

The type of mutation analysis done depends on family history. Individuals from families with known mutations or from ethnic groups with common mutations (for example, Ashkenazi Jewish women) can be tested specifically for these mutations. The sensitivity and specificity of analysis techniques are measured by individual clinical laboratories and are not publicly available. Individuals without linkages to families or groups with known mutations receive more comprehensive testing. In these cases, guidelines recommend initial testing of a relative with known breast or ovarian cancer, when possible, to check for the presence of clinically significant mutations.

Effectiveness of BRCA Mutation Testing and Early Detection and Treatment

To understand the potential benefits and harms of genetic counseling, the USPSTF reviewed 18 studies[40-57] published since its previous review. Studies generally reported positive (or no negative) psychological effects, increased accuracy of risk perception, or decreased intention to have genetic testing.

Genetic counseling significantly decreased breast cancer worry in 8 studies[44–46, 48, 50, 53–55]. Three studies[41, 44, 49] reported decreased or no changes in general anxiety and depression after genetic counseling, whereas other studies found no significant differences in anxiety scores[48, 50]. However, 1 of these studies noted an increase in state anxiety scores after genetic counseling[44]. Eight studies published since 2004 reported improved accuracy of risk perception after genetic counseling[41, 42, 44-47, 49, 50, 52]. Two studies reported decreased intention to have genetic testing after genetic counseling[45, 46].

Interventions that may reduce risk for cancer in women who are BRCA mutation carriers include: earlier, more frequent, or intensive cancer screening; use of selective estrogen receptor modulators as risk-reducing medications (for example, tamoxifen or raloxifene); and risk-reducing surgery (for example, mastectomy or salpingo-oophorectomy).

Evidence is lacking on the effect of intensive screening for BRCA-related cancer on clinical outcomes in women who are BRCA mutation carriers.

Selective estrogen receptor modulators reduced the incidence of invasive breast cancer in several randomized, controlled trials[58-64], although clinical trials of tamoxifen and raloxifene have not been conducted specifically in women who are BRCA mutation carriers. In a meta-analysis of trials published to date[26, 65], tamoxifen and raloxifene reduced the incidence of estrogen receptor–positive invasive breast cancer, with 7 fewer events per 1000 women for tamoxifen (4 trials) and 9 fewer events per 1000 women for raloxifene (2 trials), assuming 5 years of treatment. Selective estrogen receptor modulators do not reduce risk for estrogen receptor–negative breast cancer, which includes 69% of breast cancer cases associated with *BRCA1* mutations and 16% associated with *BRCA2* mutations[66].

In cohort studies of high-risk women and those who are BRCA mutation carriers, risk-reducing surgery (for example, mastectomy or salpingo-oophorectomy) substantially reduced risk for breast or ovarian cancer. Mastectomy reduced breast cancer risk by 85% to 100%, and oophorectomy or salpingo-oophorectomy reduced ovarian cancer risk by 69% to 100% and breast cancer risk by 37% to 100%[9]. In 1 fair-quality prospective cohort study[27], salpingo-oophorectomy was also associated with a 55% relative reduction in all-cause mortality (as measured during the course of the study) in women with *BRCA1* and *BRCA2* mutations without a history of breast cancer. Breast cancer risk reduction associated with oophorectomy was more pronounced in women who were premenopausal at the time of surgery[27, 67].

Potential Harms of Cancer Screening and Treatment

Intensive screening for breast and ovarian cancer is associated with false-positive results, unnecessary imaging, and unneeded surgery. In 2 studies comparing mammography with magnetic resonance imaging for breast cancer screening in which 18% to 100% of study participants were BRCA mutation carriers, mammography was associated with higher false-positive rates (14% vs. 5.5% in the first round of screening; $P < 0.001$[68]; 15% vs. 11% in another study[69]) and more false-negative results (12 vs. 1 case in the first round of screening; 12 vs. 4 cases in subsequent rounds[68]. In a retrospective analysis of a cohort of women with potentially harmful BRCA mutations or first-degree relatives with BRCA mutations, those who were

screened with mammography were more likely to have unneeded imaging than those who were screened with magnetic resonance imaging; however, rates of unneeded biopsy were similar[69].

Risk-reducing medications (for example, tamoxifen or raloxifene) can increase risk for thromboembolic events (4 to 7 events per 1000 women over 5 years). Tamoxifen increased the risk for endometrial cancer (4 to 5 cases per 1000 women) compared with placebo or raloxifene, and it also increased risk for cataracts (15 per 1000 women) compared with raloxifene[26, 65].

Data on the long-term physical harms of risk-reducing mastectomy are limited. In high-risk women having risk-reducing mastectomy with immediate reconstruction, 21% in 1 series had complications (for example, hematoma, contracture, or implant rupture)[70]. In another series, 64% reported postsurgical symptoms (for example, numbness, pain, tingling, infection, swelling, breast hardness, bleeding, organizing hematoma, failed reconstruction, breathing problems, thrombosis, and pulmonary embolism)[71]. After risk-reducing oophorectomy, 5% of women in 1 study had postsurgical complications (for example, wound infection, bladder or uterine perforation, or small-bowel obstruction)[72].

Seven observational studies provided data on psychological distress due to risk-reducing mastectomy[71, 73-76] or oophorectomy[25, 77]. In 1 study of 90 women who had risk-reducing bilateral mastectomy[73, 74], there were significant reductions in scores for anxiety and sexual pleasure and no significant differences in depression scores, body image concerns, or other measures. In another study[75], there were no significant differences in psychological measures between women who had risk-reducing mastectomy and a reference sample that did not have the procedure. Ten years after risk-reducing mastectomy, most women in another study reported that their family lives were unchanged, but 39% reported negative effects on spousal relationships because of decreased sensation and changed body appearance[76]. After risk-reducing salpingo-oophorectomy, premenopausal women reported significant worsening of vasomotor symptoms and decreased sexual function[77].

Estimate of Magnitude of Net Benefit

For women whose family history is associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, the USPSTF found adequate evidence that the benefits of testing, detection, and early intervention are moderate. For women whose family history is not associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, the USPSTF found adequate evidence that the benefits of testing, detection, and early intervention are few to none. The USPSTF found adequate evidence that the overall harms of testing, detection, and early intervention are small to moderate.

For women whose family history is associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, the USPSTF concludes with moderate certainty that the net benefit of testing, detection, and early intervention is moderate. For women whose family history is not associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes, the USPSTF concludes with moderate certainty that the net benefit of testing, detection, and early intervention ranges from minimal to potentially harmful.

How Does Evidence Fit With Biological Understanding?

The *BRCA1* and *BRCA2* genes are tumor suppressor genes. Mutations of these genes have been linked to hereditary breast and ovarian cancer. Risks for breast, ovarian, and other types of BRCA-related cancer are greatly increased in patients who have inherited potentially harmful *BRCA1* or *BRCA2* mutations. Genetic testing may identify such mutations. Several options are available to manage cancer risk in patients who are found to be mutation carriers.

Response to Public Comments

A draft version of this recommendation statement was posted for public comment on the USPSTF Web site from 2 April through 29 April 2013. In response to comments, the USPSTF clarified that this recommendation statement applies to women. It also expanded the recommendation to include women who have family members with tubal or peritoneal (in addition to breast or ovarian) cancer. The USPSTF clarified that it recognizes the potential importance of further evaluating women who have a diagnosis of breast or ovarian cancer; however, that assessment is part of disease management and is beyond the scope of this recommendation.

The USPSTF added that it found insufficient evidence to recommend one risk assessment tool over another or to support a specific risk threshold for referral for genetic counseling and BRCA testing. It also added a compilation of risk assessment tools (Tables 1 to 5). Although the preferred BRCA testing strategy is initial testing of a family member with breast or ovarian cancer, the USPSTF clarified that it is reasonable to start testing in an unaffected individual if no affected relative is available. Because of the complexity of BRCA test results, the USPSTF also suggests posttest counseling. It also clarified and updated information on BRCA testing, other resources, and recommendations of other groups.

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Update of Previous USPSTF Recommendation

In 2005, the USPSTF recommended that women whose family history is associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes be referred for genetic counseling and evaluation for BRCA testing. It also recommended against routine referral for genetic counseling or routine BRCA testing for women whose family history is not associated with an increased risk for potentially harmful mutations in the *BRCA1* or *BRCA2* genes[78].

This recommendation statement reaffirms the USPSTF's previous recommendation. Since 2005, family history risk stratification tools have been developed and validated for use in primary care practice to guide referral for BRCA genetic counseling (Tables 1 to 5). In addition, the potential benefits and harms of medications for breast cancer risk reduction have been studied for longer follow-up periods, and more information is available about the potential psychological effects of genetic counseling and risk-reducing surgery.

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Recommendations of Other Groups

The National Comprehensive Cancer Network provides specific criteria for genetic counseling and testing[1]. The American Congress of Obstetricians and Gynecologists recommends genetic risk assessment for women who have more than a 20% to 25% risk for an inherited predisposition to breast and ovarian cancer and states that it may be helpful for patients with more than a 5% to 10% risk[79]. The American Society of Clinical Oncology recommends genetic testing when there is personal or family history suggestive of genetic cancer susceptibility, the test can be adequately interpreted, and the results will aid in diagnosis or medical management of the patient or family member who has hereditary risk for cancer. It also recommends genetic testing only when pretest and posttest counseling are included[80]. The National Society of Genetic Counselors has issued practice guidelines for risk assessment and genetic counseling for hereditary breast and ovarian cancer. It recommends that genetic testing should be offered to individuals with a personal or family history suggestive of an inherited cancer syndrome, when the test can be adequately interpreted, if testing will influence medical management of the patient or relative, when potential benefits outweigh potential risks, if testing is voluntary, and when the individual seeking testing or a legal proxy can provide informed consent[81]. The European Society for Medical Oncology recommends that all patients who may be referred for BRCA testing should first complete informed consent and genetic counseling and patients who are mutation carriers should be encouraged to advise close family members to obtain genetic counseling[82]. The Society of Gynecologic Oncologists recommends genetic risk assessment for individuals with a personal risk of more than approximately 20% to 25% for an inherited predisposition to cancer and states that it may be helpful for patients with more than approximately 5% to 10% risk. Genetic testing for cancer predisposition requires informed consent that should encompass pretest education and counseling about the risks, benefits, and limitations of testing, including the implications of both positive and negative genetic test results[83].

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Members of the U.S. Preventive Services Task Force

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Table 1. Ontario Family History Assessment Tool

Table 1. Ontario Family History Assessment Tool*

Risk Factor	Points
Breast and ovarian cancer	
Mother	10
Sibling	7
Second-/third-degree relative	5
Breast cancer relative	

Parent	4
Sibling	3
Second-/third-degree relative	2
Male relative (add to above)	2
Breast cancer characteristics	
Onset at age 20–29 y	6
Onset at age 30–39 y	4
Onset at age 40–49 y	2
Premenopausal/perimenopausal	2
Bilateral/multifocal	3
Ovarian cancer relative	
Mother	7
Sibling	4
Second-/third-degree relative	3
Age at ovarian cancer onset	
<40 y	6
40–60 y	4
>60 y	2
Age at prostate cancer onset	
<50 y	1
Age at colon cancer onset	
<50 y	1
Family total	
Referral†	≥10

* From reference [19].

† Referral with a score of ≥10 corresponds to doubling of lifetime risk for breast cancer (22%).

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Table 2. Manchester Scoring System

Table 2. Manchester Scoring System*

Risk Factor	*BRCA1* Score	*BRCA2* Score
Age at onset of female breast cancer†		
<30 y	6	5

30–39 y	4	4
40–49 y	3	3
50–59 y	2	2
≥60 y	1	1
Age at onset of male breast cancer†		
<60 y	5‡	8§
≥60 y	5‡	5§
Age at onset of ovarian cancer†		
<60 y	8	5
≥60 y	5	5
Pancreatic cancer	0	1
Age at onset of prostate cancer†		
<60 y	0	2
≥60 y	0	1

* From reference [13]. Developed so that a score of 10 in either column or a combined score of 15 for both columns would be equivalent to a 10% chance of identifying a *BRCA1* or *BRCA2* mutation.

† For relatives in direct lineage.

‡ If *BRCA2* tested.

§ If *BRCA1* tested.

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Table 3. Referral Screening Tool

Table 3. Referral Screening Tool*

Risk Factor	Breast Cancer at Age ≤50 y	Ovarian Cancer at Any Age
Yourself		
Mother		
Sister		
Daughter		
Mother's side		
Grandmother		
Aunt		

Father's side		
Grandmother		
Aunt		
≥2 cases of breast cancer after age 50 y on the same side of the family		
Male breast cancer at any age in any relative		
Jewish ancestry		

* From reference [16]. A patient completes the checklist if she has a family history of breast or ovarian cancer and receives a referral if she checks ≥2 items.

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Table 4. Pedigree Assessment Tool

Table 4. Pedigree Assessment Tool*

Risk Factor	**Score†**
Breast cancer at age ≥50 y	3
Breast cancer at age <50 y	4
Ovarian cancer at any age	5
Male breast cancer at any age	8
Ashkenazi Jewish heritage	4

* From reference [17]. A score of ≥8 is the optimum referral threshold.

† For every family member with a breast or ovarian cancer diagnosis, including second- or third-degree relatives.

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Table 5. FHS-7

Did any of your first-degree relatives have breast or ovarian cancer?
Did any of your relatives have bilateral breast cancer?
Did any man in your family have breast cancer?
Did any woman in your family have breast and ovarian cancer?
Did any woman in your family have breast cancer before age 50 y?
Do you have 2 or more relatives with breast and/orovarian cancer?
Do you have 2 or more relatives with breast and/or bowel cancer?

* From reference [18]. One positive response initiates referral.

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Evidence Synthesis

Number 101

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Structured Abstract

Purpose: To review new evidence on the benefits and harms of risk assessment, genetic counseling, and genetic testing for BRCA-related cancer in women for the U.S. Preventive Services Task Force.

Data Sources: MEDLINE and PsycINFO (January 2002 to December 31, 2012), Cochrane Central Register of Controlled Trials and Cochrane Database of Systematic Reviews (4th Quarter 2012), Scopus, and reference lists were searched for English-language studies of benefits and harms of risk assessment, genetic counseling, genetic testing, and interventions to reduce BRCA-related cancer and mortality.

Data Synthesis: Thirteen general risk models, such as the Gail model, are modest predictors of individual risk for breast cancer (c-statistic, 0.55 to 0.65). Five familial risk models for nongenetics specialists to guide referrals to genetic counseling accurately predict individual risk for BRCA mutations (c-statistic, >0.80). No studies reported harms of risk assessment. Sixteen studies indicated that genetic counseling decreases cancer worry, anxiety, and depression; increases the accuracy of risk perception; and decreases intention for mutation testing.

Thirty-two new studies and 38 earlier studies provided data for meta-analysis estimates of the prevalence and penetrance of BRCA mutations. Prevalence varies by population: 0.2 to 0.3 percent in general populations, 3 percent in women with breast cancer, 6 percent in women with breast cancer onset before age 40 years, 10 percent in women with ovarian cancer, and 20 percent in high-risk families. Among Ashkenazi Jewish women, prevalence is 2 percent in unselected populations and 10 percent in high-risk families. The penetrance of BRCA mutations differs by test result. Breast cancer penetrance to age 70 years if the test is positive is 46 to 71 percent for *BRCA1* or *BRCA2*; ovarian cancer penetrance is 41 to 46 percent for *BRCA1* and 17 to 23 percent for *BRCA2*. No estimates were available for women with variants of uncertain significance. The standardized incidence rate for breast cancer is 3.81 (95% CI, 3.06 to 4.75) for uninformative negative test results and 1.13 (95% CI, 0.81 to 1.58) for true negative results. Estimates for ovarian cancer were highly heterogeneous. Breast cancer worry and anxiety increased after testing in women with positive results and decreased in others, although results differed across studies. Risk perception improved after receiving test results.

No trials of the effectiveness of intensive screening for breast or ovarian cancer in women who are mutation carriers have been published. False-positive rates, unnecessary imaging, and unneeded surgery were higher in women undergoing intensive screening. Most women experienced no anxiety after screening with magnetic resonance imaging, mammography, or clinical breast examination, although women recalled for additional testing had transient anxiety. There are no trials of risk-reducing medications specifically in women who are mutation carriers. Tamoxifen and raloxifene reduced invasive breast cancer by 30 to 68 percent in placebo-controlled trials enrolling women with various levels of risk; tamoxifen had a greater effect than raloxifene in a head-to-head trial. Results suggested that reduction was greater in women with more relatives with breast cancer, but confidence intervals overlapped and results were not specific for women who are mutation carriers. Tamoxifen and raloxifene increased thromboembolic events and tamoxifen increased endometrial cancer and cataracts. In high-risk

women and women who are mutation carriers, risk-reducing mastectomy reduced breast cancer by 85 to 100 percent and breast cancer mortality by 81 to 100 percent; risk-reducing salpingo-oophorectomy reduced breast cancer by 37 to 100 percent, ovarian cancer by 69 to 100 percent, and all-cause mortality by 55 to 100 percent. Some women experienced physical complications of surgery, postsurgical symptoms, or changes in body image; some had improved anxiety.

Limitations: Including only English-language articles and studies applicable to the United States; varying number, quality, and applicability of studies.

Conclusions: Risk assessment using familial risk models to guide referrals is accurate. Genetic counseling reduces distress, improves risk perception, and reduces intention for testing. Genetic testing provides risk estimates for specific populations depending on test results. A true negative test indicates no increased risk for breast cancer. The effectiveness of intensive screening is not known, but it increases false-positive results and procedures. Tamoxifen and raloxifene reduce risk for breast cancer, but have adverse effects. Risk-reducing mastectomy and salpingo-oophorectomy are effective in reducing breast and ovarian cancer. Several evidence gaps remain and additional studies are necessary to better inform practice.

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CHAPTER 1. INTRODUCTION

Purpose of Review and Prior USPSTF Recommendation

This systematic review is an update of the evidence for the U.S. Preventive Services Task Force (USPSTF) on the effectiveness and adverse effects of risk assessment, genetic counseling, and genetic testing for breast cancer susceptibility gene (BRCA)–related cancer in women who do not have cancer but are potentially at increased risk. Its purpose is to evaluate and summarize evidence addressing specific key questions important to the USPSTF as it considers new recommendations for primary care practice.

In 2005, based on results of a previous review,[1,2] the USPSTF recommended against routine referral for genetic counseling or routine BRCA testing for women whose family histories are not associated with increased risks for deleterious mutations in breast cancer susceptibility gene 1 (*BRCA1*) or breast cancer susceptibility gene 2 (*BRCA2*) (D recommendation).[3] The USPSTF also recommended that women whose family histories are associated with increased risks for mutations in the *BRCA1* or *BRCA2* genes be referred for genetic counseling and evaluation for BRCA testing (B recommendation).

The USPSTF concluded that the potential harms of routine referral for genetic counseling or BRCA mutation testing in women without family history risk outweigh the benefits, and that the benefits of referring women with family history risk to suitably trained health care providers outweigh the harms. Benefits included improved accuracy of risk assessment and pretest probability for testing and improved patient knowledge, risk perception, and psychological and health outcomes. Potential harms included inaccurate risk assessment; inappropriate testing; misinterpretation of test results; and ethical, legal, and social implications; among others.

The 2005 USPSTF recommendation was intended for the primary prevention of cancer and applied to women without previous diagnoses of breast or ovarian cancer, consistent with the USPSTF scope of preventive care for the general population. Recommendations for men and women with cancer were not included. The 2005 USPSTF recommendation is included in the Affordable Care Act for covered preventive services,[4] and provided the basis for a Healthy People 2020 objective to increase the proportion of women with family histories of breast or ovarian cancer who receive genetic counseling.[5]

The previous systematic review[1,2] identified several research limitations and evidence gaps. The review concluded that a primary care approach to genetic risk assessment and BRCA mutation testing had not been evaluated, and evidence was lacking to determine the benefits and harms of this approach for women without cancer. Risk assessment, genetic counseling, and mutation testing did not cause adverse psychological outcomes, and counseling improved distress and risk perception in the highly-selected populations studied. Studies of intensive cancer screening approaches, such as earlier and more frequent mammography, were inconclusive. Trials of risk-reducing medications, such as tamoxifen and raloxifene, reported reduced breast cancer incidence in women with varying baseline levels of risk compared with placebo, but also increased adverse effects. Observational studies of risk-reducing mastectomy and salpingo-

oophorectomy reported reduced breast and ovarian cancer outcomes in women who were mutation carriers.

Limitations identified by the previous review included:

- The quality and generalizability of studies varied.
- Although several risk assessment tools were available, most were designed for use by genetics specialists rather than primary care providers.
- Methods of risk stratification were subject to misclassification, and data to guide clinicians in the best approach were lacking.
- Studies of the effectiveness of genetic counseling on patient decisions and outcomes were lacking.
- Most studies of BRCA mutation testing were conducted in highly-selected samples of women, many with preexisting breast or ovarian cancer or from previously identified kindreds.
- Family history risk was often based on self-reported information; thus, the accuracy of risk stratification was limited by the accuracy of reported family history.
- In some cases, data to determine penetrance came exclusively from one study, and when multiple studies were available, they were heterogeneous and likely unreliable. (Penetrance is the probability of developing breast or ovarian cancer in women who have a known *BRCA1* or *BRCA2* mutation.)
- Most studies used research laboratory techniques to detect clinically significant mutations that differed from the DNA sequencing available clinically.
- The clinical significance of mutations was determined by each study.
- The applicability of studies based on highly-selected women in research settings to the general screening population was questionable.
- Data were not available to determine the optimal age at which to test and how age at testing influenced estimates of benefits and adverse effects.
- The long-term impact of testing was unknown, and most studies followed patients for less than 1 year.
- Studies did not evaluate psychological aspects of medical outcomes.
- Few data were available about the impact of testing on family members.
- Treatment effects were influenced by several variables that were not available and not easily factored into estimates of clinical outcomes.

Evidence gaps identified by the previous review included:

- Impact of screening in the general population.
- Patient-centered issues, such as access to testing; effectiveness of screening approaches, including risk stratification; use of system supports; and patient acceptance and education.
- Studies about who should perform risk assessment and genetic counseling services, and what skills are needed.
- Studies about what happens after patients are identified as high-risk in clinical settings.

- The consequences of genetic testing for individuals and their relatives.
- Well-designed investigations using standardized measures and enrolling subjects who reflect the general population, including minority women.
- Information about predictors of cancer, response to interventions, and other modifying factors from an expanded database or registry of patients who are counseled and tested for BRCA mutations.
- Additional research on interventions, including trials of risk-reducing medications that enroll women who are mutation carriers, evaluations of the effect of age at intervention, measurement of long-term outcomes, and factors related to acceptance of risk-reducing interventions.

Condition Definition

Clinically significant, or deleterious, mutations in the *BRCA1* and *BRCA2* genes are associated with increased risks for breast, ovarian, fallopian tube, and peritoneal cancer in women.[6,7] Often referred to as the Hereditary Breast and Ovarian Cancer syndrome, this condition is described as BRCA-related cancer in this review to explicitly include fallopian tube and peritoneal cancer. Research indicates that BRCA-related fallopian tube cancer has probably been misdiagnosed as ovarian cancer in the past.[8-10] These mutations are also associated with male breast cancer and, to a lesser degree, pancreatic and early-onset prostate cancer; *BRCA2* mutations are associated with melanoma. Although all of these types of cancer are considered during familial risk assessment, studies with these cancer outcomes are outside the scope of this review. BRCA mutations cluster in families exhibiting an autosomal dominant pattern of transmission in either the maternal or paternal lineage.

Recent estimates indicate that clinically significant mutations in either of the BRCA genes increase a woman's risk of breast cancer by age 70 years to 45 to 65 percent.[11,12] *BRCA1* mutations increase ovarian, fallopian tube, or peritoneal cancer risk to 39 percent, and *BRCA2* mutations to 10 to 17 percent.[11,12] These mutations are estimated to occur in 1 in 300 to 500 women in the general population,[13-16] and account for 5 to 10 percent of breast cancer overall.[13,17]

Specific BRCA mutations, known as founder mutations, are clustered among certain ethnic groups, including Ashkenazi Jews,[18-20] blacks,[21] and Hispanics,[22,23] and among families in the Netherlands,[24] Iceland,[25,26] and Sweden.[27] Several additional genes not included in this review are also associated with hereditary susceptibility to breast and ovarian cancer,[7,28,29] but are not commonly tested.

Specific cancer phenotypes are also associated with BRCA mutations even in the absence of family history, including triple-negative breast cancer and high-grade serous ovarian or fallopian tube cancer.[30-35] Pathologic and clinical characteristics of tumors also differ by the type of mutation. In a series of 3,797 cases of breast cancer in women who were *BRCA1* mutation carriers, 78 percent were estrogen receptor (ER)–negative, 79 percent progesterone receptor (PR)–negative, 90 percent human epidermal growth factor receptor 2 (HER2)–negative, and 69 percent triple-negative.[36] The proportion of ER-negative cases decreased with increasing age. In

a series of 2,392 cases of breast cancer in women who were *BRCA2* mutation carriers, 23 percent were ER-negative, 36 percent PR-negative, 87 percent HER2-negative, and 16 percent triple-negative.[36] These characteristics are important in determining cancer treatment and prognosis.

Prevalence and Burden of Disease

Breast cancer is the second most common cancer in women in the United States after nonmelanoma skin cancer, and is the second leading cause of cancer death after lung cancer.[37,38] In 2013, an estimated 232,340 women in the United States will be diagnosed with breast cancer and 39,620 women will die from it.[28] According to lifetime risk estimates for the general population, 12.3 percent (95% confidence interval [CI], 12.2 to 12.4) of women will develop breast cancer sometime during their lives, and 2.8 percent (95% CI, 2.76 to 2.80) will die from it.[39] The 5-year relative survival rate for all stages of breast cancer in the United States is 89 percent, but improves to 99 percent with localized disease. Five-year relative survival rates for women with regional and distant disease are 84 and 23 percent, respectively.[39]

Ovarian cancer is the fifth leading cause of cancer death in women in the United States,[38] accounting for an estimated 22,240 new cases and 14,030 deaths in 2013.[40] According to lifetime risk estimates for the general population, 1.40 percent (95% CI, 1.38 to 1.43) of women will develop ovarian cancer sometime during their lives and 1.02 percent (95% CI, 1.01 to 1.03) will die from it.[39] The 5-year relative survival rate for all stages of ovarian cancer in the United States is 44 percent, but may improve to 92 percent for women whose disease is detected and treated in early stages.[39] However, up to 75 percent of women with ovarian cancer have nonlocalized disease at the time of diagnosis because early stages are often asymptomatic. Five-year relative survival rates for women with regional and distant disease are 72 and 27 percent, respectively.[39]

Rationale for Screening/Screening Strategies

BRCA-related cancers are associated with family histories of these cancer types. Approximately 5 to 10 percent of women with breast cancer have a mother or sister with breast cancer, and up to 20 percent have either a first- or second-degree relative with breast cancer.[41-45] Although most of these women do not have BRCA mutations, some women report family history patterns that suggest their presence. Genetic risk assessment and BRCA mutation testing involve determining individual risk for clinically significant BRCA mutations followed by mutation testing of high-risk individuals. Mutation testing of appropriate candidates could lead to increased awareness of cancer risk and effective use of interventions to reduce BRCA-related cancer incidence and mortality.

Risk Assessment and Genetic Counseling

Several characteristics are associated with an increased likelihood of deleterious BRCA mutations,[46-49] including breast cancer diagnosed at an early age (before age 40 or 50 years), bilateral breast cancer, triple-negative breast cancer diagnosed before age 50 years, history of both breast and ovarian cancer, breast cancer in male relatives, multiple cases of breast cancer in

the family, both breast and ovarian cancer in the family, family members with two primary breast cancers, and Ashkenazi Jewish ancestry. These and other individual and family characteristics can be used to assess personal cancer risk and the need for referral for additional evaluation and testing. Approaches to assessing personal risk for BRCA mutation status range from simple checklists of criteria to comprehensive kindred analysis requiring expertise in cancer genetics. Practice and coverage standards in the United States generally follow the National Comprehensive Cancer Network (NCCN) referral criteria for genetic counseling (described in **Appendix A1**).[50]

Genetic counseling is the process of identifying and counseling individuals who are at risk for familial or inherited cancer and is recommended prior to BRCA mutation testing.[50-52] Services include comprehensive evaluations of familial risk for inherited disorders using kindred analysis and models to estimate risk. These include models based on logistic regression (e.g., Couch[46]), Bayesian analysis (e.g., BRCAPRO,[12,53] BOADICEA[54]), and patient data (Myriad prevalence tables[55]), among others. Some models are more appropriate for specific patients, and model accuracy varies across different populations.[56] In the course of an evaluation for BRCA-related cancer, other cancer syndromes are sometimes identified. Genetic counseling also includes identification of candidates for testing, patient education, discussion of the benefits and harms of genetic testing, interpretation of results after testing, and discussion of management options. Some genetic counseling programs offer their services by telephone.

Providers of genetic counseling may be genetic counselors,[57-59] nurse educators,[60,61] or other health professionals with comparable skills.[62] Accreditation standards from specialty groups specifically outline essential training and skills for genetics professionals.[63]

Mutation Testing

The NCCN provides specific criteria for genetic testing.[50] Guidelines recommend that mutation testing begin with a relative with known BRCA-related cancer, including male relatives, to determine if a clinically significant mutation is segregating in the family before testing individuals without cancer.[50] If an affected family member is not available, then the relative with the highest probability of mutation should be tested. Ideally, results of the initial test will guide testing decisions of other family members. However, the optimal candidate may not be available for testing, limiting the interpretation of results. Individuals without cancer meeting NCCN criteria for testing include those from families with known *BRCA1* or *BRCA2* mutations or from families with extensive cancer history (further described in **Appendix A1**).

The type of mutation analysis required also depends on family history (**Table 1**). A small number of clinically significant *BRCA1* and *BRCA2* mutations have been found repeatedly in different families, including the three founder mutations common in the Ashkenazi Jewish population. However, most identified mutations have been found in only a few families.[64] Individuals from families with known mutations or from ethnic groups with common mutations can be tested specifically for them. Several clinical laboratories in the United States test for specific mutations or sequence specific exons. The sensitivity and specificity of analytic techniques are determined by the laboratories and are not generally available.

Individuals without linkages to known mutations can determine their mutation status by direct DNA sequencing. A commercial laboratory, Myriad Genetic Laboratories, previously held a patent on this procedure and provided most of the testing in the United States. Myriad reports analytic sensitivity and specificity as both greater than 99 percent.[65] Approximately 12 percent of high-risk families without a *BRCA1* or *BRCA2* coding-region mutation may have other clinically significant genomic rearrangements.[65,66] Many of these mutations can be tested using the BRCA Rearrangement Test, now available as a subsequent step in testing.

Tests may indicate positive (i.e., BRCA mutation detected), variant of uncertain clinical significance (i.e., an abnormality of the BRCA gene, but unknown if it is associated with an increased risk for cancer), uninformative negative, or true negative results. A true negative result represents the absence of a mutation in an individual who has relatives with cancer and known BRCA mutations. An uninformative negative also indicates the absence of a mutation in an individual; however, information about her relatives is not definitive because either a mutation was not detected by their tests or they have not been tested.

Interventions

Interventions to reduce risk for cancer in women who are BRCA mutation carriers include earlier, more frequent, or intensive cancer screening; risk-reducing medications; and risk-reducing surgery. Cancer screening recommendations specifically for women who are BRCA mutation carriers are outside the scope of the USPSTF. The NCCN recommends that women who are BRCA mutation carriers conduct monthly breast self-examinations beginning by age 18 years, annual or semiannual clinician breast examinations beginning at age 25 years, and annual mammography and breast magnetic resonance imaging (MRI) beginning at age 25 years or individualized based on the earliest age of onset in the family.[50] The NCCN also recommends that women consider risk-reducing mastectomy and salpingo-oophorectomy, monitoring with transvaginal ultrasound (TVUS) and cancer antigen-125 (CA-125) levels every 6 months for women not undergoing salpingo-oophorectomy, and risk-reducing medications.

Tamoxifen, a selective estrogen receptor modulator (SERM), is considered a candidate for breast cancer risk reduction based on its effectiveness in preventing recurrences in women with breast cancer.[67] Placebo-controlled randomized, controlled trials (RCTs) of tamoxifen indicate reduced primary ER-positive breast cancer in women with family histories of breast cancer.[68-72] Raloxifene, another SERM used primarily for treating osteoporosis, also reduced risk for breast cancer in trials of women with various levels of breast cancer risk.[73,74] SERMs also have important adverse effects, including thromboembolism, endometrial cancer (tamoxifen), and vasomotor and other symptoms.[75,76] Exemestane, an aromatase inhibitor, also reduces risk for primary breast cancer in women with increased risk[77] and is in clinical use, but is not approved by the U.S. Food and Drug Administration for this indication. The USPSTF currently recommends consideration of risk-reducing medications for women who are at increased risk for breast cancer and low risk for complications, and discourages its use in average-risk women.[78]

Risk-reducing mastectomy and salpingo-oophorectomy are also options for women who are BRCA mutation carriers.[79-82] Bilateral total simple mastectomy with or without reconstruction is

currently the most common approach.[83,84] This procedure provides more complete removal of breast tissue than the previously used subcutaneous mastectomy. However, no procedure completely removes all breast tissue[85] and breast cancer can still occur postmastectomy.[86] Surgical reports indicating the potential for cancer occurrence after bilateral oophorectomy have led to more extensive procedures to remove potential tumor sites, such as bilateral salpingo-oophorectomy with or without hysterectomy.[87,88] Despite this approach, the occurrence of peritoneal carcinomatosis remains a possibility.[89-91]

Current Clinical Practice

Guidelines recommend testing for mutations only when an individual has personal or family history of cancer suggestive of inherited cancer susceptibility, the test can be adequately interpreted, and results will aid in management.[51,92]

Actual practices in the United States are unclear. The lack of effectiveness trials, differing interpretations of existing research among specialties, variability of insurance coverage, and direct-to-consumer advertising targeting patients, physicians, and health systems have resulted in highly variable clinical practices. The initial focus of mutation testing has been on patients with cancer. For women without cancer or relatives with known BRCA mutations, an integrated clinical pathway generally involves a series of sequential steps, including: 1) risk stratification and referral for genetic counseling, 2) genetic counseling for women identified with increased risk based on family history information, 3) BRCA mutation testing for women or their relatives with significant familial risk, and 4) interventions to reduce risk based on benefits, harms, and patient preferences.

In practice, these steps may not be sequential or clearly defined. In the United States, genetic testing is marketed directly to consumers, who may bypass preceding steps. In surveys, many clinicians were unfamiliar with genetic tests and criteria for referral or testing.[93,94] Some clinicians provide risk assessment, testing, and risk-reducing surgery without using comprehensive risk assessment methods or involving genetic counselors. Screening MRI is often performed based on risk criteria or other considerations that have not been evaluated for effectiveness, while risk-reducing medications are rarely used.[95]

Relevant data describing current clinical practice was collected through the Michigan Department of Community Health Cancer Genomics Program using statewide telephone surveys and a clinical genetic counseling database. Results indicated that approximately 8 percent of women without breast or ovarian cancer had two or more first- or second-degree relatives with breast or ovarian cancer.[96] Among women without cancer who had family histories indicating that they would probably benefit from genetic counseling, 35.7 percent received genetic counseling and 9.8 percent had genetic testing during 2009. Most referrals of women without cancer were made by obstetricians/gynecologists, primary care physicians, or patients themselves, comprising 44.3 percent of patients counseled. Among women without cancer who saw genetic counselors, 55.2 percent underwent genetic testing. Of these, results indicated 91.6 percent were negative, 3.9 percent were positive, and 4.5 percent were variants of unknown significance. Respondents described their top three reasons for declining testing after receiving

genetic counseling as: 1) not being the best candidate, 2) the test was not clinically indicated, and 3) inadequate insurance coverage.

The uptake of specialized services after genetic testing is generally high among women with positive test results that indicate the presence of clinically significant BRCA mutations.[97,98] In a recent study of women who had genetic testing in a U.S. university-based cancer risk program, women with positive results were significantly more likely to have risk-reducing salpingo-oophorectomy and screening with TVUS and serum CA-125 testing, while those with true negative results were less likely to have these procedures.[99] Among women with variants of uncertain significance and uninformative negative results, 12 percent had risk-reducing salpingo-oophorectomy, 37 percent had TVUS, and 34 percent had serum CA-125 testing.

Recommendations of Other Groups

Current recommendations of other professional groups are described in **Table 2**.

CHAPTER 2. METHODS

Analytic Framework and Key Questions

Based on evidence gaps identified from the previous review,[1,2] the USPSTF and Agency for Healthcare Research and Quality (AHRQ) determined the key questions for this update using the methods of the USPSTF.[100] Investigators created an analytic framework incorporating the key questions and outlining the patient populations, interventions, outcomes, and potential adverse effects (**Figure 1**). Definitions are described in **Appendix A2** and key questions are outlined in **Figure 1**. A draft research plan describing the analytic framework, key questions, scope, and systematic review approach was posted on the USPSTF Web site for public comment for 30 days in March and April 2012. A total of 213 comments from 54 respondents were received and reviewed, and the research plan was modified after discussion with investigators, the AHRQ Medical Officer, and USPSTF members. In addition, the USPSTF requested information about the impact of genetic testing on family members and the effects of direct-to-consumer marketing of BRCA genetic tests. These are described in supplementary sections of the review.

The target population included women without cancer or known deleterious BRCA mutations who are seen in clinical settings applicable to U.S. primary care practice, although the ideal candidate for mutation testing could be a male or female relative with cancer. The conditions of interest were BRCA mutation carrier status and BRCA-related cancer (predominantly breast, ovarian, fallopian tube, and peritoneal).

Search Strategies

In conjunction with a research librarian, investigators used the National Library of Medicine's Medical Subject Headings keyword nomenclature to search the Cochrane Central Register of Controlled Trials and Cochrane Database of Systematic Reviews (2005 through the 4th Quarter 2012), Health Technology Assessment, National Health Sciences Economic Evaluation Database, Database of Abstracts of Reviews of Effects (4th Quarter 2012), and MEDLINE and PsycINFO (2004 to December 31, 2012) for relevant English-language studies, systematic reviews, and meta-analyses. Search strategies are listed in **Appendix B1**. Secondary referencing involved manually reviewing reference lists of papers and reviewing citations of key studies using Scopus.

Study Selection

Investigators developed inclusion and exclusion criteria for abstracts and articles based on the target population, key questions, and outcome measures (**Appendix B2**). New research conducted in the United States or in similar populations that receive services and interventions applicable to U.S. medical practice published in 2003 or later was considered. After an initial review of abstracts, full-text articles were reviewed using additional inclusion criteria. In addition, studies from the previous review that met inclusion criteria for this update were

included in summary tables and meta-analysis in order to build on prior relevant research.

RCTs, systematic reviews, prospective and retrospective cohort studies, case-control studies, and diagnostic accuracy evaluations that addressed key questions 1, 2, and 4 were included. These include studies of the accuracy of risk assessment methods, outcomes of genetic counseling and testing, and effectiveness studies of interventions to reduce risk of BRCA-related cancer in women who are mutation carriers. Risk assessment methods were included only if they were designed for use by nongenetics specialists to guide referrals and were feasible for clinical settings (i.e., brief, nontechnical, did not require special training to administer or interpret). Evaluation of complex models used in genetic counseling was outside the scope of this review. Interventions include intensive screening (e.g., earlier and more frequent mammography, breast MRI), risk-reducing medications (e.g., tamoxifen, raloxifene), and risk-reducing surgery (e.g., mastectomy, salpingo-oophorectomy). For intensive screening interventions, when effectiveness studies were not available, studies that reported test characteristics of screening modalities, such as sensitivity and specificity, were included. Only medications approved by the U.S. Food and Drug Administration for cancer risk reduction were considered, consistent with the scope of the USPSTF.

Studies of any design were included to describe potential adverse effects of risk assessment, genetic counseling, mutation testing, and risk-reducing interventions (key questions 3 and 5). Potential adverse effects include inaccurate risk assessment; inappropriate testing; false-positive and false-negative results; false reassurance; incomplete testing; misinterpretation of the test result; anxiety; cancer worry; immediate and long-term harms associated with breast imaging, risk-reducing medications, and risk-reducing surgery; and ethical, legal, and social implications.

Data Abstraction and Quality Rating

An investigator abstracted data about the study design and setting; participant characteristics; data collection procedures; numbers enrolled and lost to followup; methods of exposure and outcome ascertainment; analytic methods, including adjustment for confounders; and outcomes. A second investigator confirmed the accuracy of key data. By using predefined criteria for RCTs, systematic reviews, cohort, case-control, and diagnostic accuracy studies developed by the USPSTF,[100,101] two investigators rated the quality of studies (good, fair, poor) and resolved discrepancies by consensus (**Appendix B3**).

Quality could not be assessed for many studies with designs that did not have predefined quality criteria, such as descriptive, cross-sectional, before-after, and case-series. For studies of penetrance (i.e., the probability of developing breast or ovarian cancer in women who have known *BRCA1* or *BRCA2* mutations) that computed a standardized incidence ratio (SIR) as the summary measure, we considered several factors to determine study quality in the absence of predefined criteria. Studies were considered high-quality if: 1) genotypes were known by direct measurement or inference from genotypes of relatives rather than probabilistically assigned; 2) breast and ovarian cancer outcomes were determined prospectively after ascertainment of the family genetic profile; 3) important covariates were measured for all individuals and accounted for in the analysis, including use of risk-reducing surgery and medications, age, Ashkenazi

Jewish ancestry, race or ethnicity, and vital status; and 4) reported family history was validated by review of medical records of family members.

The applicability of studies was determined using the population, intervention, comparator, outcomes, timing of outcomes measurement, and setting format adapted to this topic.[102]

Data Synthesis

We assessed the aggregate quality of the body of evidence for each key question (good, fair, poor) by using methods developed by the USPSTF based on the number, quality, and size of studies and consistency of results between studies.[100] Studies were considered consistent if outcomes were generally in the same direction of effect and ranges of effect sizes were narrow.

Statistical Meta-Analysis

To determine clinical outcomes related to various mutation testing results, we combined data in several meta-analyses to obtain estimates of mutation prevalence, penetrance, and relative risk for developing breast or ovarian cancer. These include estimates for women from unselected populations, high-risk cohorts, and Ashkenazi Jewish populations with tests indicating BRCA-positive (i.e., detected *BRCA1* or *BRCA2* mutations), variant of uncertain significance, uninformative negative, and true negative results using data from studies meeting inclusion criteria. Relevant studies from the previous review as well as those identified for this update were included in the meta-analyses.

To determine the appropriateness of meta-analysis, we considered clinical and methodological diversity and assessed statistical heterogeneity. We abstracted or calculated estimates of prevalence, penetrance, and relative risk (risk ratio [RR] or SIR) and their standard errors (SEs) from each study and used them in the meta-analysis. When the SIR was not reported, but the studies reported data for observed and expected numbers of cancer cases, or the study only reported the observed number of cancer cases and we could calculate the expected number of cancer cases from Surveillance Epidemiology and End Results data,[39] we calculated the SIR and its CI based on observed and expected numbers of cancer cases using the relationship between the Poisson distribution and the chi-square distribution.[103]

We assessed the presence of statistical heterogeneity among the studies by using standard chi-square tests, and the magnitude of heterogeneity by using the I^2 statistic.[104] We used a random-effects model to combine data for prevalence, penetrance, and relative risk while accounting for variation among studies. In general, when there is no variation among studies, the random-effects model yields the same results as a fixed-effects model without a study effect.[105] To account for clinical heterogeneity, we stratified analyses by clinical characteristics (e.g., breast vs. ovarian cancer, levels of risk, or methods used to select probands for BRCA-positive women) when necessary. We conducted sensitivity analyses to assess the robustness of results that considered variation from outlying studies. The results of the sensitivity analyses indicated no major differences from the main analysis. All analyses were performed using Stata/IC 12.1 (StataCorp, College Station, TX) and SAS 9.3 (SAS Institute, Cary, NC).

External Review

The draft report was reviewed by content experts, USPSTF members, AHRQ Project Officers, and collaborative partners and revised prior to finalization (**Appendix B4**).

Response to Comments Received During the Public Comment Period

A draft version of this evidence report was posted for public comment on the USPSTF Web site from April 2 to April 29, 2013. Comments were contributed by seven individuals and primarily concerned the scope of the review (i.e., include women with existing cancer, men, other types of mutations); issues that were already addressed by the systematic review, but were missed by the respondent (e.g., effect of risk-reducing salpingo-oophorectomy before and after menopause); studies or topics of interest that had no publications meeting inclusion criteria (e.g., testosterone supplements to reduce breast cancer risk); and comments about the recommendation statement. These comments did not lead to important changes in the systematic review.

CHAPTER 3. RESULTS

We reviewed 5,268 references from electronic searches, reference lists, and manual searches of recently published studies. After applying inclusion and exclusion criteria, we reviewed 1,600 full-text papers. Of these, 140 provided data addressing one or more of the key questions and were included in the systematic review. **Appendix B5** shows the results of our literature search and selection process and **Appendix B6** lists the excluded full-text papers. Included studies and quality ratings are in **Appendixes C1 to C4**.

Key Question 1. Does Risk Assessment, Genetic Counseling, and Genetic Testing Lead to Reduced Incidence of BRCA-Related Cancer and Reduced Cause-Specific and All-Cause Mortality?

No studies addressed the overarching issues of key question 1.

Key Question 2a. What Is the Accuracy of Methods to Assess Familial Cancer Risk for BRCA-Related Cancer When Performed by a Nongenetics Specialist in a Clinical Setting?

Key Question 3a. What Are the Potential Adverse Effects of Risk Assessment?

Summary

Several studies of risk stratification methods for nongenetics specialists met inclusion criteria for key question 2a, but no studies met criteria for key question 3a regarding potential adverse effects. The sensitivity of self-reported family cancer history in first-degree relatives varied between 65 and 82 percent for breast cancer and was 50 percent for ovarian cancer in validation studies, although specificity was greater than 90 percent. Referral criteria have been developed by several groups, but their accuracy has not been evaluated. A published systematic review of studies of 13 general breast cancer risk models and 11 studies of five familial risk models provided accuracy measures. Reference standards varied across studies, limiting comparisons between methods. General breast cancer risk models, such as the Gail model, are modest predictors for individuals (c-statistic, 0.55 to 0.65). Familial risk models, including the Ontario Family History Assessment Tool (FHAT), Manchester Scoring System, Referral Screening Tool (RST), Pedigree Assessment Tool (PAT), and FHS-7, predict risk specifically for BRCA mutations and are intended to guide referrals to genetic counseling. Studies indicated high accuracy for these models (c-statistic, >0.80), although some models have only been evaluated in single studies.

Evidence

This key question focuses on the evaluation of a patient's individual familial risk for BRCA-related cancer in a clinical setting by a nongenetics specialist for the purpose of initiating appropriate referrals for more comprehensive evaluations by genetic counselors and other specialists. These methods of risk stratification and referral differ from those intended for comprehensive evaluations. Risk models have been developed that predict the probability of developing breast cancer or the likelihood of having a mutation. Although the mutation probability is linked to family history, BRCA mutations explain only a small proportion of the familial aggregation of breast cancer, and even less of the hereditable variance in risk in a population.

Determination of Family History

Family history of BRCA-related cancer is important in estimating individual risk for a *BRCA1* or *BRCA2* mutation in women without cancer or known family mutations. Among women with first-degree relatives with cancer, the relative risk for cancer has been estimated in meta-analyses as 2.1 (95% CI, 2.0 to 2.2) for breast cancer[43] and 3.1 (95% CI, 2.6 to 3.7) for ovarian cancer.[106] Decisions about referral, testing, and risk-reducing interventions are often based on self-reports of family histories that include type of cancer, relationship within the family, and age of onset. Appropriate decisions rely on family histories that are accurately reported by women and correctly obtained by clinicians.

The accuracy of family cancer history information was determined in studies that validated self-reported family histories with medical records. In one study, a report of breast cancer in a first-degree relative of a healthy individual had a sensitivity of 82 percent, specificity of 91 percent, positive likelihood ratio of 8.9 (95% CI, 5.4 to 15.0), and negative likelihood ratio of 0.20 (95% CI, 0.08 to 0.49).[107] A more recent population-based study in the United States indicated the accuracy of self-reported breast cancer history in a first-degree relative as 64.9 percent sensitivity and 99.0 percent specificity.[108] In this study, the accuracy for first-degree relatives was higher than for second-degree relatives. For ovarian cancer, a report of ovarian cancer in a first-degree relative was less reliable than for breast cancer, and had a sensitivity of 50 percent, specificity of 99 percent, positive likelihood ratio of 34.0 (95% CI, 5.7 to 202.0), and negative likelihood ratio of 0.51 (95% CI, 0.13 to 2.10).[107]

Referral Guidelines

Referral guidelines have been developed by health maintenance organizations,[109] professional organizations,[51,92] cancer programs,[50,110] State and national health programs,[111-113] and investigators[114] to assist nongenetics specialists in identifying women who are at potentially increased risk for BRCA mutations. Although specific items vary among the guidelines, most include questions about personal and family history of BRCA mutations, breast and ovarian cancer, age at diagnosis, bilateral breast cancer, and Ashkenazi Jewish ancestry. Most guidelines are intended to lead to a referral for more extensive genetic evaluations and counseling, not directly to testing. Although guidelines vary, practice and coverage standards in the United States generally follow the NCCN referral criteria for genetic counseling (described in **Appendix**

A1).[50] The effectiveness of this approach in improving breast cancer outcomes has not been evaluated.

General Risk Stratification Models to Predict Individual Risk for Breast Cancer in Primary Care Settings

Although used in clinical settings, general risk stratification models predicting individual risk for breast cancer were not developed to identify women with increased probabilities of BRCA mutations.

A recent systematic review[115,116] included 19 studies[117-137] evaluating 13 risk stratification models to identify women with increased risk for breast cancer (**Table 3**). Models specifically evaluating risk for *BRCA1* and *BRCA2* mutations were outside the scope of this review and were not included.

Most general risk models are based on the Breast Cancer Risk Assessment Tool, also referred to as the Gail model. This model was derived from multivariate logistic regression analysis of identified risk factors for breast cancer,[128] and subsequently modified with Surveillance Epidemiology and End Results data.[126] Subsequent models use a similar approach, but vary in their use of reference standards and included variables. The original Gail model included age, age at menarche, age at first birth, family history of breast cancer in first-degree relatives, number of prior breast biopsies, and history of atypical hyperplasia.[128] Subsequent models include one or more of these variables in addition to other factors (**Table 3**).

Most models accurately predict breast cancer incidence in populations of women. For most models in the studies, the expected numbers of cases of breast cancer closely matched the observed numbers (calibration: estimated/observed [E/O], 0.90 to 1.10).[119,121-124,126,127,129,135,136] However, they are only modestly accurate in predicting breast cancer risk for individuals. In studies, discriminatory accuracy was expressed as concordance statistics, determined by the area under the receiver-operating characteristic curve (c-statistic). Values ranged from 0.55 to 0.65 across the studies,[117-124,126,127,129,131-135] which is comparable to age alone as a predictor.

Familial Risk Stratification Models to Predict Individual Risk for BRCA Mutations in Primary Care Settings

Familial risk stratification models for BRCA-related cancer are primarily intended for use by nongenetics specialists to guide patient referrals to genetic counselors for more definitive evaluations. Several models have been developed and evaluated, including the FHAT, Manchester Scoring System, RST, PAT, and FHS-7. Ten studies describing performance characteristics of these models met inclusion criteria for this review (**Table 4**, **Appendix C5**).[56,138-146] Included studies met criteria for fair or good quality and compared the referral models to validated risk assessment models, including BRCAPRO, Claus, Myriad, BOADICEA, Tyrer-Cuzick, and Penn II. Studies of the RST, PAT, and FHS-7 were published after the previous USPSTF systematic review.

FHAT. The FHAT is a 17-question instrument developed to assist Canadian clinicians in

selecting patients for referral to genetic counseling.[142] The referral threshold is equivalent to doubling the general population lifetime risk for breast or ovarian cancer (22%). In the FHAT, points are assigned according to the number of relatives, third-degree or closer, who are diagnosed with breast, ovarian, colon, or prostate cancer; age at diagnosis; and type of primary cancer and number of primary cancer cases. Patients with scores of 10 or more points warrant referral. FHAT results were compared with Claus and BRCAPRO estimates for 184 women with incident familial and nonfamilial breast cancer.[142] The sensitivity and specificity of the FHAT for a clinically significant *BRCA1* or *BRCA2* mutation were 94 and 51 percent, respectively. This compares with sensitivity and specificity of 74 and 79 percent for a 20 percent threshold for having a clinically significant *BRCA1* or *BRCA2* mutation using BRCAPRO, and 74 and 54 percent using Claus methods. Additional validation studies of the FHAT have replicated its accuracy,[145] and its concordance statistics range from 0.68 to 0.83 across a wide variety of conditions.[144,146]

Manchester Scoring System. The Manchester Scoring System was developed in the United Kingdom to predict *BRCA1* or *BRCA2* mutations at the 10 percent likelihood level.[141] Points are assigned depending on type of cancer (breast, ovarian, pancreatic, or prostate), affected family members, and age at diagnosis. The model provides scores for *BRCA1* and *BRCA2* mutations separately. The scoring system was validated in three sample sets in other regions of the United Kingdom and compared with other existing models. In validation studies, the Manchester model (combined *BRCA1* and *BRCA2*) had 58 to 93 percent sensitivity, 33 to 71 percent specificity, and concordance statistics of 0.75 to 0.80, comparing well with the other models tested.[56,139,141,144,145]

RST. The RST was developed to help primary care clinicians make appropriate referrals for genetic counseling in response to the USPSTF 2005 recommendation.[140] The RST is a clinical scoring tool that uses a checklist of risk information, including breast cancer at age 50 years or younger in self or relatives, ovarian cancer at any age in self or relatives, two or more breast cancer cases after age 50 years on the same side of the family, male breast cancer, and Jewish ancestry. The referral threshold is reached with two or more positive responses. It was designed for simplicity, and is the least complicated model to administer for screening purposes. In an evaluation study, the RST was administered to 2,464 unselected women undergoing screening mammography in a U.S. health care system.[140] Results were compared against validated risk assessment models, including BRCAPRO, Myriad II, BOADICEA, and FHAT. The RST demonstrated a sensitivity of 81 percent, specificity of 92 percent, and concordance statistic of 0.87. A revised model is also available online.[147]

PAT. The PAT was also designed to identify women at increased risk for BRCA-related cancer in U.S. primary care settings.[143] The PAT uses a point scoring system based on information from first-, second-, and third-degree relatives regarding breast cancer onset at ages younger or older than 50 years; ovarian cancer at any age; male breast cancer; and Ashkenazi Jewish ancestry. Performance characteristics were determined in a study of 3,906 women without cancer undergoing screening mammography at a U.S. community hospital.[143] Results were compared against the Myriad II and Gail models. The PAT had optimal sensitivity of 100 percent and specificity of 93 percent at scores of 8 or more. The PAT had a concordance statistic of 0.96, which was much higher than results using the Gail 5-year (0.39) or lifetime estimate (0.59).

FHS-7. The FHS-7 is a seven-question instrument about family history of breast, ovarian, and colorectal cancer.[138] It was developed as a simple instrument for primary care settings for screening and referral purposes. The questions include first-degree relatives with breast or ovarian cancer, any relatives age 50 years and younger with breast cancer, bilateral breast cancer, breast and ovarian cancer in the same person, male breast cancer, two or more relatives with breast and/or ovarian cancer, and two or more relatives with breast and/or colon cancer. A single positive response is the threshold for referral. In an evaluation study in Brazil, the FHS-7 was administered to 9,218 women during routine visits to primary care clinics. Results were compared with Claus, Gail, Tyrer-Cuzick, and Penn II models. The FHS-7 had a sensitivity of 88 percent, specificity of 56 percent, and concordance statistic of 0.96.[138]

Key Questions 2b, 3b. What Are the Benefits and Potential Adverse Effects of Genetic Counseling in Determining Eligibility for Genetic Testing for BRCA-Related Cancer?

Summary

Sixteen new studies evaluated the benefits and harms of genetic counseling, including a systematic review; RCTs; and cohort, case-control, and before-after studies of distress, accuracy of risk perception, and intention for testing. Results indicated that counseling decreases cancer worry, anxiety, and depression; increases the accuracy of risk perception; and decreases intention for mutation testing. Face-to-face counseling was preferred in some studies. Limitations of studies included dissimilar comparison groups and small sizes.

Evidence

Twenty-seven studies met inclusion criteria, including 16 published since the prior review[148-165] and 11 included previously[57-60,62,166-171] (**Table 5**, **Appendix C6**). Studies provided data about distress due to genetic counseling for BRCA-related cancer measured as worry, anxiety, or depression. Additional outcomes included intention for genetic testing and accuracy of risk perception. Results for key questions 2b and 3b are both presented in this section of the review because studies generally provided measures for both benefits and harms.

Eleven studies included in the previous review indicated that breast cancer worry usually decreased after genetic counseling, and women preferred personal contact over computer software or telephone counseling.[57-60,62,166-171] Also, studies showed that measures of anxiety and depression generally decreased or did not differ with counseling.[59,62,166,167,169-171] Risk perception was not well reported in previous studies and results were inconclusive.[57-59,166-171] Studies also showed that women's intention to pursue genetic testing decreased after counseling.[57,58,60]

The new studies include one fair-quality systematic review,[165] seven RCTs (six fair-quality[152,154,155,157,160,164] and one poor-quality[151]), one fair-quality prospective cohort study,[161,162] one good-quality case-control study,[148] and six studies with before-after designs for which quality rating criteria were not available.[149,150,153,156,158,159,163] Limitations of studies included inadequate

reporting of randomization technique,[151,152,154,157,160,164] noncomparable groups at baseline,[151,161,162] and no specified eligibility criteria.[151]

Studies enrolled from 64 to 1,971 women with family histories of breast and ovarian cancer who were seeking genetic counseling and were potentially interested in receiving genetic testing for BRCA mutations. Several studies compared different types of genetic counseling[152,154,155,157,164] and genetic counseling versus no counseling,[148,151,160-162] while others compared outcomes before and after genetic counseling.[149,150,153,156,158,159,163] The types of genetic counseling services provided are summarized in **Table 6**.

Studies used the Cancer Worry Scale (CWS) and the State-Trait Anxiety Inventory (STAI) to measure breast cancer worry; the Hospital Anxiety and Depression Scale (HADS), Impact of Events Scale (IES), General Health Questionnaire (GHQ), and Visual Analogue Scale to measure anxiety and depression; and general Likert scales to measure intention to undergo genetic testing and risk perception. These are described in **Table 7**.

Breast Cancer Worry

No studies reported increases in measures of breast cancer worry after women received genetic counseling; eight studies reported decreases,[150,152-155,157,158,162] while one study reported no changes.[151]

A fair-quality RCT measuring worry with the CWS reported that women who received either in-person or telephone counseling had significant decreases in worry after counseling compared with the control group who did not receive counseling (mean decrease from baseline, 0.90 in-person vs. 0.82 telephone vs. 0.38 none; $p=0.002$).[157] More women in the in-person counseling group felt they could discuss their concerns during counseling sessions compared with women who received telephone counseling (77.4% vs. 67.3%, respectively; $p<0.05$). Fewer women in the in-person counseling group said they would have preferred another type of counseling (14.9% vs. 37.0%, respectively; $p<0.001$).

A fair-quality RCT reported decreases in worry after both group and individual genetic counseling compared with a noncounseling control group (mean change from baseline, -0.7 group vs. -0.9 individual vs. +0.1 none; $p<0.001$).[152] Another study comparing a computer intervention with an in-person counseling session reported significant decreases in both groups after counseling, with no differences between groups.[154] Only one poor-quality RCT reported no significant difference in cancer worry after telephone counseling compared with a control group not receiving counseling, as measured on a three-item, 4-point Likert scale.[151]

A fair-quality prospective cohort study reported that more women receiving counseling experienced decreases in cancer-specific distress, as measured by the IES.[162] The cancer-specific distress of women with counseling decreased more from baseline to 1 year postcounseling (from 52% to 41%) compared with high-risk women referred for mammography with no genetic counseling (from 41% to 35%), or with a random sample from the general population (from 32% to 30%) with no counseling.[162] Although more women who had genetic counseling experienced a decrease in cancer-specific distress, this difference was only statistically significant when

compared with women in the general population (p=0.006).

Similarly, two before-after studies, using a modified CWS, reported reductions in cancer worry after genetic counseling compared with baseline.[153,158] One reported a reduction after 1 month, which became statistically significant after 1 year of followup (mean, 11.6 at baseline vs. 10.9 at 1 month vs. 10.8 at 1 year; p<0.001 for change from baseline to 1 year).[158] While the other reported reductions after 9 months that remained after 6 years, they were not statistically significant (mean, 11.54 at baseline vs. 10.37 at 9 months vs. 10.35 at 6 years; p=0.29), and no statistically significant difference was observed in those who did not receive counseling (mean, 11.29 at baseline vs. 10.39 at 9 months vs. 10.65 at 6 years; p=0.44).[153]

One before-after study (in two publications) using the IES reported that women's levels of worry decreased over time from initial levels, particularly after they were informed of their risks.[149,150] One fair-quality RCT reported significant reductions in cancer worry in women who were at moderate or high risk 6 months after genetic counseling compared with baseline, based on CWS scores.[155] Reductions were also significant when compared with women who only attended initial in-person precounseling sessions.

Anxiety and Depression

No studies reported significant increases in anxiety and depression after receiving genetic counseling; three studies reported significant decreases in anxiety and depression,[154,163,164] while three studies reported no changes.[150,158,162]

A good-quality RCT compared women receiving genetic counseling from a nurse specialist in addition to resources about informing at-risk relatives, a pamphlet, and a videotape versus women receiving the standard care given at the clinic, which was genetic counseling from a specialist nurse with no additional resources.[164] Both groups reported significant decreases in mean anxiety and depression scores, as measured by the HADS, at 2 weeks and 8 months after counseling (p<0.01 over time). However, there were no significant differences between groups at any time point and none of the mean scores reached the clinical threshold (score of ≥8).

Another study reported significant decreases in mean anxiety scores, as measured by the STAI, from before genetic counseling, when scores indicated high anxiety (score >22), to immediately and 6 months after genetic counseling, when scores fell below the threshold for high anxiety (22.22 vs. 18.77 vs. 16.98, respectively; p<0.001).[163] However, in a fair-quality RCT, anxiety scores at baseline indicated high anxiety and significantly increased from baseline to 3 months following counseling (Genetic Risk Assessment in the Clinical Environment [GRACE], 40.00 to 56.28 to 52.15 vs. counseling, 35.73 to 47.78 to 51.19; p<0.01 over time), as measured by the STAI.[154] While participants' scores in the GRACE group improved slightly at followup, they never returned to their baseline levels.

No significant differences in anxiety or depression scores were found in a fair-quality cohort study comparing women receiving genetic counseling with a high-risk reference sample and a random sample from the general population.[162] The number of women meeting clinical thresholds for anxiety and depression, as measured by the HADS, was low (<12% anxiety and

<2% depression). However, slightly more women in the counseling group had moderate levels of distress, as measured by the IES (12% vs. 8%). A before-after study reporting anxiety outcomes from baseline to 1 year after genetic counseling also reported no significant differences, though all mean scores were above the clinical threshold for psychiatric disorders.[158] In another before-after study, no significant changes in women's anxiety or depression scores were detected over time, regardless of their levels of risk.[150] In this study, only baseline scores indicated mild anxiety, and followup scores were below the clinical threshold for anxiety, as measured by the HADS.[150]

Risk Perception

A fair-quality systematic review of 19 studies published before February 2007 reported results of studies of risk perception after genetic counseling.[165] In these studies, risk perception was measured by changes in the proportion of women who accurately perceived their own risk, and by the degree of overestimation or underestimation of risk. Overall, the accuracy of risk perception increased from an average of 42 percent accuracy before counseling to 58 percent after counseling. Women who continued to overestimate their risks did so by approximately 18 percent (range, 6% to 40%), which was an improvement of approximately 8 percent after counseling. Seven studies indicated that counseling that delivered information about family history, heredity, and personal risk estimates positively influenced risk perception accuracy. Three of five studies showed significant improvement in risk accuracy when education about heredity was included, and three of six studies showed an improvement in risk accuracy when facilitating informed decisionmaking and adaptation to personal risk was part of counseling.

Eight studies published since 2004,[152,156-158,160,161,163,169] including four cited in the 2007 published systematic review,[152,156,158,169] were consistent in reporting improved accuracy of breast cancer risk perception after genetic counseling. One study reported less accuracy.[153] These findings differ from the prior USPSTF review, in which results were inconclusive.[57-59,166-171] The recent studies measured risk perception using subjects' self-rated lifetime risk of breast cancer compared with the general population (0- to 100-point scale), lifetime likeliness of developing breast cancer on a 5-point Likert scale, and comparisons between risk estimates of subjects and counselors.

A fair-quality RCT measuring perceived breast cancer risk on a 5-point scale and rating chances of diagnosis from 0 to 100 percent reported that women overestimated their risks of breast cancer by an average of 25 percentage points.[151] The proportion of women underestimating their risks was larger among women with perceived lower risks (40%) than in those who perceived it as the same (16%), higher (10%), or much higher (5%) than the risks of other women ($p=0.009$). Women with the highest overestimations were more likely to improve their accuracy with counseling ($p<0.0001$), although counseling was effective in improving accuracy only in women age 50 years or younger ($p=0.0040$).

A fair-quality RCT reported no differences in risk accuracy between telephone and in-person counseling.[157] Accuracy significantly improved over time for both groups ($p<0.001$), and was better than in a control group that did not receive genetic counseling ($p<0.001$).

A before-after study measured risk perception using a 5-point scale ranging from 1 (chances of breast cancer much lower than the average woman) to 5 (chances much higher than the average woman). There was a significant decrease from baseline to 1 week (mean, 4.29 vs. 3.83; p=0.00) and at 1 week compared with a control group (mean, 3.83 vs. 3.97; p=0.01). However, perception of risk increased at 9 months (mean, 3.99) and after 6 years (mean, 4.08), without returning to baseline levels.[153]

Only one before-after study assessed the accuracy of risk perception for developing ovarian cancer.[159] In this study, all women underestimated their risks of developing ovarian cancer by 5 percent 6 months after counseling.

Intent to Participate in Genetic Testing

Two studies reported decreased intention to undergo genetic testing after genetic counseling.[152, 157] A study comparing telephone counseling versus in-person counseling versus no counseling used a four-question measure to determine women's intentions to pursue genetic testing.[157] Participants' combined baseline scores for their intention to pursue genetic testing was 2.22 and there were no significant differences between groups at baseline. After counseling, the control group had increased intention scores, while the two counseling groups had decreased scores (mean change from baseline, +0.51 control vs. -0.61 in-person vs. -0.52 telephone; p<0.001).

A fair-quality RCT reported decreased interests in genetic testing 6 months after group and individual counseling.[152] Interests in testing for both counseling groups decreased significantly more than in the control group (mean decrease from baseline, 0.7 group vs. 0.6 individual vs. 0.2 control; p<0.01).

Key Question 2c. What Is the Clinical Validity of Genetic Testing for Deleterious Mutations in Women With Increased Risk for BRCA-Related Cancer?

Summary

In the context of this key question, clinical validity is how consistently and accurately BRCA mutation status predicts risk for BRCA-related cancer. This review describes clinical validity using the measures of prevalence and penetrance of BRCA mutations. Thirty-two new cohort, cross-sectional, and descriptive studies were combined with 38 earlier studies for meta-analysis estimates of the prevalence and penetrance of BRCA mutations in various groups of women. Limitations include heterogeneity of studies, differences between laboratory techniques for research and clinical care, lack of studies outside of high-risk populations, bias in estimates from women or families with cancer, and no studies of penetrance in women with test results indicating variants of uncertain significance.

Prevalence is the frequency of BRCA mutations in the population. Estimates of prevalence in high-risk populations overestimate assumptions of prevalence in unselected populations, but

inform an individual's likelihood of carrying a BRCA mutation and candidacy for testing. Estimates of the prevalence of BRCA mutations vary by population: 0.2 to 0.3 percent in unselected women; 1.8 percent for *BRCA1* and 1.3 percent for *BRCA2* in women with breast cancer; 6 percent in women with breast cancer onset at age 40 years or younger; 4.4 percent for *BRCA1* and 5.6 percent for *BRCA2* in women with ovarian cancer; and 13.6 percent for *BRCA1*, 7.9 percent for *BRCA2*, and 19.8 percent for both combined in women with high-risk families. For Ashkenazi Jewish women, prevalence is 2.1 percent in unselected populations and 10.2 percent in those with high-risk families.

Penetrance is the likelihood of developing breast or ovarian cancer for a given BRCA genotype, and is age dependent. Estimates of the penetrance of BRCA mutations differ by test result. In high-risk women with positive test results, risks for breast cancer to age 70 years include 46 percent for *BRCA1* and 50 percent for *BRCA2* when a single family member is tested, and 70 percent for *BRCA1* and 71 percent for *BRCA2* when multiple family members are tested. Risks for ovarian cancer to age 70 years in high-risk women with positive test results are 41 percent for *BRCA1* and 17 percent for *BRCA2* when a single family member is tested, and 46 percent for *BRCA1* and 23 percent for *BRCA2* when multiple family members are tested. Risks for Ashkenazi Jewish women to age 75 years is 34 percent for breast cancer and 21 percent for ovarian cancer.

In women with uninformative negative test results, the SIR for breast cancer is 3.81 (95% CI, 3.06 to 4.75). In women with true negative test results, the SIR for breast cancer is 1.13 (95% CI, 0.81 to 1.58). Estimates for ovarian cancer are highly heterogeneous and cannot be combined in meta-analysis.

Evidence

A total of 32 studies of prevalence and penetrance not included in the prior review met inclusion criteria,[21,172-202] in addition to 38 studies included in the prior review[13,15,16,19,20,46,47,122,188,203-231] (**Appendixes C7** and **C8**). Studies estimated prevalence for high-risk and Ashkenazi Jewish populations and penetrance for BRCA-positive, uninformative negative, and true negative results (**Figure 2**). No studies provided risk estimates for women with variants of uncertain significance. Most studies used a variety of research laboratory techniques to detect clinically significant mutations that differ from the DNA sequencing that is clinically available.

Prevalence

Unselected Populations. No direct measures of the prevalence of clinically significant *BRCA1* or *BRCA2* mutations in the general, nonJewish U.S. population have been published. Models estimate it to be about 0.2 to 0.3 percent.[13-16]

High-Risk Populations. Studies provide prevalence estimates for three different types of high-risk groups: 1) women with early-onset breast or ovarian cancer (e.g., before age 45 years), 2) women with breast or ovarian cancer from selected high-risk cohorts (e.g., consecutive cases from cancer registries or surgical units), and 3) women from high-risk families based on family history of breast and/or ovarian cancer (**Table 8**). Prevalence estimates based on high-risk groups

overestimate prevalence in unselected or general populations.[15] However, women from high-risk groups are the most likely candidates for BRCA testing and identifying them can guide testing decisions within a family.

Early-Onset Breast or Ovarian Cancer. Eleven studies reported prevalence estimates for women with early-onset breast or ovarian cancer.[13,16,174,195,199,204,207,208,218,220,223]

For *BRCA1*, the meta-analysis indicated a prevalence of 4.26 percent (95% CI, 2.61 to 6.87; 10 studies)[13,16,174,195,204,207,208,218,220,223] in women diagnosed with breast cancer at age 40 years or younger, and 5.17 percent (95% CI, 2.39 to 9.59; 2 studies)[13,195] in those diagnosed with ovarian cancer at age 40 years or younger (**Table 1**). For *BRCA2*, prevalence was 2.90 percent (95% CI, 1.35 to 6.14; 5 studies)[13,16,174,195,220] in women diagnosed with breast cancer at age 40 years or younger, and 0.64 percent (95% CI, 0.02 to 3.50) in those diagnosed with ovarian cancer at age 40 years or younger, based on only one study.[195] For *BRCA1* or *BRCA2*, the combined prevalence estimate was 5.98 percent (95% CI, 1.87 to 17.47)[16,208,220] in women diagnosed with breast cancer at age 40 years or younger. Additional estimates are described in **Table 9** and suggest higher prevalence rates in women with younger ages of cancer onset. While subject selection for the youngest age group (≤35 years) in these studies was based primarily on age at diagnosis of breast or ovarian cancer, some studies used family history information to select subjects for the older age group (≤45 years).

High-Risk Cohorts. Results of a meta-analysis of four studies based on data from breast cancer case series indicated a combined prevalence estimate for *BRCA1* of 1.84 percent (95% CI, 0.72 to 4.63).[13,194,204,223] The prevalence of *BRCA2* was 1.31 percent (95% CI, 0.67 to 1.95), based on one study.[13]

Results of a meta-analysis of four studies based on data from ovarian cancer case series indicated a combined prevalence estimate for *BRCA1* of 4.41 percent (95% CI, 2.47 to 7.74),[195,204,216, 228] with substantial heterogeneity among studies (I^2=70%; p=0.006). The prevalence of *BRCA2* was 5.61 percent (95% CI, 4.13 to 7.09), based on one study.[195]

Prevalence was also reported for racial and ethnic minorities in three studies;[195,204,223] however, the studies were small, few mutations were detected, and results were not conclusive.

High-Risk Families. Additional prevalence estimates for women from referral populations with various levels of family history range from 3.66 percent[174] to 30.8 percent[193] for *BRCA1* and from 6.1 percent[174] to 15.4 percent[193] for *BRCA2* in white, nonHispanic, nonAshkenazi Jewish women.

In 11 studies in which recruitment was based on family history of breast and/or ovarian cancer, results of the meta-analysis indicated *BRCA1* prevalence of 13.58 percent (95% CI, 10.09 to 17.07),[174,183,188,193,194,199,200,202,207,211,232] with significant heterogeneity among studies (I^2=86%; p<0.001). Heterogeneity remained high in a sensitivity analysis that excluded an outlier[193] (I^2=89%; p<0.001). Estimates were similar in sensitivity analyses that excluded two studies with mixed populations of race/ethnicity.[46,194] One study reported a *BRCA1* prevalence of 35.71 percent (95% CI, 26.92 to 44.51) in families with two or more cases of ovarian cancer.[212]

For *BRCA2*, meta-analysis results of eight studies in which recruitment was based on family history of breast and/or ovarian cancer indicated a prevalence of 7.90 percent (95% CI, 5.30 to 10.50).[174,183,188,193,194,199,202,211] One study reported a prevalence estimate of 7.14 percent (95% CI, 2.13 to 12.15) in families with histories of two or more cases of ovarian cancer.[212]

For *BRCA1* and *BRCA2* combined, the prevalence was 19.78 percent (95% CI, 12.98 to 26.57).[46, 174,183,188,193,199,200,207,211] In a study in which subjects were ascertained based on family histories of two or more cases of ovarian cancer, the estimate was 42.86 percent (95% CI, 33.79 to 51.92).[212] In a sensitivity analysis excluding an outlier,[193] prevalence decreased to 15.93 percent (95% CI, 9.21 to 22.66), although there was significant heterogeneity (I^2=94%; p<0.001). Estimates were similar in sensitivity analyses that excluded one study with mixed populations of race/ethnicity.[47]

Prevalence was also reported for racial and ethnic groups from referral populations with various levels of family history risk. One study reported a prevalence of 22.7 percent for *BRCA1* and 8.1 percent for *BRCA2* in 110 Hispanic individuals in a hereditary cancer registry.[21] No *BRCA1* or *BRCA2* mutations were detected in three Hispanic individuals tested in another study.[193] Black individuals presenting for BRCA testing in high-risk clinics had a prevalence of 16.3 percent for *BRCA1* and 11.6 percent for *BRCA2*.[193]

Ashkenazi Jewish. Five studies provided estimates of *BRCA1* prevalence in Ashkenazi Jewish populations unselected by personal or family history of breast cancer,[19,20,191,209,214] and six studies provided estimates for *BRCA2* prevalence[19,20,191,209,214,224] (**Table 10**). These studies reported the prevalence of the three founder mutations, including mutations 5382insC and 185delAG in *BRCA1* and 6174delT in *BRCA2*.

Based on the meta-analysis, prevalence for *BRCA1* was 1.2 percent (95% CI, 0.98 to 1.42)[20,209, 214] and for *BRCA2* was 1.17 percent (95% CI, 0.95 to 1.38)[20,209,214,224] (**Table 11**). For *BRCA1* and *BRCA2* combined, prevalence was 2.08 percent (95% CI, 1.28 to 2.88).[20,191,209, 214] There was significant heterogeneity among studies (I^2=89%; p<0.001), with the most recent publication[191] estimating prevalence at about half the rates of previous studies for both *BRCA1* and *BRCA2*. The new study included fewer women with family or personal histories of breast or ovarian cancer compared with other studies (e.g., personal history, 0.8% vs. 8%).[214] Also, secular trends may have influenced prevalence estimates over time. For example, high-risk families who have already been tested may not have responded to advertisements recruiting participants to more recent studies. In a sensitivity analysis that excluded results from the most recent study, prevalence for the founder mutations was 2.46 percent (95% CI, 2.13 to 2.78),[20,209,214] without significant heterogeneity among studies (I^2=0%; p=0.496).

No new studies provided prevalence estimates for Ashkenazi Jews selected for personal or family histories of breast cancer. From the previous review,[1,2] results of the meta-analysis indicated an estimated prevalence of founder mutations of 10.2 percent (95% CI, 4.2 to 22.9), including 6.4 percent (95% CI, 1.1 to 29) for *BRCA1* and 1.1 percent (95% CI, 0.6 to 2.0) for *BRCA2* in women with family histories of breast or ovarian cancer.[19,47,219]

Penetrance

Penetrance is the probability of developing BRCA-related cancer in women who have a given *BRCA1* or *BRCA2* genotype, and is reported as the cumulative risk to a specified age. The meta-analysis results reflect the age parameters and cancer outcomes provided by the studies for positive, true negative, and uninformative negative test results. There were no studies of penetrance in women with variants of uncertain significance.

BRCA-Positive Results in High-Risk Populations. There were significant methodological differences across studies that reported penetrance in women who were *BRCA1*or *BRCA2* mutation carriers. Results are reported separately depending on whether a single person (**Table 11**) or multiple individuals (**Table 12**) in a family were tested.

Eight studies reported breast cancer penetrance based on testing a single individual per family.[13,15,176,187,188,190,195,225] For *BRCA1* mutations, breast cancer penetrance was 46 percent (95% CI, 40 to 51) to age 70 years[13,15,176,188,190] (**Table 13**); for *BRCA2*, penetrance was 50 percent (95% CI, 40 to 60) to age 70 years.[13,15,176,188,190]

Eight studies reported estimates based on testing multiple individuals per family.[172,173,178,185,192,201,206,210] For *BRCA1* mutations, breast cancer penetrance was 70 percent (95% CI, 61 to 79) to age 70 years;[173,178,185,192,201,206] for *BRCA2*, penetrance was 71 percent (95% CI, 59 to 83) to age 70 years.[173,178,192,201,210] Between-study heterogeneity was significant.

Estimates were not combined across the two types of studies because of significant heterogeneity and large differences between estimates with nonoverlapping CIs. A published meta-analysis that combined all types of studies reported breast cancer penetrance in BRCA-positive women to age 70 years as 57 percent (95% CI, 47 to 66) for *BRCA1* and 49 percent (95% CI, 40 to 57) for *BRCA2*.[12] A second meta-analysis that included 22 studies based on case-series unselected for family history reported estimates of 65 percent (95% CI, 44 to 78) for *BRCA1* and 45 percent (95% CI, 31 to 56) for *BRCA2*.[11] This meta-analysis also reported significant between-study heterogeneity. The results of published meta-analyses differ from the results of this review because they included studies of women with Ashkenazi Jewish ancestry or studies in which only Ashkenazi Jewish founder mutations were tested. These populations were excluded from the meta-analysis reported in this review.

Seven studies reported ovarian cancer penetrance based on testing a single individual per family.[13,15,176,188,190,195,225] For *BRCA1* mutations, ovarian cancer penetrance was 41 percent (95% CI, 32 to 49) to age 70 years;[13,15,176,188,190] for *BRCA2*, penetrance was 17 percent (95% CI, 11 to 24) to age 70 years.[13,15,176,188] There was no significant heterogeneity between studies.

Six studies reported estimates based on testing multiple individuals per family.[173,178,192,201,206,210] For *BRCA1* mutations, ovarian cancer penetrance was 46 percent (95% CI, 35 to 57) to age 70 years;[173,178,192,201,206] for *BRCA2*, penetrance was 23 percent (95% CI, 12 to 34) to age 70 years.[173,178,192,201,210] There was significant heterogeneity between studies.

Estimates for ovarian cancer from studies of testing a single person or multiple individuals per

family were very similar and all studies were combined in additional meta-analyses. Combined measures for *BRCA1* mutations include penetrance of 45 percent (95% CI, 37 to 52) to age 70 years and for *BRCA2*, 19 percent (95% CI, 13 to 25) to age 70 years. These estimates are similar to a published meta-analysis that reported penetrance in BRCA-positive women to age 70 years as 49 percent (95% CI, 40 to 57) for *BRCA1* and 18 percent (95% CI, 13 to 23) for *BRCA2*.[12] A second meta-analysis that included 22 studies based on case-series unselected for family history reported estimates of 39 percent (95% CI, 18 to 54) for *BRCA1* and 11 percent (95% CI, 2.4 to 19) for *BRCA2*.[11]

Studies had several limitations and biases. Many studies selected families for analysis based on personal histories of breast or ovarian cancer (probands). Probands and their family members are more likely to have other risk factors for breast or ovarian cancer that may affect penetrance,[233] and breast or ovarian cancer survivors may have a different spectrum of mutations compared with women with newly diagnosed cancer. Penetrance may also depend on the specific mutation within the gene, and only one study reported penetrance estimates stratified by exons.[172]

BRCA-Positive Results in Ashkenazi Jewish Populations. Several studies described in previous sections of this review provided estimates that included Ashkenazi Jewish along with nonAshkenazi Jewish families. Only one new study reported penetrance in Ashkenazi Jewish families specifically, and these estimates combined women who were *BRCA1* and *BRCA2* mutation carriers.[187] Estimates specifically for *BRCA1* and *BRCA2* were provided in the prior review[1,2] and are similar to a published meta-analysis.[234]

In the previous meta-analysis of 10 studies,[203,204,208,209,213,214,217,226,227,231] breast cancer penetrance was 33.7 percent (95% CI, 24.1 to 44.9) to age 75 years in Ashkenazi Jewish women without family histories of breast or ovarian cancer. In those with family histories, penetrance was 34.7 percent (95% CI, 17.6 to 57.0) to age 75 years, based on nine studies.[47,203,208,209,213,214,217,224,226]

From the previous meta-analysis of five studies,[203,205,221,222,225] ovarian cancer penetrance was 21.4 percent (95% CI, 14.9 to 29.7) to age 75 years in Ashkenazi Jewish women without family histories of breast or ovarian cancer. In those with family histories, penetrance was 18.1 percent (95% CI, 7.6 to 37.3) to age 75 years, based on two studies.[47,222]

Uninformative Negative Results

An uninformative negative result can occur for several reasons, including other family members have not been tested; the family carries a BRCA mutation, but it was not detected because of limitations of the test; the family carries a high-risk mutation in another gene; or no high-risk mutation is segregating in the family.

Three studies provided data to estimate the SIR for the development of breast cancer in women with uninformative negative results compared with estimates for the general population (**Table 15**).[182,189,230] Estimates across studies were very similar, ranging from 3.25 to 3.32. The overall estimate for the SIR for breast cancer was 3.81 (95% CI, 3.06 to 4.75) (**Figure 3**).

The same three studies provided data for SIRs for the development of ovarian cancer in women

with uninformative negative results compared with estimates for the general population (**Figure 3**, **Table 14**).[182,189,230] However, these estimates varied widely across studies (0.85 to 11.6), and could not be combined because of significant heterogeneity (I^2=77.4%; p=0.012). This heterogeneity likely reflects the differing ascertainment criteria for study recruitment. The study with the lowest SIR (0.85 [95% CI, 0.23 to 3.12]) included only first-degree relatives of breast cancer cases.[189] The other studies included families with breast cancer (SIR, 3.88 [95% CI, 0.05 to 21.6])[182] and families with at least two first-degree relatives with ovarian cancer (SIR, 11.6 [95% CI, 3.12 to 29.7]).[230]

True Negative Results

A true negative result is possible for individuals who have relatives with cancer and a known BRCA mutation segregating in the family, but their own results are negative.

Ten studies provided data for the meta-analysis of SIRs for the development of breast cancer in women with true negative results compared with estimates for the general population (**Table 15**).[175,177,180,181,184-186,196,198,201] Although SIR estimates ranged from 0.39 to 2.9 across studies, the CI for all studies included the value 1.0, indicating that the estimated risk was not statistically significantly different from that in the general population. The overall combined SIR estimate for breast cancer is 1.13 (95% CI, 0.81 to 1.58) (**Figure 4**).

Most studies included women as true negatives only if their genotype was known by direct testing or could be inferred from the known genotypes of their relatives (e.g., descendants of an individual who tested negative were inferred to also be mutation negative). However, two studies probabilistically assigned genotypes for a portion of women who were untested and whose genotypes were unknown.[180,186] This approach would bias the results toward the null hypothesis of no difference between groups because of misassignment of genotypes. Also, all studies except one[180] used a prospective design that included only newly diagnosed cancer cases after the identification of the family. A study design that includes cancer diagnoses known prior to the identification of the family could falsely increase the risk estimate in relatives because the family may be more likely to seek testing. Bias could also be introduced in studies that did not control for risk-reducing salpingo-oophorectomy in the analysis.[175,177,180,198]

Two studies provided data for the SIR for the development of ovarian cancer in women with true negative results compared with estimates for the general population, although results differed (**Figure 4**, **Table 15**).[198,201] One study reported an SIR of 0 (95% CI, 0 to 12) for *BRCA1* and 0 (95% CI, 0 to 24) for *BRCA2*.[201] A second study reported an increased risk of ovarian cancer with a SIR of 4.6 (95% CI, 1.2 to 11.7).[198] However, this analysis was not conducted prospectively, and its ascertainment of families with strong family histories of breast and ovarian cancer could bias results. For this same study, the SIR estimate for breast cancer decreased from 5.3 (95% CI, 3.5 to 7.7) to 2.1 (95% CI, 0.4 to 6.2) after accounting for prospectively identified breast cancer cases only.

Key Question 3c. What Are the Potential Adverse Effects of Genetic Testing?

Summary

Thirteen cohort, case-control, and before-after studies reported distress measures and risk perception related to BRCA testing. Limitations of studies included high loss to followup and differences between comparison groups. In these studies, breast cancer worry and anxiety increased for women with positive results and decreased for others, although results differed across studies. Risk perception improved after receiving test results.

Evidence

Thirteen new observational studies met inclusion criteria,[235-249] as well as one included previously.[250] Studies provided data about distress due to BRCA testing measured as worry, anxiety, depression, or other psychosocial outcomes (**Table 16**, **Appendix C9**). No studies described other adverse effects of testing, such as false-positive or false-negative results or unnecessary risk-reducing interventions.

Of eight included cohort studies, five met criteria for good-quality,[239,240,242,245,248,250] two for fair-quality,[238,244] and one for poor-quality.[243] The remaining studies included a fair-quality case-control study[236,247] and five studies with before-after designs for which quality rating criteria were not available.[235,237,241,246,249] Limitations of studies included unclear enrollment of the cohort,[238,243,244] high loss to followup,[244] and significant differences between groups at baseline or lack of reporting of baseline participant characteristics.[238,243,244]

The studies varied in size from 17 to 10,244 women; however, the largest study was dominated by the control group (n=10,000).[240] Studies enrolled women with family histories of breast and ovarian cancer seeking genetic testing for *BRCA1* or *BRCA2* mutations. Several studies reported outcomes by mutation status,[236,238-240,242-245,247,248,250] while others compared outcomes before and after genetic testing.[235,237,241,246,249]

Descriptions of the outcome measures are provided in **Table 7**. The studies used the IES, Cancer-Related Worry scale, and CWS-R to measure breast cancer worry; the STAI, IES, Post-Traumatic Growth Inventory, HADS, GHQ, Swedish Short-Form 36-Item Health Survey, Emotional Approach Coping Scale, Multidimensional Fatigue Symptom Inventory-Short Form, Beck Hopelessness Scale, Brief Symptom Inventory, Beck Depression Inventory, and Center for Epidemiologic Studies-Depression Scale to measure anxiety and depression; and the Pittsburgh Sleep Quality Index to measure sleep disturbances.

Breast Cancer Worry

Five studies reported significant increases in breast cancer worry after receiving BRCA test results.[236,241,248-250] A good-quality prospective cohort study used a single question to measure worry on a four-item Likert scale: "During the last 2 weeks, how often did you worry about

developing breast cancer?"[248] Women who were mutation carriers had a significant increase in worry compared with women with true negative or uninformative results 1 and 7 months after disclosure of genetic testing results ($p<0.05$). A fair-quality case-control study found no differences in worry between women who were carriers and women who were noncarriers with high-risk family history, as reported by the Cancer-Related Worry scale.[236] However, when results were combined for both groups, their levels of worry were significantly higher than that of low-risk women who were not tested ($p=0.022$).

A decrease in breast cancer worry for both women who were carriers and women who were noncarriers from baseline to 3 years after disclosure of genetic test results was reported in one study (mean decrease of 1.3 and 2.2, respectively), as measured by the CWS-R.[238] This decrease was significant for women who were mutation carriers ($p=0.03$) and did not differ between groups. A study of 17 women who were mutation carriers reported an increase in breast cancer worry from baseline to 1 year after disclosure of genetic test results and a decrease at 2 years, though scores remained in the mild distress range, as measured by the IES (5.2 vs. 23.8 vs. 17.2; $p=0.05$).[249] In a good-quality cohort study, women who were carriers had higher breast cancer worry, as measured by the IES, compared with women who did not get tested (mean, 16.1 vs. 12.3, respectively; $p=0.045$).[250] One cohort study included a logistic regression bivariate analysis of responses of women undergoing genetic testing. In women without cancer, a positive genetic test result was associated with distress ($p=0.03$), while a negative result was associated with pleasant experiences with the testing process ($p=0.008$).[241]

Anxiety

Two studies reported significant decreases in anxiety scores after women received genetic test results compared with pretest evaluations, based on HADS and IES scores.[235,243] One study reported a significant decrease regardless of mutation status (mean, 5.6 pretest vs. 4.2 at 1 year posttest; $p<0.001$),[235] while the other reported a significant decrease only in women who were noncarriers ($p=0.001$).[243] A fair-quality prospective cohort study reported an increase in anxiety scores over time on the GHQ.[238] In this study, 18 percent of women who were carriers and 17 percent of women who were noncarriers were identified as having anxiety, based on the GHQ 3 years after receiving genetic test results.

Two prospective cohort studies, one good-quality[248] and one fair-quality,[244] reported significantly higher anxiety scores ($p<0.05$ in both studies), as measured by the IES or IES-R, in women receiving a positive genetic test result compared with women receiving a true negative or uninformative test result. Only one of these studies reported results in the moderate distress range on the IES at baseline for all groups; women with a true negative or uninformative test result had scores decreasing to below case threshold by 7 months.[248] One good-quality prospective cohort study reported higher anxiety scores, as measured by the HADS, in women who did not get genetic testing, but had a family history of breast cancer, compared with women who received a positive genetic test result (mean, 5.3 vs. 4.2, respectively; $p<0.05$).[239,240] However, there were no differences between groups in the prevalence of HADS-defined anxiety (24% in both groups).

In a good-quality cohort study, women who were noncarriers had lower anxiety scores on the

STAI at 7 to 10 days followup (mean, 31.6 vs. 38.5 vs. 36.8, respectively; p=0.024) compared with women who were carriers and women who did not get tested, though all scores indicated high anxiety.[250] Four studies reported no differences in anxiety either over time[237,246] or between women who were carriers, noncarriers, and age-matched controls,[236,245] with all below the case cutoff threshold.

Depression

Only one good-quality prospective cohort study reported higher depression scores, as measured by the HADS, in women who did not get genetic testing, but had a family history of breast cancer, compared with women receiving positive BRCA test results (mean, 2.9 vs. 1.7, respectively; p<0.05), though scores did not reach the threshold for clinical depression.[240] Four studies reported no differences in depression either over time[235,246] or between women who were carriers, noncarriers, and age-matched controls,[236,245] with all scores below the case cutoff threshold. In a good-quality cohort study, women who were noncarriers had lower depression scores, as measured by the Beck Depression Inventory, at 4 months followup (mean, 3.6 vs. 6.2 vs. 6.4, respectively; p=0.024) compared with women who were carriers and women who did not get tested, though scores did not reach the threshold for clinical depression.[250]

Sleep Disturbances

A fair-quality case-control study reported more subjective sleep problems, as measured by the Pittsburgh Sleep Quality Index, in women who were carriers compared with women who were noncarriers and age-matched controls (mean, 7.29 vs. 3.94 vs. 4.21, respectively; p=0.013).[247] However, actual sleep duration, latency, and wakefulness, as measured by a wrist monitor, showed no differences between groups.

Other Outcomes

Two small (n=13 and n=7) descriptive case-series studies did not meet eligibility criteria, but provided outcomes relevant to harms to familial relationships.[251,252]

A study of women with true negative test results reported that they were relieved to find out they were not carriers, and several women described feeling particularly reassured that their children would also not have the mutation.[251] Most women (10/13 [67%]) believed their risk of developing breast or ovarian cancer continued to be slightly higher than that of the general population and therefore chose to undergo intensive screening. These women also decreased their communication about mutation status with other family members, especially those who were BRCA-positive.

A study of women with test results indicating the presence of BRCA mutations indicated that women were still grappling with how to live with their carrier status 3 years after disclosure of test results.[252] Some women felt comforted by other mutation carriers in the family, but felt less comforted by the noncarriers. Several women had undergone risk-reducing mastectomy, oophorectomy, or both, and although they felt assured knowing they had done everything they could to reduce their risks of developing cancer, they also felt a loss of their natural breasts and

ovarian hormones. This study also described that women struggled with what to tell their daughters, and how and when to tell them about their mutation status.

Risk Perception

A good-quality prospective cohort study reported an 18 percent increase in the number of women who perceived their risk of breast cancer to be high or very high 5 years after receiving a positive test result for a BRCA mutation versus before receiving results (p=0.016).[242] Women who were noncarriers had a corresponding 47 percent decrease (p<0.001). Also, 20 percent more women who were mutation carriers perceived their risk of ovarian cancer to be high or very high (p=0.007), while 27 percent of women who were noncarriers perceived their risk to be low (p<0.001).

Supplemental Information on the Impact of Genetic Testing on Family Members

Testing for BRCA mutations and disclosure of mutation status can have an impact beyond the patient in the clinician's office. While there are conflicting opinions and rulings on a clinician's ethical and legal duty to warn a patient's family about hereditary disease risk,[253] patients may want to inform family members themselves.[254,255] Studies indicate that most patients feel a responsibility to share their BRCA test results with family members in order to benefit them.[256,257]

A descriptive study of 162 women who were tested for BRCA mutations and 444 relatives indicated that 69.4 percent of tested women shared their test results with at-risk relatives, but more often with female (sisters or daughters) rather than male relatives (brothers and sons) (79.9% vs. 60.4%; p<0.001).[258] More women who tested positive for a BRCA mutation indicated that they had a difficult time explaining the results compared with those with true negative or indeterminate results (14.6% vs. 0% vs. 1.4%, respectively; p<0.001). In addition, women who tested positive were more likely to indicate that they and their relatives were upset when communicating the results compared with women who had true negative or indeterminate results (upset relatives, 52.4% vs. 10.0% vs. 7.4%, respectively; p<0.001; upset patient, 19.5% vs. 0% vs. 1.9%, respectively; p<0.001).

A descriptive study of 115 women who were BRCA mutation carriers reported that all participants disclosed test results to some at-risk relatives, and 88 percent disclosed to all at-risk relatives.[259] However, only 56.8 percent of at-risk relatives subsequently underwent testing, although female relatives were more likely to have testing compared with male relatives (73% vs. 49%, respectively; p<0.01).

Four descriptive studies focused on disclosure of BRCA test results to children. Two small studies indicated that women who were mutation carriers who disclosed their positive test results to their children did so because of their concern about passing along the gene.[260,261] In a study of 13 tested parents and 22 adult children, 77 percent of children felt the disclosure had no significant impact on their emotional health, while 18 percent reported a negative impact.[262] Only 31.8 percent of children had undergone BRCA testing by the time of the survey, but 87

percent of those who had not undergone testing indicated intention to do so. A small study of children ages 11 to 17 years who had mothers with BRCA mutations reported normal scores on anxiety and depression measures (STAI) after hearing of their mother's test results.[263] However, 70 percent of children had mothers with breast cancer, and 57 percent of them had worrisome thoughts about their mother's cancer that affected their feelings at least some of the time. Children who worried about their own cancer risk were more likely to be withdrawn (p=0.02) and have somatic problems (p=0.003), and children who worried about a family member's cancer risk were more likely to have thought problems (p=0.02).

Supplemental Information on the Effects of Direct-to-Consumer Marketing of BRCA Mutation Testing

Until the U.S. Supreme Court decision against DNA patents in June 2013,[264,265] Myriad Genetics held patents on the direct DNA sequencing of *BRCA1* and *BRCA2* mutations and was the exclusive provider of clinical testing in the United States.[266] Myriad allowed other laboratories to conduct direct DNA sequencing for research purposes under strict constraints. Testing for specific known mutations, including previously identified familial types and Ashkenazi Jewish founder mutations, does not require full sequence testing and has been provided by other laboratories. Although other types of genetic tests were patented in the United States, they were nonexclusively licensed. For example, genetic testing for familial colorectal cancer has been available from multiple laboratories.[267]

Myriad launched its initial direct-to-consumer advertising campaign in 2002, targeting potential patients in specific U.S. markets. Advertising included print and electronic media to raise awareness of breast cancer susceptibility genes and encourage women to speak to their physicians about testing. A study to determine the impact of the marketing campaign on patients and physicians was conducted by the Centers for Disease Control and Prevention.[268] This study surveyed randomly selected women from the community as well as family physicians, internists, obstetrician/gynecologists, and oncologists in 2003, comparing two pilot cities with marketing campaigns (Atlanta and Denver) with two control cities that had no marketing (Seattle and Raleigh-Durham).

In pilot cities, women reported increased awareness of the BRCA test ($p<0.05$) and seeing an advertisement for the test ($p<0.05$).[268] Cities did not differ by women's interests in having the test, overall knowledge about genetic testing for breast and ovarian cancer, and if they had ever talked to health care providers or friends/family about the test.[268] Physicians' knowledge did not differ between sites.[269] In pilot cities, there were increases in patients asking about testing (adjusted odds ratio [AOR], 2.1 [95% CI, 1.6 to 2.9]), asking for referrals (AOR, 1.6 [95% CI, 1.1 to 2.4]), and asking directly for testing (AOR, 2.1 [95% CI, 1.5 to 3.0]).[269] In pilot cities, 14 percent of physicians reported an increase in the number of times they ordered BRCA testing in the previous 6 months compared with 7 percent of physicians in control cities (AOR, 1.9 [95% CI, 1.2 to 3.1]).[269]

A telephone survey to assess the impact of direct-to-consumer marketing among women of varying genetic risk was conducted in 315 women enrolled in a registry of families with cancer in Denver, a Myriad marketing site.[270] In this study, high-risk women were more knowledgeable

about the test and more likely to recall media advertisements than low-risk women (60% vs. 39%; p<0.01). Approximately 40 percent of women were interested in testing and 10 percent had increased worry about cancer after viewing the advertisements. However, women across all risk groups overstated the benefits of testing, and equal numbers of high- and low-risk women thought they would benefit from testing (51% vs. 60%).

Another study in Denver surveyed 750 low-risk women, 100 high-risk women, and 180 primary care providers in a managed care organization.[271] Sixty-two percent of patient respondents described exposure to the Myriad advertisements, and 63 percent with exposure reported that the advertisements caused no anxiety. However, some women reported anxiety from the advertisements, including women with high levels of perceived breast cancer risk (AOR, 3.23 [95% CI, 1.35 to 7.73]) and Hispanic women (AOR, 4.19 [95% CI, 1.48 to 11.83]). Women who viewed the advertisements had greater knowledge about testing. Eighty-four percent of physicians reported that the advertisements caused no strain on the doctor-patient relationship, and 80 percent reported no effect on daily clinical practice.

A study of referrals to genetic counseling in the same managed care organization in Denver was compared with a similar organization in a nonmarketed city.[272] Results indicated a 244 percent increase in referrals during the marketing campaign compared with the previous year (p<0.001), although the proportion of referrals of high-risk women declined from 69 percent to 48 percent (p<0.001) during the campaign. No changes in practice were detected in the nonmarketed organization.

Myriad has recently launched a new campaign directly targeting mammography imaging centers and primary care, obstetrician/gynecology, and surgery practices. This strategy involves risk stratification using a simple checklist administered by a physician or nonphysician (e.g., mammography technician), patient consent, and specimen collection with subsequent testing by Myriad. Results are then sent to the ordering physician who follows up as needed. The impact of this approach has not yet been evaluated.

Key Question 4. Do Interventions Reduce the Incidence of BRCA-Related Cancer and Mortality in Women With Increased Risk?

Summary

No trials of the effectiveness of intensive screening for breast or ovarian cancer in women who are BRCA mutation carriers with cancer or mortality outcomes have been published. Six observational studies that reported test characteristics of breast and ovarian cancer screening are described. Overall, the sensitivity of screening for breast cancer with MRI was higher than with mammography (71% vs. 41%), while specificity was comparable (90% vs. 95%). Sensitivity of screening for ovarian cancer was 43 percent for TVUS and 71 percent for serum CA-125 testing, and specificity was 99 percent.

There are no trials of risk-reducing medications specifically in women who are BRCA mutation carriers. A systematic review and meta-analysis of four tamoxifen and two raloxifene placebo-controlled RCTs and one head-to-head trial (Study of Tamoxifen and Raloxifen Trial [STAR]) provided efficacy outcomes for women who had various risk levels. Trials were limited by heterogeneity, and data on doses, duration, and timing of use were lacking. Tamoxifen and raloxifene reduced invasive breast cancer by 30 to 68 percent compared with placebo (7 to 9/1,000 women over 5 years); tamoxifen had a greater effect than raloxifene in the STAR trial (5/1,000 women over 5 years). Reduction was greater in women with family history of breast cancer, but CIs were overlapping. Reduction was significant for ER-positive but not ER-negative breast cancer. Noninvasive breast cancer and mortality were not significantly reduced and did not differ between medications.

Four studies reported descriptive outcomes of risk-reducing mastectomy, one study reported outcomes after salpingo-oophorectomy, and three studies reported outcomes after oophorectomy. Comparison groups varied between studies, although results were consistent. Risk-reducing bilateral mastectomy reduced breast cancer by 85 to 100 percent in high-risk women and women who were mutation carriers; oophorectomy or salpingo-oophorectomy reduced breast cancer 37 to 100 percent and ovarian cancer 69 to 100 percent in high-risk women and women who were mutation carriers. Breast cancer–specific mortality was reduced by 81 to 100 percent after risk-reducing mastectomy in one study and all-cause mortality was reduced by 55 to 100 percent after risk-reducing salpingo-oophorectomy in another study.

Evidence

Intensive Screening

Breast Cancer. No studies from the previous review met inclusion criteria for the updated review. No RCTs of the effectiveness of intensive screening to reduce breast cancer incidence or mortality in women who are at increased risk were identified by searches. Four observational studies, including three prospective studies[273-275] and one retrospective analysis of a prospective study,[276] provided descriptive information about test characteristics of screening modalities (**Table 17**, **Appendix C10**). In these studies, prevalent cases were defined as women with cancer detected on the first round of screening and incident cases were those detected on subsequent rounds.[273,277,278]

The Dutch MRI Screening Study (MRISC), a prospective study, evaluated performance characteristics of breast cancer screening in 2,157 women with 15 percent or higher cumulative lifetime risks of breast cancer, including 594 women who were BRCA mutation carriers.[278] Screening included biannual clinical breast examinations and annual concurrent contrast enhanced MRI and mammography. Digital mammography replaced film during the study period. In this study, women were categorized by mutation status or as high- or moderate-risk based on their family histories and risk factors as applied to modified Claus tables. The average age of participants at study entry was 40 years, and they were followed for a mean of 4 years. There were 97 breast cancer cases (78 invasive, 19 ductal carcinoma in situ [DCIS]) detected in 94 women, including 78 screen-detected cancer cases (15 prevalent, 63 incident), six of which were

detected at risk-reducing mastectomy, and 13 interval cancer cases detected by the woman between screening rounds after initial negative results.

Analysis of results of 75 women with breast cancer indicated significantly higher sensitivity of MRI versus mammography (71% vs. 41%; p=0.0016). Both modalities had high specificity (MRI, 90%; mammography, 95%). Including only women with invasive cancer increased the sensitivity of MRI to 77 percent and decreased that of mammography to 36 percent (MRI vs. mammography, p=0.00005). In women who were *BRCA1* carriers, the sensitivity of MRI was 67 percent versus 25 percent for mammography (p=0.0129), and for *BRCA2*, 69 percent versus 62 percent (p=1.0). Additional comparisons of the sensitivity of modalities between risk groups and by carrier status were not statistically significant. At diagnosis, 80 percent of invasive tumors were 2 cm or less in size, 39 percent were grade 3, and 31 percent were node positive. Women who were *BRCA1* carriers were more likely to experience interval cancer, were younger at diagnosis, and had larger, higher grade tumors at diagnosis compared with other risk groups ($p<0.05$ for comparisons between all subgroups).

The Magnetic Resonance Imaging Breast Screening study was a prospective multicenter study conducted in the United Kingdom that evaluated screening of high-risk women using annual contrast enhanced MRI and mammography.[274] The study enrolled 649 women, including 120 who were BRCA mutation carriers, with a median age at entry of 40 years. The duration of followup varied, but each woman completed at least two annual screenings. Thirty-five cancer cases (29 invasive, six DCIS) were detected, including two interval cancer cases.

The sensitivity of screening all women using mammography plus MRI (94%) was higher than that of using either method alone (MRI, 77%; mammography, 40%), though specificity was reduced when the methods were combined (77%) compared with either MRI alone (81%) or mammography (93%) alone. Including only invasive cancer cases increased MRI sensitivity to 86 percent, reduced mammography sensitivity to 31 percent, and increased the sensitivity of combined methods to 97 percent.

In women who were *BRCA1* mutation carriers or were related to carriers, the sensitivity of screening with MRI alone (92%) or combined with mammography (92%) was higher than that of mammography alone (23%). However, the specificity of MRI alone (79%) or MRI plus mammography (74%) was less than that of mammography alone (92%). In women who were *BRCA2* mutation carriers or were related to carriers, the sensitivity of screening with MRI plus mammography (92%) was higher than that of either method alone (MRI, 58%; mammography, 50%). The specificity of mammography alone (94%) was higher than that of MRI alone (82%) or MRI plus mammography (78%). At diagnosis, invasive cancer cases were an average 15 mm in size, 66 percent were grade 3, and 19 percent were node positive.

A prospective study of 1,325 high-risk Italian women, including 48 who were BRCA mutation carriers, evaluated a breast cancer screening program of mammography, ultrasound, and clinical breast examinations.[273] MRI screening was introduced later in the study for women who were mutation carriers. Screening intervals varied by risk category, age, and modality and ranged between 6 months and 2 years. After a median followup of 55 months, 44 breast cancer cases (28 invasive, 16 DCIS) were detected, including 36 screen-detected cases and eight interval cases. In

four women who were mutation carriers with screen-detected breast cancer, the sensitivity of screening with mammography was 50 percent, ultrasound 75 percent, ultrasound plus mammography 75 percent, and MRI 100 percent. At diagnosis, 61 percent of invasive breast cancer cases were stage I, 64 percent were less than 15 mm in size, and 36 percent were node positive.

A retrospective chart review of a prospective study of 73 women at a single institution in the United States evaluated outcomes after screening using MRI alternating with mammography every 6 months in addition to six monthly clinical breast examinations.[276] Participants were mutation carriers or first-degree relatives at a high-risk cancer clinic with a median age of 44 years who had two screening cycles and were followed for a median of 2 years. Thirteen breast cancer cases (10 invasive, three DCIS) were detected in 11 patients. The sensitivity and specificity of MRI was 92 and 87 percent respectively.

Ovarian Cancer. The previous review included a descriptive study of TVUS screening in 1,610 women with family histories of ovarian cancer and reported that only six of 61 women with abnormal scans had ovarian cancer.[279] A recently published large U.S. screening RCT, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, reported no mortality benefit of screening average-risk women ages 55 to 74 years with TVUS and serum CA-125 testing compared with usual care after a median followup of 12.4 years.[280] This trial did not report outcomes specifically for high-risk women, including those who were BRCA mutation carriers.

One new descriptive study identified in updated searches reported test characteristics of TVUS and serum CA-125 testing (**Appendix C10**).[281] A European prospective descriptive study evaluated the use of annual CA-125 measurement and TVUS from ages 30 to 35 years in women who were at increased risk. In 459 women who were BRCA carriers with complete data amounting to 1,116 annual screening visits, the sensitivity of serum CA-125 testing alone was 71 percent, TVUS alone was 43 percent, and combined modalities was 71 percent. Corresponding specificities were 99 percent for each modality alone and combined.[281] The positive predictive value was 33 percent for serum CA-125 testing alone, 20 percent for TVUS alone, and 23 percent for combined modalities. Three percent of women had abnormalities detected by one or both screening modalities, and seven ovarian cancer cases were diagnosed.

Risk-Reducing Medications

The previous review identified no trials that evaluated the use of risk-reducing medications specifically in women who are mutation carriers, although the National Surgical Adjuvant Breast and Bowel Project (NSABP P-1) trial of tamoxifen described results for 288 women who were mutation carriers and who developed breast cancer during the trial.[282] Of the eight women with breast cancer who had *BRCA1* mutations, five received tamoxifen and three received placebo (RR, 1.67 [95% CI, 0.32 to 10.70]). Of 11 women with breast cancer and *BRCA2* mutations, three received tamoxifen and eight received placebo (RR, 0.38 [95% CI, 0.06 to 1.56]). Also, 86 percent (6/7) of women with *BRCA1* mutations had ER-negative breast cancer, and 67 percent (6/9) with *BRCA2* mutations had ER-positive breast cancer.

The updated review identified no RCTs that evaluated use of risk-reducing medications

specifically in women who are BRCA mutation carriers, although several RCTs of women who had various levels of risk have been published and summarized in meta-analyses.[116,283] Four placebo-controlled trials of tamoxifen include the NSABP P-1 trial,[284] Royal Marsden trial,[285] Italian Randomized Tamoxifen Prevention Trial,[286] and the International Breast Cancer Intervention Study (IBIS-I).[287] Placebo-controlled trials of raloxifene include the Raloxifene Use for the Heart Trial (RUTH)[73] and the Multiple Outcomes of Raloxifene Evaluation trial, with its followup study, Continuing Outcomes Relevant to Evista.[288] The STAR[289] trial was a head-to-head trial that compared raloxifene with tamoxifen. Inclusion criteria varied between trials, duration of active treatment ranged from 4 to 8 years, and followup ranged from 6 to 13 years. Additional details of the trials are provided in **Appendix C11**. Trials meeting fair-quality criteria were limited by incomplete reporting of followup,[284,285,287] inadequate maintenance of comparable groups,[284,285,287] high (>30%) crossover between groups,[284] and low (<65%) numbers of participants completing all treatment years.[285,286]

Results of a published meta-analysis indicate that women randomized to either tamoxifen (RR, 0.70 [95% CI, 0.59 to 0.82]; 4 trials; 7 cases/1,000 women over 5 years) or raloxifene (RR, 0.44 [95% CI, 0.27 to 0.71]; 2 trials; 9/1,000 women) had reduced risks for invasive breast cancer compared with women randomized to placebo (**Table 18**).[116,283] Updated results of the head-to-head trial indicated greater risk reduction with tamoxifen compared with raloxifene (RR for raloxifene, 1.24 [95% CI, 1.05 to 1.47]; 5/1,000 women).[289] Tamoxifen and raloxifene reduced ER-positive but not ER-negative or noninvasive cancer in placebo-controlled trials, and had similar effects in the STAR trial. All-cause mortality was not reduced in placebo trials and was similar in the STAR trial.

Although no trials evaluated breast cancer incidence specifically in women who were BRCA mutation carriers, all trials evaluated breast cancer incidence by family history, except IBIS-I, in which 97 percent of participants reported some degree of family history.[287] No trials evaluated breast cancer or all-cause mortality outcomes based on familial risk. Trials defined a positive family history as breast cancer in any first-degree relative, except the Royal Marsden trial, which also included second-degree relatives.[285]

In women randomized to tamoxifen, invasive breast cancer risk was further reduced for those with the highest numbers of affected relatives in the NSABP P-1 (RR for no relatives, 0.54 [95% CI, 0.34 to 0.83]; RR for ≥3 relatives, 0.49 [95% CI, 0.16 to 1.34]), although CIs were overlapping[284] (**Figure 5**). The Royal Marsden trial reported similar findings (RR for 0–2 relatives, 0.51 [95% CI, 0.27 to 0.96]; RR for ≥3 relatives, 0.43 [95% CI, 0.19 to 0.95]).[285] The Italian trial reported increased breast cancer risk in women with familial risk using tamoxifen, but the risk estimate was not statistically significant (RR, 1.43 [95% CI, 0.65 to 3.15]).[286]

In women randomized to raloxifene, the Multiple Outcomes of Raloxifene Evaluation and Continuing Outcomes Relevant to Evista trials indicated a greater reduction in breast cancer risk in women with at least one affected first-degree relative (adjusted hazard ratio [HR] for no relatives, 0.55 [95% CI, 0.36 to 0.84]; HR for ≥1 relative, 0.16 [95% CI, 0.06 to 0.42])[288] (**Figure 6**). RUTH indicated no significant effect of family history.[73] The raloxifene trials were primarily designed to determine its effect on osteoporosis and heart disease outcomes and only a minority of participants reported family histories of breast cancer. In the STAR trial comparing

tamoxifen and raloxifene, the effect of family history was not statistically significant.[289]

Risk-Reducing Surgery

Mastectomy. Four studies met inclusion criteria; one from the previous review[290,291] and three from updated searches.[292-294] The prior evidence review included a retrospective descriptive study based on data from patients' medical records.[290,291] In women who underwent risk-reducing mastectomy, breast cancer was reduced by 92 percent in high-risk women compared with sister controls, and by 89.5 percent in moderate-risk women compared with Gail model-based expected incidence.[291] Postmastectomy breast cancer–related deaths were reduced by 81 percent in high-risk women compared with sister controls, and by 100 percent in moderate-risk women compared with expected rates.[290] When the high-risk group was evaluated for BRCA status, none of the 18 women who were mutation carriers developed postmastectomy breast cancer compared with the 4.5 (low-penetrance model) and 6.1 (high-penetrance model) cases that were expected.[295]

Since the prior review, three new prospective studies reported breast cancer outcomes after risk-reducing bilateral mastectomy[292-294] (**Table 19**, **Appendix C12**). Cohort studies met criteria for good-quality[294] or fair-quality,[292] and one descriptive study could not be rated for quality.[293] The fair-quality study was limited by a lack of information about groups at baseline, attrition, and followup.[292]

A study enrolling women from 22 North American and European centers evaluated outcomes for women with BRCA mutations.[292] During 2.7 years of followup, no women who had risk-reducing mastectomies were diagnosed with breast cancer compared with 34 of 585 (5.8%) women who did not have mastectomies.

Another study compared observed with expected breast cancer cases in women with BRCA mutations or who were otherwise considered at high risk. Results indicated that none of 307 women who had bilateral mastectomies were diagnosed with breast cancer, while 21.3 cases were expected.[293] In a study of women who were mutation carriers in Denmark, three of 96 (0.8% per person-year) women who underwent mastectomy were diagnosed with breast cancer versus 16 of 211 (1.7% per person-year) who did not (HR, 0.39 [95% CI, 0.12 to 1.36]), although the study was inadequately powered for this outcome.[294]

Salpingo-Oophorectomy or Oophorectomy. Four studies met inclusion criteria; one from the previous review[229] and three from updated searches.[185,292,296] The previous evidence review included a prospective cohort study of women from families with high ovarian cancer risk who had risk-reducing oophorectomy compared with first-degree relatives who were at similar risk and did not have surgery.[229] Eight ovarian cancer cases occurred in 346 relatives without surgery (2.3%) versus two cases of carcinomatosis in 44 women with surgery (4.5%). Also, 14 cases of breast cancer occurred in relatives without surgery (4.0%) versus three cases in women with surgery (6.8%). Mean followup time was not reported for this study, but person-years ranged from 460 to 1,665. This study met criteria for poor-quality and was limited by a lack of information about groups at baseline, methods for ascertaining exposures and outcomes, followup, and attrition.[229]

One new study of risk-reducing salpingo-oophorectomy[292] and two new studies of oophorectomy[185,296] in high-risk women and women who were mutation carriers met inclusion criteria (**Table 19**, **Appendix C12**). Two studies[185,292] met criteria for fair-quality and one was descriptive.[296] The fair-quality studies were limited by a lack of information about groups at baseline, attrition, and followup.[185,292]

In a prospective cohort study evaluating the outcomes of women who were BRCA mutation carriers at 22 North American and European centers, salpingo-oophorectomy was significantly associated with reduced incidence of ovarian or primary peritoneal cancer (1.3% vs. 5.8%; HR, 0.28 [95% CI, 0.12 to 0.69]).[292] In addition, salpingo-oophorectomy was associated with reduced breast cancer incidence (11.6% vs. 21.6%; HR, 0.54 [95% CI, 0.37 to 0.79]) and all-cause mortality (1.8% vs. 5.9%; HR, 0.45 [95% CI, 0.21 to 0.95]). Reductions in breast cancer–specific (0.5% vs. 2.3%; HR, 0.27 [95% CI, 0.05 to 1.33]) and ovarian cancer–specific mortality (0.7% vs. 2.5%; HR, 0.39 [95% CI, 0.12 to 1.29]) were not statistically significant.

In a prospective cohort study of women from families with known *BRCA1* mutation carriers, oophorectomy was associated with reduced breast cancer incidence (18% vs. 42%; HR, 0.38 [95% CI, 0.15 to 0.97]).[185] Risk reduction was most pronounced in women who had the procedure at a younger age.

A retrospective study compared observed versus expected breast cancer incidence rates in women who underwent oophorectomy.[296] In this study, oophorectomy was associated with reduced risks that were more pronounced in women who were younger than age 50 years and premenopausal at time of surgery (O/E = 1/3.9; RR, 0.26 [95% CI, 0.001 to 0.99]) compared with older postmenopausal women (O/E = 3/5.4; RR, 0.56 [95% CI, 0.11 to 1.33]).

Key Question 5. What Are the Potential Adverse Effects of Interventions to Reduce Risk for BRCA-Related Cancer?

Summary

For breast cancer screening, the adverse effects of intensive screening were described in three studies of physical harms and two studies of anxiety. Results indicated that false-positive rates, unnecessary imaging, and unneeded surgeries were higher in women undergoing intensive screening using MRI versus mammography. Most women experienced no anxiety after breast cancer screening with MRI, mammography, or clinical breast examination. Two studies described harms of ovarian cancer screening; one reported an unneeded diagnostic surgery rate of 55 percent in women who were mutation carriers screened with TVUS and serum CA-125 testing.

There are no trials of risk-reducing medications specifically in women who are BRCA mutation carriers. A systematic review and meta-analysis of four tamoxifen and two raloxifene placebo-controlled RCTs and one head-to-head trial provided adverse event outcomes for women who had various levels of risk. Trials were limited by heterogeneity and data on long-term effects were incomplete. Tamoxifen and raloxifene increased thromboembolic events compared with

placebo (4 to 7/1,000 women over 5 years) and tamoxifen had a greater effect than raloxifene (4/1,000 women over 5 years). Tamoxifen increased endometrial cancer compared with placebo (4/1,000 women over 5 years) and raloxifene (5/1,000 women over 5 years), and increased cataracts compared with raloxifene (15/1,000 women over 5 years). Both caused undesirable side effects in some women, such as vasomotor symptoms.

Case-series and before-after studies described surgical complications, physical effects, and distress measures related to risk-reducing surgery. Studies lacked important outcomes, enrolled small numbers of participants, and had no comparison groups. Some women experienced physical complications of surgery, had postsurgical symptoms, or changes in body image, while some women had improved anxiety.

Evidence

Intensive Screening

Breast Cancer. The previous review identified no studies with information about the harms of intensive screening for breast cancer. The updated review includes three studies, in four publications, reporting false-positive or false-negative results, unneeded procedures, or recall rates (**Table 20**, **Appendix C13**),[274,276,277,297] and two studies about discomfort, pain, or anxiety (**Table 21**, **Appendix C14**).[275,298]

In studies of false-positive or false-negative results, unneeded procedures, or recall rates, women with increased familial risk of breast cancer were recruited from the Netherlands, the United Kingdom, and the United States. Two studies used prospective designs,[274,277,297] and one retrospectively analyzed data from a completed prospective study.[276] Sample sizes ranged from 73 to 1,909, and 18 to 100 percent of participants were BRCA mutation carriers. Mean/median age at entry was 40 to 44 years, and mean/median followup was approximately 2 years or at least two annual scans by the time of analysis.[277,297]

Two studies reported false-positive rates of mammography compared with MRI.[276,297] The Dutch MRISC reported results by screening round, and defined the false-positive rate as the number of positive test results in women who did not have cancer. The false-negative rate was defined as the number of negative test results in women who had cancer. This study reported significantly higher false-positive rates for MRI compared with mammography in the first and subsequent imaging rounds (first round with prior mammography, 14% vs. 5.5%; subsequent rounds, 8.2% vs. 4.6%; $p<0.001$ for both rounds).[297] False-negative results for MRI were lower than for mammography, although numbers were small.

In a study of six monthly breast cancer screenings using MRI alternating with mammography, a result was considered a false-positive if initial findings on screening appeared suspicious, but followup clinical examination, imaging, or biopsy resulted in a final benign assessment. This study reported similar false-positive results for both modalities (MRI, 11%; mammography, 15%), and did not report false-negative findings.[276]

Two studies reported the number of unneeded additional imaging procedures or biopsies.[276,277]

These procedures were considered unneeded because final results were benign and women may never have undergone the procedures if the original screening test had not been performed. The Dutch MRISC determined the need for additional procedures using the Breast Imaging Reporting and Data System (BI-RADS) score from the screening examination. Women with BI-RADS scores of 3 (probably benign) or 0 (need additional imaging evaluation) underwent further evaluations using ultrasound with or without fine-needle aspiration or repeat mammography or MRI. Women with BI-RADS scores of 4 (suspicious abnormality) or 5 (highly suggestive of malignancy) underwent biopsy. Results indicated that 43 percent of women with unneeded biopsies had preceding screening MRI and 28 percent had mammography.[277]

A study that retrospectively analyzed data from a prospectively followed cohort of women who were BRCA mutation carriers or their first-degree relatives found that alternating MRI with mammography screening every 6 months yielded a greater proportion of unneeded imaging procedures (targeted ultrasound) in women screened with mammography (8/11) than with MRI (4/8).[276] However, rates of unneeded biopsies were similar (3/11 for mammography and 2/8 for MRI).

Recall rates for annual MRI were higher than for annual mammography in a descriptive study conducted in the United Kingdom that included women who were mutation carriers (MRI, 11% per woman-year; mammography, 3.9% per woman-year; combined, 13% per woman-year).[274] In this study, 245 of 279 recalls were for benign findings, amounting to 8.5 recalls per cancer detected.

A fair-quality prospective cohort study of women with a mean age of 40.9 years compared discomfort, pain, and anxiety of women undergoing intensive screening with annual mammography, MRI, and biannual clinical breast examinations with women only receiving biannual clinical breast examinations.[275] These outcomes did not differ between groups, as measured by the Medical Outcomes Study 36-Item Short Form (**Table 21**, **Appendix C14**).[275] Most women experienced no anxiety after each type of screening intervention (72% after mammography, 63% after MRI, 78% after clinical breast examination).

In a before-after study of MRI plus mammography, ultrasound, and clinical breast examination, women who were recalled reported higher anxiety scores compared with women who were not recalled at 4 to 6 weeks after screening (8.8 vs. 5.9, respectively; p=0.03).[298] These represent midrange scores, as measured by the HADS. Between-group differences were not significant by 6 months (7.1 vs. 5.9, respectively).

Ovarian Cancer. Two studies met inclusion criteria, one from the previous review[279] and one from updated searches.[281] A prospective descriptive study included in the previous review estimated false-positive results for TVUS when used for screening for ovarian cancer in 1,601 self-referred asymptomatic women with at least one relative who was diagnosed with ovarian cancer.[279] Forty-three percent of women were screened with only one ultrasound. In this study, 3.8 percent (61/1,601) of screened women had suspicious findings on TVUS and were referred to surgery. Cancer was detected in six of 61 referred cases, yielding a false-positive rate of 3.4 percent (95% CI, 2.6 to 4.5). Addition of color flow imaging to ultrasound reduced the number of false-positive cases from 55 to six.

The updated review identified a descriptive study conducted in the Netherlands that reported the number of unneeded diagnostic surgeries associated with ovarian cancer screening using annual serum CA-125 measurements and annual TVUS in 459 women who were BRCA mutation carriers[281] (**Appendix C13**). Abnormalities were detected in 9 percent (40/459) of women with complete data, which included 3 percent (38/1,116) of screening visits, as well as visits for symptomatic complaints. Of 26 diagnostic procedures, cancer was not detected in 67 percent (4/6) following abnormal CA-125 measurement compared with 100 percent (9/9) following abnormal TVUS findings. Combined modalities resulted in an unneeded diagnostic surgery rate of 55 percent (6/11).

Risk-Reducing Medications

No studies evaluated the adverse effects of risk-reducing medications specifically in women who are BRCA mutation carriers, although adverse effects were reported in several RCTs of women who had various levels of risk and have been summarized in meta-analyses.[116,283] Studies include four placebo-controlled trials of tamoxifen,[284-287] two placebo-controlled trials of raloxifene,[73,288] and a head-to-head RCT of tamoxifen versus raloxifene.[289] No adverse effect outcomes were provided specifically by mutation status or family history risk in these trials. Details of the trials are provided in **Appendix C11**. Fair-quality trials were limited by incomplete reporting of followup,[284,285,287] inadequate maintenance of comparable groups,[284,285,287] high (>30%) crossover between groups,[284] and low (<65%) numbers of participants completing all treatment years.[285,286]

In these trials, thromboembolic events were increased for tamoxifen (RR, 1.93 [95% CI, 1.41 to 2.64]; 4 trials; 4 cases/1,000 women over 5 years) and raloxifene (RR, 1.60 [95% CI, 1.15 to 2.23]; 2 trials; 7/1,000 women over 5 years) compared with placebo (**Table 22**).[116,283] Raloxifene caused fewer events than tamoxifen in STAR (RR, 0.77 [95% CI, 0.60 to 0.93]; 4/1,000 women over 5 years).[289] Coronary heart disease events or stroke were not increased in placebo-controlled trials, and did not differ in STAR, although women randomized to raloxifene had higher stroke mortality than placebo in RUTH (RR, 1.49 [95% CI, 1.00 to 2.24]).[299]

Tamoxifen caused more cases of endometrial cancer (RR, 2.13 [95% CI, 1.36 to 3.32]; 3 trials; 4/1,000 women over 5 years), and was related to more benign gynecologic conditions, surgical procedures (including hysterectomy), and uterine bleeding than placebo.[116,283] Raloxifene did not increase risk for endometrial cancer or uterine bleeding.[116,283] In the STAR trial, raloxifene caused fewer cases of endometrial cancer (RR, 0.55 [95% CI, 0.36 to 0.83]; 5/1,000 women over 5 years), hyperplasia, and procedures than tamoxifen.[289] Women using tamoxifen had more cataract surgeries than placebo in the NSABP P-1 trial.[284] Raloxifene did not increase risk for cataracts or cataract surgery compared with placebo, and caused fewer cataracts than tamoxifen in STAR (RR, 0.80 [95% CI, 0.72 to 0.95]; 15/1,000 women).[289]

Most common side effects were vasomotor symptoms and vaginal discharge, itching, or dryness for tamoxifen and vasomotor symptoms and leg cramps for raloxifene. In STAR, raloxifene users reported more musculoskeletal problems, dyspareunia, and weight gain, while tamoxifen users had more gynecological problems, vasomotor symptoms, leg cramps, and bladder control symptoms.[289]

Risk-Reducing Surgery

Mastectomy. The prior review found no studies that met inclusion criteria for the physical harms of mastectomy, though it described a series of 112 high-risk women, including 79 who were mutation carriers, undergoing risk-reducing mastectomy with immediate reconstruction. Twenty-one percent had physical complications, including hematomas, contracture, or implant rupture.[300]

Four descriptive studies about surgical complications, physical effects, or distress related to risk-reducing surgery met inclusion criteria for the updated review.[301-305] Three studies reported information on physical harms of risk-reducing mastectomy.

In a case-series of 122 women who had undergone mastectomy, 64.4 percent reported postsurgical symptoms of numbness, pain, tingling, infection, swelling, breast hardness, bleeding, organizing hematoma, failed reconstruction, breathing problems, thrombosis, and pulmonary embolism.[301] In a study of pain after surgery using the Health-Related Quality of Life tool, there were no significant differences between women's scores obtained before mastectomy and either 6 months or 1 year postmastectomy.[302]

A case-series from the Karolinska University evaluated the physical effects of risk-reducing mastectomy and immediate breast reconstruction in 59 high-risk women.[303] Questionnaires were sent to study subjects at least 2 years after the mastectomy and at least 1 year after any corrective procedures. Eleven patients had postoperative infections and three of them needed implant extraction, four reported hematomas, two needed revisions of flap necrosis, and 35 required corrective procedures. Of the 55 patients who completed the questionnaires, 48 reported postmastectomy pain and discomfort. Of these, five required occasional pain medication and 12 reported that pain affected their daily lives.

Four descriptive studies, in five publications, provided data about distress due to mastectomy to reduce risk for BRCA-related cancer in women who were at increased risk because of family history or BRCA mutation (**Table 23**, **Appendix C14**).[301-305]

A before-after study enrolled 90 high-risk women who had risk-reducing bilateral mastectomies, including 41 percent (37/90) *BRCA1* mutation carriers, 14 percent (13/90) *BRCA2* mutation carriers, 29 percent (26/30) with 50 percent lifetime risk, and 9 percent (98/90) with 25 percent lifetime risk. Results indicated significant decreases in anxiety scores, as measured by the HADS, 6 months and 1 year after surgery compared with before surgery (mean, 3.80 vs. 3.83 vs. 5.59, respectively; p=0.0004).[302,304] The study also reported decreased pleasure in sexual activity, as measured by the pleasure subscale of the Sexual Activity Questionnaire (SAQ), 1 year after surgery compared with 6 months after surgery and before surgery (mean, 11.18 vs. 12.21 vs. 12.28, respectively; p=0.005). Depression scores, body image concerns, or any other portion of the SAQ were not significantly different.

A case-series study of 59 women undergoing risk-reducing mastectomy compared with a reference sample of 1,725 women from a previous study of women considering risk-reducing mastectomy reported no significant differences on any psychological or sexual activity measures.[303] These measures also did not differ in a separate case-series of women undergoing

risk-reducing mastectomy that compared women younger versus older than age 50 years.[301]

A descriptive case-series study, utilizing semistructured interviews, described physical and psychological effects in 13 women 10 years after risk-reducing mastectomy. Most women reported that their family lives were unchanged (8/13 [62%]), although 39 percent (5/13) reported a negative effect on their relationship with their spouse, due to decreased sensation and changed body appearance.[305] Most women considered the cosmetic results positive (10/13 [77%]) and most had discussed breast cancer risk with their daughters (10/11 [91%]).

Salpingo-Oophorectomy. The prior review found no studies that met inclusion criteria, though it included a descriptive study of risk-reducing salpingo-oophorectomy in women who were mutation carriers that included 70 percent of participants with personal histories of breast cancer. Four out of 80 women who underwent salpingo-oophorectomy without hysterectomy experienced complications of wound infection, bladder perforation, small bowel obstruction, and uterine perforation.[79]

Only one new study was identified for the updated review. A before-after study of women who were mutation carriers with a mean age of 47.5 years included 47 women with personal histories of breast cancer and 67 women without. Most women reported significant worsening of vasomotor symptoms ($p<0.01$), as measured by the Menopause-Specific Quality of Life-Intervention scale, and decreased sexual functioning ($p<0.05$), as measured by the SAQ, after risk-reducing salpingo-oophorectomy.[306]

CHAPTER 4. DISCUSSION

Summary of Review Findings

A summary of the findings of the systematic review and meta-analysis is provided in **Table 24**. No studies directly addressed the overarching question regarding the effectiveness of risk assessment, genetic counseling, and genetic testing in reducing cancer incidence and mortality (key question 1).

Several studies of the accuracy of methods to assess familial risk for BRCA-related cancer by nongenetics specialists met inclusion criteria for key question 2a, but no studies met criteria for key question 3a regarding potential adverse effects (**Figure 7**). Although various clinical criteria for referral to genetic counseling have been developed, their accuracy in predicting mutation or cancer risk has not been evaluated. A published systematic review of studies of 13 general breast cancer risk models, such as the Gail model, indicated that they are modest predictors of individual risk (c-statistic, 0.55 to 0.65). Ten studies evaluated the accuracy of five familial risk models that predict risk specifically for BRCA mutations and are intended to guide referrals to genetic counseling. These include the FHAT, Manchester Scoring System, RST, PAT, and FHS-7. Results indicated high accuracy (c-statistic, >0.80), although some models have only been evaluated in single studies. Reference standards and study designs varied across studies, limiting comparisons between models. Risk was most often based on self-reported information; thus, the accuracy of risk models was limited by the accuracy of reported family history in each study.

A new systematic review and several new RCTs and cohort, case-control, and before-after studies of distress, accuracy of risk perception, and intention for genetic testing evaluated benefits and harms of genetic counseling (key questions 2b and 3b). No studies reported increased measures of breast cancer worry after women received genetic counseling; seven studies reported decreased worry, while one study reported no changes. Also, no studies reported significant increases in anxiety or depression after receiving genetic counseling, while three studies reported significant decreases and three reported no changes. In most studies, anxiety and depression scores were below clinical thresholds.

Eight new studies reported that the accuracy of a woman's perception of her breast cancer risk improved after genetic counseling. Two new studies reported decreased intention to undergo genetic testing after genetic counseling. The new studies expand and support the results of 11 studies included in the previous evidence review (**Figure 7**). Studies were limited by differences in their designs and measures, use of dissimilar comparison groups, and enrollment of small numbers of women from specialty clinics.

Key question 2c concerns how consistently and accurately BRCA mutation status predicts risk for BRCA-related cancer (clinical validity). To address this question, 31 new cohort, cross-sectional, and descriptive studies were combined with 39 earlier studies for meta-analysis estimates of the prevalence and penetrance of BRCA mutations in various groups of women (**Figure 8**). Prevalence varied by population, including 0.2 to 0.3 percent in unselected women; 1.8 percent for *BRCA1* and 1.3 percent for *BRCA2* in women with breast cancer; 6 percent in

women with breast cancer onset at age 40 years or younger; 4.4 percent for *BRCA1* and 5.6 percent for *BRCA2* in women with ovarian cancer; and 13.6 percent for *BRCA1*, 7.9 percent for *BRCA2*, and 19.8 percent for combined *BRCA1* and *BRCA2* in women with high-risk families. In Ashkenazi Jewish women, prevalence was 2.1 percent in unselected populations and 10.2 percent in those with high-risk families.

In high-risk women with positive test results, risk for breast cancer to age 70 years included 46 to 70 percent for *BRCA1* and 50 to 71 percent for *BRCA2*; risk for ovarian cancer was 41 percent for *BRCA1* and 17 percent for *BRCA2* (**Figure 8**). In Ashkenazi Jewish women, risk to age 75 years was 34 percent for breast cancer and 21 percent for ovarian cancer. No estimates are available for women with variants of uncertain significance. In women with uninformative negative test results, the SIR for breast cancer was 3.81 (95% CI, 3.06 to 4.75). In women with true negative test results, the SIR for breast cancer was 1.13 (95% CI, 0.81 to 1.58). Estimates for ovarian cancer were highly heterogeneous. Limitations included differences between laboratory techniques for research and clinical care, lack of studies outside of high-risk populations, and bias in estimates from women or families with cancer.

Studies of potential adverse effects of genetic testing (key question 3c) reported that breast cancer worry and anxiety increased for women with positive results and decreased for others, although results differed (**Figure 8**). Risk perception improved after receiving test results. Studies were limited by high loss to followup and differences between comparison groups. Other relevant outcomes were not studied, including false-negative or false-positive results, genetic discrimination, and insurability.

Interventions to reduce the incidence of BRCA-related cancer and mortality in women with increased risk include intensive screening, risk-reducing medications, and risk-reducing surgery (key question 4). No trials evaluated the effectiveness of intensive screening. Although no trials of risk-reducing medications specifically in women who are BRCA mutation carriers were available, several RCTs that included women with various levels of risk are relevant. Tamoxifen and raloxifene reduced invasive breast cancer by 30 to 68 percent compared with placebo, and tamoxifen had a greater effect than raloxifene in a head-to-head trial (**Figure 9**). Results suggested that reduction was greater in women with more relatives with breast cancer, but CIs overlapped. Reduction was significant for ER-positive but not ER-negative breast cancer. Noninvasive breast cancer and mortality were not significantly reduced and did not differ between medications. Trials were limited by heterogeneity and data were lacking on doses, duration, and timing of use.

For high-risk women and women who are mutation carriers, observational studies indicated that risk-reducing bilateral mastectomy reduced breast cancer by 85 to 100 percent, and oophorectomy or salpingo-oophorectomy reduced breast cancer by 37 to 100 percent and ovarian cancer by 69 to 100 percent. Breast cancer–specific mortality was reduced by 81 to 100 percent after risk-reducing mastectomy in one study, and all-cause mortality was reduced by 55 to 100 percent after risk-reducing salpingo-oophorectomy in another. Comparison groups varied between studies, although results were consistent.

Studies of the potential adverse effects of intensive screening for breast cancer (key question 5)

indicated that false-positive rates, unnecessary imaging, and unneeded surgery were higher in women undergoing intensive screening using MRI compared with mammography (**Figure 9**). In one study, most women experienced no anxiety after breast cancer screening with MRI, mammography, or clinical breast examination. Studies of ovarian cancer screening reported high unneeded diagnostic surgery rates after screening with TVUS and serum CA-125 testing.

Trials of risk-reducing medications indicated that tamoxifen and raloxifene increased thromboembolic events compared with placebo and tamoxifen had a greater effect than raloxifene. Tamoxifen increased endometrial cancer and cataracts. Both caused undesirable side effects for some women, such as vasomotor symptoms.

Case-series and before-after studies described surgical complications, physical effects, and distress measures related to risk-reducing surgery. Some women experienced physical complications of surgery, postsurgical symptoms, or changes in body image, while some women had improved anxiety. Studies lacked important outcomes, and the few available studies had small numbers of participants and no comparison groups.

Limitations

Limitations of this review include using only English-language articles and studies applicable to the United States, although this focus improves its relevance to the USPSTF recommendation. Also, the number, quality, and applicability of studies evaluated in the evidence review varied widely. Limitations of studies specific to each key question are briefly described in **Table 24**.

Most studies in this review were conducted in highly-selected samples of women, many with preexisting breast or ovarian cancer, from high-risk groups, or from previously identified kindreds. How the results of studies based on these highly-selected women in research settings translate to a general screening population is unknown. In some cases, data to determine penetrance came exclusively from one study, and when multiple studies were available, they were heterogeneous. Estimates may therefore be unreliable. Most studies used research laboratory techniques to detect clinically significant mutations that differ from the DNA sequencing available clinically. The clinical significance of mutations was determined by each study, and was based on likely functional significance and/or previous evidence of increased cancer risk.

Data are not available to determine the optimal age at which to test and how age at testing influences benefits and harms. Whether testing for BRCA mutations reduces cause-specific or all-cause mortality and improves quality of life is unknown. The harms associated with receiving a false-negative test result or a result indicating mutations of unknown significance are not known.

The systematic review focused on five key questions that limited its scope. Several relevant issues were not addressed. These include the impact of modifier genes on estimates of penetrance[307-311] and estimates for cancer susceptibility genes other than *BRCA1* and *BRCA2*.[312-315] The prevalence of *BRCA1* and *BRCA2* mutations outside of U.S. or European populations was

also not evaluated. Indications for testing in women who have previously been diagnosed with breast or ovarian cancer, or estimation of their risk of contralateral breast cancer,[201,316-319] were not considered because the review focused on women without cancer. For example, women with triple-negative (i.e., HER2-negative, ER-negative, and PR-negative) breast cancer may be more likely to carry *BRCA1* mutations.[320] Also, the review did not consider indications for use of the BRCA Rearrangement Test as an adjunct to standard clinical testing, an emerging practice in the United States. The clinical utility of genetic testing is determined by outcomes following testing. Clinical utility was not explicitly included in the key questions, although the review considered use of risk-reducing interventions after genetic testing. Most studies relating to clinical utility are descriptive case-series and important outcomes are lacking. Finally, men were not included in the scope of this review except as family members of the women under evaluation.

Evidence of harms often relied on observational studies with designs that lacked quality rating criteria. Existing studies show that most women do not experience adverse effects from BRCA risk assessment, counseling, and testing. However, the long-term impact is unknown because most studies followed patients for less than 1 year. Studies used several types of measures and scales that limited comparisons between studies and prohibited meta-analysis. Measures of anxiety or depression often lacked clinical thresholds, and when available, few studies reported results based on the number of individuals who met them. No studies measured genetic discrimination as a harm of testing.

Treatment effects are influenced by several factors that were not evaluated in studies. The effectiveness of salpingo-oophorectomy in reducing risk for breast cancer depends on the age at which the procedure is performed, and it becomes less effective when performed after menopause. However, it is not clear how and when the benefit/harm ratio shifts for individuals facing this decision. Also, the type of risk-reducing intervention selected by women who are mutation carriers may depend on the specific mutation; for example, women with *BRCA1* mutations have a higher risk of ovarian cancer than those with *BRCA2* mutations. Medications are most effective in reducing risk for ER-positive breast tumors, although they have not been specifically evaluated in women with BRCA mutations. The proportion of ER-positive tumors varies from 28 percent in women with *BRCA1* mutations to 63 percent in women with *BRCA2* mutations. How these factors influence patient decisionmaking and eventual clinical outcomes is unknown.

Emerging and Future Research

In order to determine the appropriateness of risk assessment and testing for BRCA mutations in primary care, more information is needed about mutation prevalence and impact in the general population. Research has focused on highly-selected women in referral centers and generally reported short-term outcomes. Issues such as access to testing; effectiveness of screening approaches, including risk stratification; use of system supports; and patient acceptance and education require additional study. Who should perform risk assessment and genetic counseling services, how it should be done, and what skills are needed are unresolved questions. Trials comparing types of providers and protocols could address these issues. What happens after patients are identified as high-risk in clinical settings is also unknown. The consequences of

genetic testing for individuals and their relatives require more study. Well-designed investigations that use standardized measures and enroll subjects who reflect the general population, including minority women, are needed.

An expanded database or registry of patients who receive genetic counseling and testing for BRCA mutations would provide essential information about predictors of cancer, response to interventions, and other modifying factors. Traditionally, all clinical testing through direct DNA sequencing in the United States was done by a single private laboratory, and patient data were inaccessible. Developing a centralized accessible database with key variables to address these issues as testing practices change in the wake of the recent U.S. Supreme Court decision on DNA patents[265] would be a major advance in this field. Additional data from women of varying socioeconomic, racial, and ethnic groups are needed. Currently available risk prediction tools and interventions may not apply to these populations.

Additional research on interventions is needed. Without effectiveness trials of intensive screening, practice standards have preceded supporting evidence. For example, while intensive screening with annual TVUS and serum CA-125 testing is recommended for high-risk women, there are no trials of screening effectiveness, and a descriptive study of 3,532 European women who were at increased risk of ovarian cancer, receiving TVUS and serum CA-125 testing, and followed for up to 16 years indicated no stage shifts in disease incidence.[321] Trials of risk-reducing medications in women who are mutation carriers that include aromatase inhibitors, evaluation of the effect of age at intervention on outcomes, and measurement of long-term outcomes are also needed. Comparisons of salpingo-oophorectomy versus more limited surgery would inform current practice. Studies of factors related to acceptance of risk-reducing interventions based on genetic information would be useful, such as determining if cancer incidence in relatives is reduced because they adopt risk-reducing interventions. This information could improve patient decisionmaking and lead to better health outcomes.

Conclusions

Risk assessment by nongenetics specialists using familial risk models to determine individual risks for BRCA mutations can accurately guide referrals for genetic counseling. Comprehensive risk evaluations by genetic counselors provide estimates of individual risks for mutations and identify optimal candidates for genetic testing. Genetic counseling reduces distress, improves patients' risk perception, and reduces their intentions for genetic testing. Results of genetic testing provide estimates of an individual's chances of developing BRCA-related cancer depending on the specific test results. Women with positive test results have a 34 to 71 percent chance of developing breast cancer and 17 to 41 percent chance of developing ovarian cancer by age 70 years. Estimates for women with variants of uncertain significance are not available. Women with uninformative negative results have nearly a four-fold increase in risk for breast cancer; those with true negative results have no increased risk for breast cancer, while estimates for ovarian cancer are uncertain.

Although intensive screening for breast and ovarian cancer with MRI, TVUS, and serum CA-125 testing are recommended by experts for women who are mutation carriers, their effectiveness has

not been evaluated. Intensive breast cancer screening with MRI increases sensitivity, but also causes more false-positive results and procedures; screening for ovarian cancer is not accurate and leads to more procedures. Tamoxifen and raloxifene reduce risk for breast cancer in women with varying levels of risk, but increase risk for thromboembolic events. Tamoxifen also increases risk for endometrial cancer. Risk-reducing mastectomy and salpingo-oophorectomy are effective in reducing breast and ovarian cancer in women who are mutation carriers and high-risk women.

The process of familial risk assessment by nongenetics specialists, referral and evaluation by genetic counselors, genetic testing, and use of intensive screening and risk-reducing medications and surgeries is complex. Each step of the pathway requires careful interpretation of information, consideration of future risks, and shared decisionmaking before moving on to the next step. Services must be well integrated and highly individualized in order to optimize benefits and minimize harms for patients as well as their families. Additional studies are necessary to better inform practice.

REFERENCES

1. Nelson HD, Huffman LH, Fu R, et al. Genetic Risk Assessment and BRCA Mutation Testing for Breast and Ovarian Cancer Susceptibility. Evidence Synthesis No. 37. Rockville, MD: Agency for Healthcare Research and Quality; 2005. PMID: 20722133
2. Nelson HD, Huffman LH, Fu R, et al. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: systematic evidence review for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2005;143(5):362-79. PMID: 16144895
3. U.S. Preventive Services Task Force. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: recommendation statement. *Ann Intern Med.* 2005;143(5):355-61. PMID: 16144894
4. U.S. Department of Health and Human Services. What Are My Preventive Care Benefits? Washington, DC: U.S. Department of Health and Human Services; 2013. Accessed at https://www.healthcare.gov/what-are-my-preventive-care-benefits/ on 25 November 2013.
5. U.S. Department of Health and Human Services. Healthy People 2020. Washington, DC: U.S. Department of Health and Human Services; 2013. Accessed at http://www.healthypeople.gov/2020/topicsobjectives2020/overview.aspx?topicid=15 on 25 November 2013.
6. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*. 1994;266(5182):66-71. PMID: 7545954
7. Wooster R, Weber BL. Breast and ovarian cancer. *N Engl J Med.* 2003;348(23):2339-47. PMID: 12788999
8. Zweemer R, van Diest P, Verheijen R, et al. Molecular evidence linking primary cancer of the fallopian tube to BRCA1 germline mutations. *Gynecol Oncol.* 2000;76(1):45-50. PMID: 10620440
9. Medeiros F, Muto M, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am J Surg Pathol.* 2006;30(2):230-6. PMID: 16434898
10. Tone A, Begley H, Sharma M, et al. Gene expression profiles of luteal phase fallopian tube epithelium from BRCA mutation carriers resemble high-grade serous carcinoma. *Clin Cancer Res.* 2008;14(13):4067-78. PMID: 18593983
11. Antoniou A, Pharoah P, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet.* 2003;72(5):1117-30. PMID: 12677558
12. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol.* 2007;25(11):1329-33. PMID: 17416853
13. Anglian Breast Cancer Study Group. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. *Br J Cancer.* 2000;83(10):1301-8. PMID: 11044354
14. Antoniou AC, Gayther SA, Stratton JF, et al. Risk models for familial ovarian and breast cancer. *Genet Epidemiol.* 2000;18(2):173-90. PMID: 10642429

15. Antoniou AC, Pharoah PD, McMullan G, et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. *Br J Cancer.* 2002;86(1):76-83. PMID: 11857015
16. Peto J, Collins N, Barfoot R, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst.* 1999;91(11):943-9. PMID: 10359546
17. Whittemore AS, Gong G, John EM, et al. Prevalence of BRCA1 mutation carriers among U.S. non-Hispanic whites. *Cancer Epidemiol Biomarkers Prev*. 2004;13(12):2078-83. PMID: 15598764
18. Neuhausen S, Gilewski T, Norton L, et al. Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nat Genet*. 1996;13(1):126-8. PMID: 8673092
19. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med.* 1997;336(20):1401-8. PMID: 9145676
20. Roa BB, Boyd AA, Volcik K, et al. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nat Genet*. 1996;14:185-7. PMID: 8841191
21. Weitzel JN, Lagos V, Blazer KR, et al. Prevalence of BRCA mutations and founder effect in high-risk Hispanic families. *Cancer Epidemiol Biomarkers Prev*. 2005;14(7):1666-71. PMID: 16030099
22. Weitzel J, Lagos V, Blazer K, et al. Evidence for common ancestral origin of a recurring BRCA1 genomic rearrangement identified in high-risk Hispanic families. *Cancer Epidemiol Biomarkers Prev.* 2007;16(8):1615-20. PMID: 17646271
23. Mefford H, Baumbach L, Panguluri R. Evidence for BRCA1 founder mutation in families of West African ancestry. *Am J Hum Genet.* 1999;65(2):575-8. PMID: 10417303
24. Peelen T, van Vliet M, Petrij-Bosch A, et al. A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. *Am J Hum Genet.* 1997;60(5):1041-9. PMID: 9150151
25. Arason A, Jonasdottir A, Barkardottir RB, et al. A population study of mutations and LOH at breast cancer gene loci in tumours from sister pairs: two recurrent mutations seem to account for all BRCA1/BRCA2 linked breast cancer in Iceland. *J Med Genet*. 1998;35(6):446-9. PMID: 9643283
26. Thorlacius S, Olafsdottir G, Tryggvadottir L, et al. A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat Genet.* 1996;13(1):117-9. PMID: 8673089
27. Einbeigi Z, Bergman A, Kindblom LG, et al. A founder mutation of the BRCA1 gene in Western Sweden associated with a high incidence of breast and ovarian cancer. *Eur J Cancer.* 2001;37(15):1904-9. PMID: 11576847
28. National Cancer Institute. Breast Cancer Treatment (PDQ®). Bethesda, MD: National Cancer Institute; 2013. Accessed at http://www.cancer.gov/cancertopics/pdq/treatment/breast/healthprofessional on 25 November 2013.
29. Lindor NM, Greene MH; Mayo Familial Cancer Program. The concise handbook of family cancer syndromes. *J Natl Cancer Inst.* 1998;90(14):1039-71. PMID: 9672254
30. Lakhani S, Manek S, Penault-Llorca F. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. *Clin Cancer Res.* 2004;10(7):2473-81. PMID: 15073127

31. Evans DG, Young K, Bulman M, et al. Probability of BRCA1/2 mutation varies with ovarian histology: results from screening 442 ovarian cancer families. *Clin Genet*. 2008;73(4):338-45. PMID: 18312450
32. Tonin P, Maugard C, Perret C. A review of histopathological subtypes of ovarian cancer in BRCA-related French Canadian cancer families. *Fam Cancer*. 2007;6(4):491-7. PMID: 17636423
33. Bolton K, Chenevix-Trench G, Goh C. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. *JAMA*. 2012;307(4):382-90. PMID: 22274685
34. Crum C, Drapkin R, Kindelberger DW. Lessons from BRCA: the tubal fimbria emerges as an origin for pelvic serous cancer. *Clin Med Res*. 2007;5(1):35-44. PMID: 17456833
35. Levine D, Argenta P, Yee C. Fallopian tube and primary peritoneal carcinomas associated with BRCA mutations. *J Clin Oncol*. 2013;21(22):4222-7. PMID: 14615451
36. Mavaddat N, Barrowdale D, Andrulis I. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *Cancer Epidemiol Biomarkers Prev*. 2012;21(1):134-47. PMID: 22144499
37. Centers for Disease Control and Prevention. Breast Cancer Statistics. Atlanta: Centers for Disease Control and Prevention; 2013. Accessed at http://www.cdc.gov/cancer/breast/statistics/ on 25 November 2013.
38. U.S. Cancer Statistics Working Group. United States Cancer Statistics: 1999–2007 Incidence and Mortality Web-Based Report. Atlanta: Centers for Disease Control and Prevention; 2013. Accessed at http://apps.nccd.cdc.gov/uscs/toptencancers.aspx on 25 November 2013.
39. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2010. Bethesda, MD: National Cancer Institute; 2013. Accessed at http://seer.cancer.gov/csr/1975_2010/ on 25 November 2013.
40. National Cancer Institute. Ovarian Epithelial Cancer Treatment (PDQ®). Bethesda, MD: National Cancer Institute; 2013. Accessed at http://cancer.gov/cancertopics/pdq/treatment/ovarianepithelial/HealthProfessional on 25 November 2013.
41. Colditz GA, Willett WC, Hunter DJ. Family history, age, and risk of breast cancer. Prospective data from the Nurses' Health Study. *JAMA*. 1993;270(3):338-43. PMID: 8123079
42. Johnson N, Lancaster T, Fuller A. The prevalence of a family history of cancer in general practice. *Fam Pract*. 1995;12(3):287-9. PMID: 8536831
43. Pharoah PD, Day NE, Duffy S, et al. Family history and the risk of breast cancer: a systematic review and meta-analysis. *Int J Cancer*. 1997;71(5):800-9. PMID: 9180149
44. Slattery ML, Kerber RA. A comprehensive evaluation of family history and breast cancer risk. The Utah Population Database. *JAMA*. 1993;270(13):1563-8. PMID: 8371466
45. Yang Q, Khoury MJ, Rodriguez C, et al. Family history score as a predictor of breast cancer mortality: prospective data from the Cancer Prevention Study II, United States, 1982-1991. *Am J Epidemiol*. 1998;147(7):652-9. PMID: 9554604
46. Couch FJ, DeShano ML, Blackwood MA, et al. BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. *N Engl J Med*. 1997;336(20):1409-15. PMID: 9145677

47. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol.* 2002;20(6):1480-90. PMID: 11896095
48. Shattuck-Eidens D, Oliphant A, McClure M, et al. BRCA1 sequence analysis in women at high risk for susceptibility mutations: risk factor analysis and implications for genetic testing. *JAMA.* 1997;278(15):1242-50. PMID: 9333265
49. Srivastava A, McKinnon W, Wood ME. Risk of breast and ovarian cancer in women with strong family histories. *Oncology (Williston Park).* 2001;15(7):889-902. PMID: 11499690
50. National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Breast and Ovarian. Fort Washington, PA: National Comprehensive Cancer Network; 2012.
51. American College of Medical Genetics. Genetic Susceptibility to Breast and Ovarian Cancer: Assessment, Counseling, and Testing Guidelines. Bethesda, MD: American College of Medical Genetics; 1999. Accessed at http://www.ncbi.nlm.nih.gov/books/NBK56955/ on 25 November 2013.
52. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer Screening and Diagnosis. Fort Washington, PA: National Comprehensive Cancer Network; 2011.
53. Berry D, Parmigiani G, Sanchez J, et al. Probability of carrying a mutation of breast-ovarian cancer gene BRCA1 based on family history. *J Natl Cancer Inst.* 1997;89(3):227-38. PMID: 9017003
54. Antoniou A, Cunningham A, Peto J. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br J Ca*. 2008;98(8):1457-66. PMID: 18349832
55. Myriad Genetic Laboratories. Mutation Prevalence Tables. Salt Lake City, UT: Myriad Genetic Laboratories; 2010. Accessed at http://d1izdzz43r5o67.cloudfront.net/brac/brca-prevalence-tables.pdf on 26 November 2013.
56. Antoniou AC, Hardy R, Walker L, et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. *J Med Genet*. 2008;45(7):425-31. PMID: 18413374
57. Bowen DJ, Burke W, Yasui Y, et al. Effects of risk counseling on interest in breast cancer genetic testing for lower risk women. *Genet Med*. 2002;4(5):359-65. PMID: 12394349
58. Burke W, Culver JO, Bowen DJ, et al. Genetic counseling for women with an intermediate family history of breast cancer. *Am J Med Genet*. 2000;90(5):361-8. PMID: 10706356
59. Cull A, Miller H, Porterfield T, et al. The use of videotaped information in cancer genetic counselling: a randomized evaluation study. *Br J Cancer.* 1998;77(5):830-7. PMID: 9514066
60. Lerman C, Hughes C, Benkendorf JL, et al. Racial differences in testing motivation and psychological distress following pretest education for BRCA1 gene testing. *Cancer Epidemiol Biomarkers Prev.* 1999;8(4 Pt 2):361-7. PMID: 10207641

61. Lerman C, Narod S, Schulman K, et al. BRCA1 testing in families with hereditary breast-ovarian cancer. A prospective study of patient decision making and outcomes. *JAMA.* 1996;275(24):1885-92. PMID: 8648868
62. Bowen DJ, Burke W, McTiernan A, et al. Breast cancer risk counseling improves women's functioning. *Patient Educ Couns*. 2004;53:79-86. PMID: 15062908
63. American College of Surgeons. Cancer Program Standards 2012, Version 1.2: Ensuring Patient-Centered Care. Chicago: American College of Surgeons; 2013. Accessed at http://www.facs.org/cancer/coc/programstandards2012.html on 26 November 2013.
64. Rahman N, Stratton MR. The genetics of breast cancer susceptibility. *Annu Rev Genet*. 1998;32:95-121. PMID: 9928476
65. Myriad Genetic Laboratories. BRAC*Analysis*® Technical Specifications. Salt Lake City, UT: Myriad Genetic Laboratories; 2013. Accessed at https://s3.amazonaws.com/myriad-library/technical-specifications/BRCA+Tech+Specs_Integrated.pdf on 26 November 2013.
66. Unger MA, Nathanson KL, Calzone KA, et al. Screening for genomic rearrangements in families with breast and ovarian cancer identifies BRCA1 mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. *Am J Hum Genet.* 2000;67(4):841-50. PMID: 10978226
67. Dhingra K. Antiestrogens—tamoxifen, SERMs and beyond. *Invest New Drugs*. 1999;7(3):285-311. PMID: 10665480
68. Cuzick J, Powels T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. *Lancet.* 2003;361(9354):296-300. PMID: 2559863
69. IBIS Investigators. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. *Lancet.* 2002;360(9336):817-24. PMID: 12243915
70. Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet.* 1998;352(9122):98-101. PMID: 9672274
71. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst.* 1998;90(18):1371-88. PMID: 9747868
72. Veronesi U, Maisonneuve P, Costa A, et al. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among hysterectomised women. *Lancet.* 1998;352(9122):93-7. PMID: 9672273
73. Grady D, Cauley JA, Geiger MJ, et al. Reduced incidence of invasive breast cancer with raloxifene among women at increased coronary risk. *J Natl Cancer Inst.* 2008;100(12):854-61. PMID: 18544744
74. Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. *JAMA.* 1999;281(23):2189-97. PMID: 10376571
75. Braithwaite RS, Chlebowski RT, Lau J, et al. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *J Gen Intern Med*. 2003;18(11):937-47. PMID: 14687281
76. Curtis MG. Comparative tolerability of first-generation selective estrogen receptor modulators in breast cancer treatment and prevention. *Drug Saf*. 2001;24(14):1039-53. PMID: 11735660

77. Goss PE, Ingle JN, Ales-Martinez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med.* 2011;364(25):2381-91. PMID: 21639806
78. U.S. Preventive Services Task Force. Chemoprevention of breast cancer: recommendations and rationale. *Ann Intern Med.* 2002;137(1):56-8. PMID: 12093249
79. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med.* 2002;346(21):1609-15. PMID: 12023992
80. Meijers-Heijboer H, van Geel B, van Putten WL, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med.* 2001;345(3):159-64. PMID: 1463009
81. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. *J Clin Oncol.* 2004;22(6):1055-62. PMID: 14981104
82. Rebbeck TR, Levin AM, Eisen A, et al. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. *J Natl Cancer Inst.* 1999;91(17):1475-9. PMID: 10469748
83. Wapnir IL, Rabinowitz B, Greco RS. A reappraisal of prophylactic mastectomy. *Surg Gynecol Obstet*. 1990;171(2):171-84. PMID: 2200150
84. Woods JE. Breast reconstruction: current state of the art. *Mayo Clin Proc*. 1986;61:579-85. PMID: 3713262
85. Goodnight JE, Quagliana JM, Morton DL. Failure of subcutaneous mastectomy to prevent the development of breast cancer. *J Surg Oncol*. 1984;26:198-201. PMID: 6330460
86. Pennisi VR, Capozzi A. Subcutaneous mastectomy data. *Aesthetic Plast Surg*. 1989;13:15-21. PMID: 2728994
87. Aziz S, Kuperstein G, Rosen B, et al. A genetic epidemiological study of carcinoma of the fallopian tube. *Gynecol Oncol.* 2001;80:341-5. PMID: 11263928
88. Paley PJ, Swisher EM, Garcia RL, et al. Occult cancer of the fallopian tube in BRCA-1 germline mutation carriers at prophylactic oophorectomy: a case for recommending hysterectomy at surgical prophylaxis. *Gynecol Oncol.* 2001;80:176-80. PMID: 11161856
89. Bandera CA, Muto MG, Schorge JO, et al. BRCA1 gene mutations in women with papillary serous carcinoma of the peritoneum. *Obstet Gynecol*. 1998;92(4):596-600. PMID: 9764635
90. Piver MS, Jishi MF, Tsukada Y, et al. Primary peritoneal carcinoma after prophylactic oophorectomy in women with a family history of ovarian cancer. A report of the Gilda Radner Familial Ovarian Cancer Registry. *Cancer*. 1993;71(9):2751-5. PMID: 8467455
91. Tobacman JK, Tucker MA, Kase RG, et al. Intra-abdominal carcinomatosis after prophylactic oophorectomy in ovarian-cancer-prone families. *Lancet.* 1982;2(8302):795-7. PMID: 6126666
92. American Society of Clinical Oncology. Statement of the American Society of Clinical Oncology: genetic testing for cancer susceptibility. *J Clin Oncol.* 1996;14(5):1730-6. PMID: 8622094
93. Bellcross CA, Kolor K, Goddard KA, et al. Awareness and utilization of BRCA1/2 testing among U.S. primary care physicians. *Am J Prev Med*. 2011;40(1):61-6. PMID: 21146769

94. Cox SL, Zlot AI, Silvey K, et al. Patterns of cancer genetic testing: a randomized survey of Oregon clinicians. *J Cancer Epidemiol*. 2012;2012:294730. PMID: 23150730
95. Waters E, McNeel T, Stevens W, et al. Use of tamoxifen and raloxifene for breast cancer chemoprevention in 2010. *Breast Cancer Res Treat*. 2012;134:875-80. PMID: 22622807
96. Michigan Department of Community Health. BRCA 1/2 Surveillance in Michigan, 2008–2012. Lansing, MI: Michigan Department of Community Health; 2012. Accessed at http://www.michigan.gov/documents/mdch/11-12_BRCAsurveillance2012_FINAL_403992_7.pdf on 26 November 2013.
97. Metcalfe KA, Birenbaum-Carmeli D, Lubinski J, et al. International variation in rates of uptake of preventive options in BRCA1 and BRCA2 mutation carriers. *Int J Cancer*. 2008;122(9):2017-22. PMID: 18196574
98. Beattie MS, Crawford B, Lin F, et al. Uptake, time course, and predictors of risk-reducing surgeries in BRCA carriers. *Genet Test Mol Biomarkers*. 2009;13(1):51-6. PMID: 19309274
99. Mannis G, Fehniger J, Creasman J, et al. Risk-reducing salpingo-oophorectomy and ovarian cancer screening in 1077 women after BRCA testing. *JAMA.* 2013;173(2):96-103. PMID: 23247828
100. Harris RP, Helfand M, Woolf SH, et al. Current methods of the U.S. Preventive Services Task Force: a review of the process. *Am J Prev Med.* 2001;20(3 Suppl 1):21-35. PMID: 11306229
101. Leeflang MM, Deeks JJ, Gatsonis C, et al. Systematic reviews of diagnostic test accuracy. *Ann Intern Med.* 2008;149(12):889-97. PMID: 19075208
102. Atkins D, Chang SM, Gartlehner G, et al. Assessing applicability when comparing medical interventions: AHRQ and the Effective Health Care Program. *J Clin Epidemiol*. 2011;64(11):1198-207. PMID: 21463926
103. Sahai H, Khurshid A. Confidence intervals for the mean of a Poisson distribution: a review. *Biom J*. 1993;35:857-67.
104. Higgins J, Thompson S. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21:1539-58. PMID: 12111919
105. DerSimonian R, Laird NM. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7:177-88. PMID: 12111919
106. Stratton JF, Pharoah P, Smith SK, et al. A systematic review and meta-analysis of family history and risk of ovarian cancer. *Br J Obstet Gynaecol*. 1998;105(5):493-9. PMID: 9637117
107. Kerber RA, Slattery ML. Comparison of self-reported and database-linked family history of cancer data in a case-control study. *Am J Epidemiol.* 1997;146(3):244-8. PMID: 9247008
108. Mai PL, Garceau AO, Graubard BI, et al. Confirmation of family cancer history reported in a population-based survey. *J Natl Cancer Inst.* 2011;103(10):788-97. PMID: 21562245
109. Mouchawar J, Valentine Goins K, Somkin C, et al. Guidelines for breast and ovarian cancer genetic counseling referral: adoption and implementation in HMOs. *Genet Med*. 2003;5(6):444-50. PMID: 14614396
110. de Silva D, Gilbert F, Needham G, et al. Identification of women at high genetic risk of breast cancer through the National Health Service Breast Screening Programme (NHSBSP). *J Med Genet.* 1995;32(11):862-6. PMID: 8592328

111. National Breast and Ovarian Cancer Centre. Advice About Familial Aspects of Breast Cancer and Epithelial Ovarian Cancer: A Guide for Health Professionals. New South Wales, Australia: National Breast and Ovarian Cancer Centre; 2010. Accessed at http://canceraustralia.gov.au/sites/default/files/publications/nbocc-bog-2010-web-a4-printable_504af02a673fd.pdf on 26 November 2013.
112. de Bock GH, Vliet Vlieland TP, Hageman GC, et al. The assessment of genetic risk of breast cancer: a set of GP guidelines. *Fam Pract*. 1999;16(1):71-7. PMID: 10321400
113. Pichert G, Stahel RA. Organizing cancer genetics programs: the Swiss model. *J Clin Oncol.* 2000;18(21 Suppl):65S-9. PMID: 11060330
114. Hampel H, Sweet K, Westman JA, et al. Referral for cancer genetics consultation: a review and compilation of risk assessment criteria. *J Med Genet.* 2004;41:81-91. PMID: 14757853
115. Nelson HD, Fu R, Griffin JC, et al. Systematic review: comparative effectiveness of medications to reduce risk for primary breast cancer. *Ann Intern Med.* 2009;151(10):703-15. PMID: 19920271
116. Nelson H, Smith M, Griffin J, et al. Use of medications to reduce risk for primary breast cancer: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med.* 2013;158(8):604-14. PMID: 23588749
117. Adams-Campbell LL, Makambi KH, Palmer JR, et al. Diagnostic accuracy of the Gail model in the Black Women's Health Study. *Breast J*. 2007;13(4):332-6. PMID: 17593036
118. Amir E, Evans DG, Shenton A, et al. Evaluation of breast cancer risk assessment packages in the family history evaluation and screening programme. *J Med Genet.* 2003;40(11):807-14. PMID: 14627668
119. Barlow WE, White E, Ballard-Barbash R, et al. Prospective breast cancer risk prediction model for women undergoing screening mammography. *J Natl Cancer Inst.* 2006;98(17):1204-14. PMID: 16954473
120. Boughey JC, Hartmann LC, Anderson SS, et al. Evaluation of the Tyrer-Cuzick (International Breast Cancer Intervention Study) model for breast cancer risk prediction in women with atypical hyperplasia. *J Clin Oncol.* 2010;28(22):3591-6. PMID: 20606088
121. Boyle P, Mezzetti M, La Vecchia C, et al. Contribution of three components to individual cancer risk predicting breast cancer risk in Italy. *Eur J Cancer Prev*. 2004;13(3):183-91. PMID: 15167217
122. Chen J, Pee D, Ayyagari R, et al. Projecting absolute invasive breast cancer risk in white women with a model that includes mammographic density. *J Natl Cancer Inst.* 2006;98(17):1215-26. PMID: 16954474
123. Chlebowski RT, Anderson GL, Lane DS, et al. Predicting risk of breast cancer in postmenopausal women by hormone receptor status. *J Natl Cancer Inst.* 2007;99(22):1695-705. PMID: 18000216
124. Colditz GA, Rosner B. Cumulative risk of breast cancer to age 70 years according to risk factor status: data from the Nurses' Health Study. *Am J Epidemiol.* 2000;152(10):950-64. PMID: 11092437
125. Colditz GA, Rosner BA, Chen WY, et al. Risk factors for breast cancer according to estrogen and progesterone receptor status. *J Natl Cancer Inst.* 2004;96(3):218-28. PMID: 14759989

126. Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. *J Natl Cancer Inst.* 1999;91(18):1541-8. PMID: 10491430
127. Decarli A, Calza S, Masala G, et al. Gail model for prediction of absolute risk of invasive breast cancer: independent evaluation in the Florence-European Prospective Investigation Into Cancer and Nutrition cohort. *J Natl Cancer Inst.* 2006;98(23):1686-93. PMID: 17148770
128. Gail M, Brinton L, Byar D, et al. Projecting individualized probablities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst.* 1989;81(24):1879-86. PMID: 2593165
129. Gail MH, Costantino JP, Pee D, et al. Projecting individualized absolute invasive breast cancer risk in African American women. *J Natl Cancer Inst.* 2007;99(23):1782-92. PMID: 18042936
130. Lee EO, Ahn SH, You C, et al. Determining the main risk factors and high-risk groups of breast cancer using a predictive model for breast cancer risk assessment in South Korea. *Cancer Nurs*. 2004;27(5):400-6. PMID: 15525868
131. Petracci E, Decarli A, Schairer C, et al. Risk factor modification and projections of absolute breast cancer risk. *J Natl Cancer Inst.* 2011;103(13):1037-48. PMID: 21705679
132. Rockhill B, Spiegelman D, Byrne C, et al. Validation of the Gail, et al model of breast cancer risk prediction and implications for chemoprevention. *J Natl Cancer Inst.* 2001;93:358-66. PMID: 11238697
133. Rockhill B, Byrne C, Rosner B, et al. Breast cancer risk prediction with a log-incidence model: evaluation of accuracy. *J Clin Epidemiol.* 2003;56(9):856-61. PMID: 14505770
134. Tamimi RM, Rosner B, Colditz GA. Evaluation of a breast cancer risk prediction model expanded to include category of prior benign breast disease lesion. *Cancer*. 2010;116(21):4944-53. PMID: 20645399
135. Tice JA, Cummings SR, Smith-Bindman R, et al. Using clinical factors and mammographic breast density to estimate breast cancer risk: development and validation of a new predictive model. *Ann Intern Med.* 2008;148(5):337-47. PMID: 18316752
136. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. *Stat Med*. 2004;23(7):1111-30. PMID: 15057881
137. Vacek PM, Skelly JM, Geller BM. Breast cancer risk assessment in women aged 70 and older. *Breast Cancer Res Treat*. 2011;130(1):291-9. PMID: 21604157
138. Ashton-Prolla P, Giacomazzi J, Schmidt AV, et al. Development and validation of a simple questionnaire for the identification of hereditary breast cancer in primary care. *BMC Cancer*. 2009;9:283. PMID: 19682358
139. Barcenas CH, Hosain GM, Arun B, et al. Assessing BRCA carrier probabilities in extended families. *J Clin Oncol.* 2006;24(3):354-60. PMID: 16421416
140. Bellcross CA, Lemke AA, Pape LS, et al. Evaluation of a breast/ovarian cancer genetics referral screening tool in a mammography population. *Genet Med*. 2009;11(11):783-9. PMID: 19752737
141. Evans DG, Eccles DM, Rahman N, et al. A new scoring system for the chances of identifying a BRCA 1/2 mutation outperforms existing models including BRCAPRO. *J Med Genet.* 2004;41:474-80. PMID: 15173236

142. Gilpin CA, Carson N, Hunter AG. A preliminary validation of a family history assessment form to select women at risk for breast or ovarian cancer for referral to a genetics center. *Clin Genet.* 2000;58(4):299-308. PMID: 11076055
143. Hoskins KF, Zwaagstra A, Ranz M. Validation of a tool for identifying women at high risk for hereditary breast cancer in population-based screening. *Cancer*. 2006;107(8):1769-76. PMID: 16967460
144. Oros KK, Ghadirian P, Maugard CM, et al. Application of BRCA1 and BRCA2 mutation carrier prediction models in breast and/or ovarian cancer families of French Canadian descent. *Clin Genet.* 2006;70(4):320-9. PMID: 16965326
145. Panchal SM, Ennis M, Canon S, et al. Selecting a BRCA risk assessment model for use in a familial cancer clinic. *BMC Med Genet.* 2008;9:116. PMID: 19102775
146. Parmigiani G, Chen S, Iversen ES Jr, et al. Validity of models for predicting BRCA1 and BRCA2 mutations. *Ann Intern Med.* 2007;147(7):441-50. PMID: 17909205
147. Bellcross C. Further development and evaluation of a breast/ovarian cancer genetics referral screening tool. *Genet Med.* 2010;12(4):240. PMID: 20395744
148. Armstrong K, Micco E, Carney A, et al. Racial differences in the use of BRCA1/2 testing among women with a family history of breast or ovarian cancer. *JAMA.* 2005;293(14):1729-36. PMID: 15827311
149. Bennett P, Wilkinson C, Turner J, et al. Factors associated with intrusive cancer-related worries in women undergoing cancer genetic risk assessment. *Fam Cancer*. 2009;8(2):159-65. PMID: 19011994
150. Bennett P, Wilkinson C, Turner J, et al. Psychological factors associated with emotional responses to receiving genetic risk information. *J Genet Couns*. 2008;17(3):234-41. PMID: 18259848
151. Bloom JR, Stewart SL, Chang S, et al. Effects of a telephone counseling intervention on sisters of young women with breast cancer. *Prev Med*. 2006;43(5):379-84. PMID: 16916540
152. Bowen DJ, Burke W, Culver JO, et al. Effects of counseling Ashkenazi Jewish women about breast cancer risk. *Cultur Divers Ethni Minor Psychol*. 2006;12(1):45-56. PMID: 16594854
153. Brain K, Parsons E, Bennett P, et al. The evolution of worry after breast cancer risk assessment: 6-year follow-up of the TRACE study cohort. *Psychooncology*. 2011;20(9):984-91. PMID: 20677331
154. Braithwaite D, Sutton S, Mackay J, et al. Development of a risk assessment tool for women with a family history of breast cancer. *Cancer Detect Prev*. 2005;29(5):433-9. PMID: 16055276
155. Fry A, Cull A, Appleton S, et al. A randomised controlled trial of breast cancer genetics services in South East Scotland: psychological impact. *Br J Cancer.* 2003;89(4):653-9. PMID: 12915873
156. Gurmankin AD, Domchek S, Stopfer J, et al. Patients' resistance to risk information in genetic counseling for BRCA1/2. *Arch Intern Med.* 2005;165(5):523-9. PMID: 15767527
157. Helmes AW, Culver JO, Bowen DJ. Results of a randomized study of telephone versus in-person breast cancer risk counseling. *Patient Educ Couns*. 2006;64(1-3):96-103. PMID: 16427245

158. Hopwood P, Wonderling D, Watson M, et al. A randomised comparison of UK genetic risk counselling services for familial cancer: psychosocial outcomes. *Br J Cancer.* 2004;91(5):884-92. PMID: 15305197
159. Kelly KM, Senter L, Leventhal H, et al. Subjective and objective risk of ovarian cancer in Ashkenazi Jewish women testing for BRCA1/2 mutations. *Patient Educ Couns*. 2008;70(1):135-42. PMID: 17988821
160. Matloff ET, Moyer A, Shannon KM, et al. Healthy women with a family history of breast cancer: impact of a tailored genetic counseling intervention on risk perception, knowledge, and menopausal therapy decision making. *J Womens Health*. 2006;15(7):843-56. PMID: 16999640
161. Mikkelsen EM, Sunde L, Johansen C, et al. Risk perception among women receiving genetic counseling: a population-based follow-up study. *Cancer Epidemiol Biomarkers Prev.* 2007;31(6):457-64. PMID: 18061369
162. Mikkelsen EM, Sunde L, Johansen C, et al. Psychosocial consequences of genetic counseling: a population-based follow-up study. *Breast J*. 2009;15(1):61-8. PMID: 19120380
163. Pieterse AH, Ausems MG, Spreeuwenberg P, et al. Longer-term influence of breast cancer genetic counseling on cognitions and distress: smaller benefits for affected versus unaffected women. *Patient Educ Couns*. 2011;85(3):425-31. PMID: 21316181
164. Roshanai AH, Rosenquist R, Lampic C, et al. Does enhanced information at cancer genetic counseling improve counselees' knowledge, risk perception, satisfaction and negotiation of information to at-risk relatives? A randomized study. *Acta Oncol*. 2009;48(7):999-1009. PMID: 19636983
165. Smerecnik CM, Mesters I, Verweij E, et al. A systematic review of the impact of genetic counseling on risk perception accuracy. *J Genet Couns*. 2009;18(3):217-28. PMID: 19291376
166. Brain K, Norman P, Gray J, et al. A randomized trial of specialist genetic assessment: psychological impact on women at different levels of familial breast cancer risk. *Br J Cancer.* 2002;86(2):233-8. PMID: 11870512
167. Hopwood P, Keeling F, Long A, et al. Psychological support needs for women at high genetic risk of breast cancer: some preliminary indicators. *Psychooncology*. 1998;7(5):402-12. PMID: 9809331
168. Lerman C, Schwartz MD, Miller SM, et al. A randomized trial of breast cancer risk counseling: interacting effects of counseling, educational level, and coping style. *Health Psychol*. 1996;15(2):75-83. PMID: 8681923
169. Lobb EA, Butow PN, Barratt A, et al. Communication and information-giving in high-risk breast cancer consultations: influence on patient outcomes. *Br J Cancer.* 2004;90(2):321-7. PMID: 14735171
170. Watson M, Lloyd S, Davidson J, et al. The impact of genetic counselling on risk perception and mental health in women with a family history of breast cancer. *Br J Ca*. 1999;79(5-6):868-74. PMID: 10070883
171. Watson M, Duvivier V, Wade Walsh M, et al. Family history of breast cancer: what do women understand and recall about their genetic risk? *J Med Genet.* 1998;35(9):731-8. PMID: 9733031
172. Al-Mulla F, Bland JM, Serratt D, et al. Age-dependent penetrance of different germline mutations in the BRCA1 gene. *J Clin Pathol*. 2009;62(4):350-6. PMID: 19329713

173. Antoniou AC, Durocher F, Smith P, et al. BRCA1 and BRCA2 mutation predictions using the BOADICEA and BRCAPRO models and penetrance estimation in high-risk French-Canadian families. *Breast Cancer Res*. 2006;8(1):R3. PMID: 16417652
174. Beristain E, Martinez-Bouzas C, Guerra I, et al. Differences in the frequency and distribution of BRCA1 and BRCA2 mutations in breast/ovarian cancer cases from the Basque country with respect to the Spanish population: implications for genetic counselling. *Breast Cancer Res Treat*. 2007;106(2):255-62. PMID: 17262179
175. Bernholtz S, Laitman Y, Kaufman B, et al. Phenocopy breast cancer rates in Israeli BRCA1 BRCA2 mutation carrier families: is the risk increased in non-carriers? *Breast Cancer Res Treat*. 2012;132(2):669-73. PMID: 22113258
176. Chen S, Iversen ES, Friebel T, et al. Characterization of BRCA1 and BRCA2 mutations in a large United States sample. *J Clin Oncol.* 2006;24(6):863-71. PMID: 16484695
177. Domchek SM, Gaudet MM, Stopfer JE, et al. Breast cancer risks in individuals testing negative for a known family mutation in BRCA1 or BRCA2. *Breast Cancer Res Treat.* 2010;119(2):409-14. PMID: 19885732
178. Evans DG, Shenton A, Woodward E, et al. Penetrance estimates for BRCA1 and BRCA2 based on genetic testing in a clinical cancer genetics service setting: risks of breast/ovarian cancer quoted should reflect the cancer burden in the family. *BMC Cancer*. 2008;8:155. PMID: 18513387
179. Finkelman BS, Rubinstein WS, Friedman S, et al. Breast and ovarian cancer risk and risk reduction in Jewish BRCA1/2 mutation carriers. *J Clin Oncol.* 2012;30(12):1321-8. PMID: 22430266
180. Gronwald J, Cybulski C, Lubinski J, et al. Phenocopies in breast cancer 1 (BRCA1) families: implications for genetic counselling. *J Med Genet.* 2007;44(4):e76. PMID: 17400795
181. Harvey SL, Milne RL, McLachlan SA, et al. Prospective study of breast cancer risk for mutation negative women from BRCA1 or BRCA2 mutation positive families. *Breast Cancer Res Treat.* 2011;130(3):1057-61. PMID: 21850394
182. Kauff ND, Mitra N, Robson ME, et al. Risk of ovarian cancer in BRCA1 and BRCA2 mutation-negative hereditary breast cancer families. *J Natl Cancer Inst.* 2005;97(18):1382-4. PMID: 16174860
183. Konecny M, Milly M, Zavodna K, et al. Comprehensive genetic characterization of hereditary breast/ovarian cancer families from Slovakia. *Breast Cancer Res Treat.* 2011;126(1):119-30. PMID: 21203900
184. Korde LA, Mueller CM, Loud JT, et al. No evidence of excess breast cancer risk among mutation-negative women from BRCA mutation-positive families. *Breast Cancer Res Treat.* 2011;125(1):169-73. PMID: 20458532
185. Kramer JL, Velazquez IA, Chen BE, et al. Prophylactic oophorectomy reduces breast cancer penetrance during prospective, long-term follow-up of BRCA1 mutation carriers. *J Clin Oncol.* 2005;23(34):8629-35. PMID: 16314625
186. Kurian AW, Gong GD, John EM, et al. Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: findings from the Breast Cancer Family Registry. *J Clin Oncol.* 2011;29(34):4505-9. PMID: 22042950
187. Lubinski J, Huzarski T, Byrski T, et al. The risk of breast cancer in women with a BRCA1 mutation from North America and Poland. *Int J Cancer*. 2012;131(1):229-34. PMID: 21834074

188. Marroni F, Aretini P, D'Andrea E, et al. Penetrances of breast and ovarian cancer in a large series of families tested for BRCA1/2 mutations. *Eur J Hum Genet*. 2004;12(11):899-906. PMID: 15340362
189. Metcalfe KA, Finch A, Poll A, et al. Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation. *Br J Cancer*. 2009;100(2):421-5. PMID: 19088722
190. Metcalfe K, Lubinski J, Lynch HT, et al. Family history of cancer and cancer risks in women with BRCA1 or BRCA2 mutations. *J Natl Cancer Inst.* 2010;102(24):1874-8. PMID: 21098759
191. Metcalfe KA, Poll A, Royer R, et al. Screening for founder mutations in BRCA1 and BRCA2 in unselected Jewish women. *J Clin Oncol.* 2010;28(3):387-91. PMID: 20008623
192. Milne RL, Osorio A, Cajal TR, et al. The average cumulative risks of breast and ovarian cancer for carriers of mutations in BRCA1 and BRCA2 attending genetic counseling units in Spain. *Clin Cancer Res.* 2008;14(9):2861-9. PMID: 18451254
193. Nanda R, Schumm LP, Cummings S, et al. Genetic testing in an ethnically diverse cohort of high-risk women: a comparative analysis of BRCA1 and BRCA2 mutations in American families of European and African ancestry. *JAMA.* 2005;294(15):1925-33. PMID: 16234499
194. Neuhausen SL, Ozcelik H, Southey MC, et al. BRCA1 and BRCA2 mutation carriers in the Breast Cancer Family Registry: an open resource for collaborative research. *Breast Cancer Res Treat.* 2009;116(2):379-86. PMID: 18704680
195. Risch HA, McLaughlin JR, Cole DE, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J Natl Cancer Inst.* 2006;98(23):1694-706. PMID: 17148771
196. Rowan E, Poll A, Narod SA. A prospective study of breast cancer risk in relatives of BRCA1/BRCA2 mutation carriers. *J Med Genet.* 2007;44(8):e89. PMID: 17673443
197. Seymour IJ, Casadei S, Zampiga V, et al. Results of a population-based screening for hereditary breast cancer in a region of North-Central Italy: contribution of BRCA1/2 germ-line mutations. *Breast Cancer Res Treat.* 2008;112(2):343-9. PMID: 18092194
198. Smith A, Moran A, Boyd MC, et al. Phenocopies in BRCA1 and BRCA2 families: evidence for modifier genes and implications for screening. *J Med Genet.* 2007;44:10-5. PMID: 17079251
199. Tamboom K, Kaasik K, Aršavskaja J, et al. BRCA1 mutations in women with familial or early-onset breast cancer and BRCA2 mutations in familial cancer in Estonia. *Hered Cancer Clin Pract*. 2010;8(1):4. PMID: 20380699
200. Tommasi S, Crapolicchio A, Lacalamita R, et al. BRCA1 mutations and polymorphisms in a hospital-based consecutive series of breast cancer patients from Apulia, Italy. *Mutat Res*. 2005;578(1-2):395-405. PMID: 16026807
201. van der Kolk DM, de Bock GH, Leegte BK, et al. Penetrance of breast cancer, ovarian cancer and contralateral breast cancer in BRCA1 and BRCA2 families: high cancer incidence at older age. *Breast Cancer Res Treat.* 2010;124(3):643-51. PMID: 20204502
202. Vaziri SA, Krumroy LM, Rostai M, et al. Frequency of BRCA1 and BRCA2 mutations in a clinic-based series of breast and ovarian cancer families. *Hum Mutat*. 2001;17(1):74. PMID: 11139249

203. Abeliovich D, Kaduri L, Lerer I, et al. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. *Am J Hum Genet.* 1997;60(3):505-14. PMID: 9042909
204. Anton-Culver H, Cohen PF, Gildea ME, et al. Characteristics of BRCA1 mutations in a population-based case series of breast and ovarian cancer. *Eur J Cancer.* 2000;36(10):1200-8. PMID: 10882857
205. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. *JAMA.* 2000;283(17):2260-5. PMID: 10807385
206. Brose MS, Rebbeck TR, Calzone KA, et al. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. *J Natl Cancer Inst.* 2002;94(18):1365-72. PMID: 12237282
207. Eccles DM, Englefield P, Soulby MA, et al. BRCA1 mutations in southern England. *Br J Cancer.* 1998;77(12):2199-203. PMID: 9649133
208. FitzGerald MG, MacDonald DJ, Krainer M, et al. Germ-line BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med.* 1996;334(3):143-9. PMID: 8531968
209. Fodor FH, Weston A, Bleiweiss IJ, et al. Frequency and carrier risk associated with common BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer patients. *Am J Hum Genet.* 1998;63(1):45-51. PMID: 9634504
210. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. *Am J Hum Genet.* 1998;62(3):676-89. PMID: 9497246
211. Gayther SA, Mangion J, Russell P, et al. Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. *Nat Genet.* 1997;15(1):103-5. PMID: 8988179
212. Gayther SA, Russell P, Harrington P, et al. The contribution of germline BRCA1 and BRCA2 mutations to familial ovarian cancer: no evidence for other ovarian cancer-susceptibility genes. *Am J Hum Genet.* 1999;65(4):1021-9. PMID: 10486320
213. Gershoni-Baruch R, Dagan E, Fried G, et al. Significantly lower rates of BRCA1/BRCA2 founder mutations in Ashkenazi women with sporadic compared with familial early onset breast cancer. *Eur J Cancer.* 2000;36(8):983-6. PMID: 10885601
214. Hartge P, Struewing JP, Wacholder S, et al. The prevalence of common BRCA1 and BRCA2 mutations among Ashkenazi Jews. *Am J Hum Genet.* 1999;64(4):963-70. PMID: 10090881
215. Hopper JL, Southey MC, Dite GS, et al. Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in BRCA1 and BRCA2: Australian Breast Cancer Family Study. *Cancer Epidemiol Biomarkers Prev*. 1999;8(9):741-7. PMID: 10498392
216. Janezic SA, Ziogas A, Krumroy LM, et al. Germline BRCA1 alterations in a population-based series of ovarian cancer cases. *Hum Mol Genet*. 1999;8(5):889-97. PMID: 0196379
217. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science*. 2003;302(5645):643-6. PMID: 14576434
218. Langston AA, Malone KE, Thompson JD, et al. BRCA1 mutations in a population-based sample of young women with breast cancer. *N Engl J Med.* 1996;334:137-42. PMID: 8531967

219. Liede A, Karlan BY, Baldwin RL, et al. Cancer incidence in a population of Jewish women at risk of ovarian cancer. *J Clin Oncol.* 2002;20(6):1570-7. PMID: 11896106
220. Malone KE, Daling JR, Neal C, et al. Frequency of BRCA1/BRCA2 mutations in a population-based sample of young breast carcinoma cases. *Cancer*. 2000;88(6):1393-402. PMID: 10717622
221. Modan B, Hartge P, Hirsh-Yechezkel G, et al. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a BRCA1 or BRCA2 mutation. *N Engl J Med.* 2001;345(4):235-40. PMID: 11474660
222. Moslehi R, Chu W, Karlan B, et al. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. *Am J Hum Genet.* 2000;66(4):1259-72. PMID: 10739756
223. Newman B, Mu H, Butler LM, et al. Frequency of breast cancer attributable to BRCA1 in a population-based series of American women. *JAMA.* 1998;279(12):915-21. PMID: 9544765
224. Oddoux C, Struewing JP, Clayton CM, et al. The carrier frequency of the BRCA2 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. *Nat Genet.* 1996;14(2):188-90. PMID: 8841192
225. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *Am J Hum Genet.* 2001;68(3):700-10. PMID: 11179017
226. Robson M, Gilewski T, al. BRCA-associated breast cancer in young women. *J Clin Oncol.* 1998;16:1642-9. PMID: 9586873
227. Satagopan JM, Offit K, Foulkes W, et al. The lifetime risks of breast cancer in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. *Cancer Epidemiol Biomarkers Prev*. 2001;10(5):467-73. PMID: 11352856
228. Stratton JF, Gayther SA, Russell P, et al. Contribution of BRCA1 mutations to ovarian cancer. *N Engl J Med.* 1997;336(16):1125-30. PMID: 9099656
229. Struewing JP, Watson P, Easton DF, et al. Prophylactic oophorectomy in inherited breast/ovarian cancer families. *J Natl Cancer Inst Monogr*. 1995(17):33-5. PMID: 8573450
230. Sutcliffe S, Pharoah PD, Easton DF, et al. Ovarian and breast cancer risks to women in families with two or more cases of ovarian cancer. *Int J Cancer*. 2000;87:110-17. PMID: 10861460
231. Warner E, Foulkes W, Goodwin P, et al. Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. *J Natl Cancer Inst.* 1999;91(14):1241-7. PMID: 10413426
232. Couch FJ, Sinilnikova O, Vierkant RA, et al. AURKA F31I polymorphism and breast cancer risk in BRCA1 and BRCA2 mutation carriers: a consortium of investigators of modifiers of BRCA1/2 study. *Cancer Epidemiol Biomarkers Prev.* 2007;16(7):1416-21. PMID: 17627006
233. Begg CB. On the use of familial aggregation in population-based case probands for calculating penetrance. *J Natl Cancer Inst.* 2002;94(16):1221-6. PMID: 12189225
234. Antoniou AC, Pharoah PD, Narod S, et al. Breast and ovarian cancer risks to carriers of the BRCA1 5382insC and 185delAG and BRCA2 6174delT mutations: a combined analysis of 22 population based studies. *J Med Genet.* 2005;42(7):602-3. PMID: 15994883

235. Arver B, Haegermark A, Platten U, et al. Evaluation of psychosocial effects of pre-symptomatic testing for breast/ovarian and colon cancer pre-disposing genes: a 12-month follow-up. *Fam Cancer*. 2004;3(2):109-16. PMID: 15340261
236. Dagan E, Shochat T. Quality of life in asymptomatic BRCA1/2 mutation carriers. *Prev Med*. 2009;48(2):193-6. PMID: 19084553
237. Ertmanski S, Metcalfe K, Trempala J, et al. Identification of patients at high risk of psychological distress after BRCA1 genetic testing. *Genet Test Mol Biomarkers*. 2009;13(3):325-30. PMID: 19405874
238. Foster C, Watson M, Eeles R, et al. Predictive genetic testing for BRCA1/2 in a UK clinical cohort: three-year follow-up. *Br J Cancer.* 2007;96(5):718-24. PMID: 17285126
239. Geirdal AO, Dahl AA. The relationship between coping strategies and anxiety in women from families with familial breast-ovarian cancer in the absence of demonstrated mutations. *Psychooncology*. 2008;17(1):49-57. PMID: 17385192
240. Geirdal AO, Reichelt JG, Dahl AA, et al. Psychological distress in women at risk of hereditary breast/ovarian or HNPCC cancers in the absence of demonstrated mutations. *Fam Cancer*. 2005;4(2):121-6. PMID: 15951962
241. Graves KD, Vegella P, Poggi EA, et al. Long-term psychosocial outcomes of BRCA1/BRCA2 testing: differences across affected status and risk-reducing surgery choice. *Cancer Epidemiol Biomarkers Prev.* 2012;21(3):445-55. PMID: 22328347
242. Julian-Reynier C, Mancini J, Mouret-Fourme E, et al. Cancer risk management strategies and perceptions of unaffected women 5 years after predictive genetic testing for BRCA1/2 mutations. *Eur J Hum Genet*. 2011;19(5):500-6. PMID: 21267012
243. Kinney AY, Bloor LE, Mandal D, et al. The impact of receiving genetic test results on general and cancer-specific psychologic distress among members of an African-American kindred with a BRCA1 mutation. *Cancer*. 2005;104(11):2508-16. PMID: 16222692
244. Low CA, Bower JE, Kwan L, et al. Benefit finding in response to BRCA1/2 testing. *Ann Behav Med*. 2008;35(1):61-9. PMID: 18347905
245. Reichelt JG, Heimdal K, Møller P, et al. BRCA1 testing with definitive results: a prospective study of psychological distress in a large clinic-based sample. *Fam Cancer*. 2004;3(1):21-8. PMID: 15131402
246. Reichelt JG, Moller P, Heimdal K, et al. Psychological and cancer-specific distress at 18 months post-testing in women with demonstrated BRCA1 mutations for hereditary breast/ovarian cancer. *Fam Cancer*. 2008;7(3):245-54. PMID: 18219587
247. Shochat T, Dagan E. Sleep disturbances in asymptomatic BRCA1/2 mutation carriers: women at high risk for breast-ovarian cancer. *J Sleep Res*. 2010;19(2):333-40. PMID: 20337906
248. van Dijk S, Timmermans DR, Meijers-Heijboer H, et al. Clinical characteristics affect the impact of an uninformative DNA test result: the course of worry and distress experienced by women who apply for genetic testing for breast cancer. *J Clin Oncol.* 2006;24(22):3672-7. PMID: 16877736
249. Metcalfe KA, Mian N, Enmore M, et al. Long-term follow-up of Jewish women with a BRCA1 and BRCA2 mutation who underwent population genetic screening. *Breast Cancer Res Treat.* 2012;133(2):735-40. PMID: 22240989

250. Meiser B, Butow P, Friedlander M, et al. Psychological impact of genetic testing in women from high-risk breast cancer families. *Eur J Cancer*. 2002;38(15):2025-31. PMID: 12376208
251. Bakos AD, Hutson SP, Loud JT, et al. BRCA mutation-negative women from hereditary breast and ovarian cancer families: a qualitative study of the BRCA-negative experience. *Health Expect*. 2008;11(3):220-31. PMID: 18816319
252. Hamilton R, Williams JK, Skirton H, et al. Living with genetic test results for hereditary breast and ovarian cancer. *J Nurs Scholarsh*. 2009;41(3):276-83. PMID: 19723276
253. Offit K, Groeger E, Turner S, et al. The "duty to warn" a patient's family members about hereditary disease risks. *JAMA*. 2004;292(12):1469-73. PMID: 15383518
254. DeMarco TA, McKinnon WC. Life after BRCA1/2 testing: family communication and support issues. *Breast Dis*. 2007;27:127-36. PMID: 17917144
255. Hamilton RJ, Bowers BJ, Williams JK. Disclosing genetic test results to family members. *J Nurs Scholarsh*. 2005;37(1):18-24. PMID: 15813582
256. Etchegary H, Miller F, deLaat S, et al. Decision-making about inherited cancer risk: exploring dimensions of genetic responsibility. *J Genet Couns*. 2009;18(3):252-64. PMID: 19294336
257. Dancyger C, Wiseman M, Jacobs C, et al. Communicating BRCA1/2 genetic test results within the family: a qualitative analysis. *Psychol Health*. 2011;26(8):1018-35. PMID: 21797732
258. Wagner Costalas J, Itzen M, Malick J, et al. Communication of BRCA1 and BRCA2 results to at-risk relatives: a cancer risk assessment program's experience. *Am J Med Genet C Semin Med Genet*. 2003;119C(1):11-8. PMID: 12704633
259. Finlay E, Stopfer JE, Burlingame E, et al. Factors determining dissemination of results and uptake of genetic testing in families with known BRCA1/2 mutations. *Genet Test*. 2008;12(1):81-91. PMID: 18373407
260. Clarke S, Butler K, Esplen MJ. The phases of disclosing BRCA1/2 genetic information to offspring. *Psychooncology*. 2008;17(8):797-803. PMID: 18646247
261. Segal J, Esplen MJ, Toner B, et al. An investigation of the disclosure process and support needs of BRCA1 and BRCA2 carriers. *Am J Med Genet A*. 2004;125A(3):267-72. PMID: 14994235
262. Bradbury AR, Patrick-Miller L, Pawlowski K, et al. Learning of your parent's BRCA mutation during adolescence or early adulthood: a study of offspring experiences. *Psychooncology*. 2009;18(2):200-8. PMID: 18702049
263. Tercyak KP, Peshkin BN, Streisand R, et al. Psychological issues among children of hereditary breast cancer gene (BRCA1/2) testing participants. *Psychooncology*. 2001;10(4):336-46. PMID: 11462232
264. ACLU and PUBPAT ask Supreme Court to rule that patents on breast cancer genes are invalid [press release]. New York: American Civil Liberties Union; September 25, 2012.
265. *Association for Molecular Pathology v Myriad Genetics*, 569 US 12-398 (2013).
266. Myriad Genetics Laboratory. BRAC*Analysis*®: Hereditary Cancer Testing for Hereditary Breast and Ovarian Cancer. Salt Lake City, UT: Myriad Genetics Laboratory; 2013. Accessed at https://www.myriad.com/products/bracanalysis/ on 26 November 2013.
267. Cook-Deegan R, DeRienzo C, Carbone J, et al. Impact of gene patents and licensing practices on access to genetic testing for inherited susceptibility to cancer: comparing

breast and ovarian cancers with colon cancers. *Genet Med.* 2010;12(4 Suppl):S15-38. PMID: 20393305
268. Centers for Disease Control and Prevention. Genetic testing for breast and ovarian cancer susceptibility: evaluating direct-to-consumer marketing—Atlanta, Denver, Raleigh-Durham, and Seattle, 2003. *MMWR Morb Mortal Wkly Rep*. 2004;53(27):603-6. PMID: 15254451
269. Myers MF, Chang MH, Jorgensen C, et al. Genetic testing for susceptibility to breast and ovarian cancer: evaluating the impact of a direct-to-consumer marketing campaign on physicians' knowledge and practices. *Genet Med.* 2006;8(6):361-70. PMID: 16778598
270. Lowery JT, Byers T, Axell L, et al. The impact of direct-to-consumer marketing of cancer genetic testing on women according to their genetic risk. *Genet Med.* 2008;10(12):888-94. PMID: 19092441
271. Mouchawar J, Laurion S, Ritzwoller DP, et al. Assessing controversial direct-to-consumer advertising for hereditary breast cancer testing: reactions from women and their physicians in a managed care organization. *Am J Manag Care*. 2005;11(10):601-8. PMID: 16232001
272. Mouchawar J, Hensley-Alford S, Laurion S, et al. Impact of direct-to-consumer advertising for hereditary breast cancer testing on genetic services at a managed care organization: a naturally-occurring experiment. *Genet Med.* 2005;7(3):191-7. PMID: 15775755
273. Cortesi L, Turchetti D, Marchi I, et al. Breast cancer screening in women at increased risk according to different family histories: an update of the Modena Study Group experience. *BMC Cancer*. 2006;6:210. PMID: 16916448
274. Leach MO. Screening with magnetic resonance imaging and mammography of a UK population at high familial risk of breast cancer: a prospective multicentre cohort study (MARIBS). *Lancet.* 2005;365(9473):1769-78. PMID: 15910949
275. Rijnsburger AJ, Essink-Bot ML, Van Dooren S, et al. Impact of screening for breast cancer in high-risk women on health-related quality of life. *Br J Cancer.* 2004;91(1):69-76. PMID: 15199386
276. Le-Petross HT, Whitman GJ, Atchley DP, et al. Effectiveness of alternating mammography and magnetic resonance imaging for screening women with deleterious BRCA mutations at high risk of breast cancer. *Cancer*. 2011;117(17):3900-7. PMID: 21365619
277. Kriege M, Brekelmans CT, Boetes C, et al. Efficacy of MRI and mammography for breast-cancer screening in women with a familial or genetic predisposition. *N Engl J Med.* 2004;351(5):427-37. PMID: 15282350
278. Rijnsburger AJ, Obdeijn IM, Kaas R, et al. BRCA1-associated breast cancers present differently from BRCA2-associated and familial cases: long-term follow-up of the Dutch MRISC screening study. *J Clin Oncol.* 2010;28(36):5265-73. PMID: 21079137
279. Bourne TH, Campell S, Reynolds KM, et al. Screening for early familial ovarian cancer with transvaginal ultrasonography and colour blood flow imaging. *BMJ*. 1993;306(3884):1025-9. PMID: 8490496
280. Buys SS, Partridge E, Black A, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening randomized controlled trial. *JAMA.* 2011;305(22):2295-302. PMID: 21642681

281. Hermsen BB, Olivier RI, Verheijen RH, et al. No efficacy of annual gynaecological screening in BRCA1/2 mutation carriers: an observational follow-up study. *Br J Cancer.* 2007;96:1335-42. PMID: 17426707
282. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2 National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) breast cancer prevention trial. *JAMA.* 2001;286(18):2251-6. PMID: 11710890
283. Nelson HD, Fu R, Humphrey L, et al. Comparative Effectiveness of Medications to Reduce Risk of Primary Breast Cancer in Women. Rockville, MD: Agency for Healthcare Research and Quality; 2009. PMID: 20704040
284. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst.* 2005;97(22):1652-62. PMID: 16288118
285. Powles TJ, Ashley S, Tidy A, et al. Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. *J Natl Cancer Inst.* 2007;99(4):283-90. PMID: 17312305
286. Veronesi U, Maisonneuve P, Rotmensz N, et al. Tamoxifen for the prevention of breast cancer: late results of the Italian randomized tamoxifen prevention trial among women with hysterectomy. *J Natl Cancer Inst.* 2007;99(9):727-37. PMID: 17470740
287. Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer—96-month follow-up of the randomized IBIS-I trial. *J Natl Cancer Inst.* 2007;99(4):272-82. PMID: 17312304
288. Lippman ME, Cummings SR, Disch DP, et al. Effect of raloxifene on the incidence of invasive breast cancer in postmenopausal women with osteoporosis categorized by breast cancer risk. *Clin Cancer Res.* 2006;12(17):5242-7. PMID: 16951244
289. Vogel VG, Costantino JP, Wickerham DL, et al. Update of the National Surgical Adjuvant Breast and Bowel Project Study of Tamoxifen and Raloxifene (STAR) P-2 Trial: preventing breast cancer. *Cancer Prev Res (Phila).* 2010;3(6):696-706. PMID: 20404000
290. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. *N Engl J Med.* 1999;340(2):77-84. PMID: 9887158
291. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. *J Natl Cancer Inst.* 2001;93(21):1633-7. PMID: 11698567
292. Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. *JAMA.* 2010;304(9):967-75. PMID: 20810374
293. Evans DG, Baildam AD, Anderson E, et al. Risk reducing mastectomy: outcomes in 10 European centres. *J Med Genet.* 2009;46(4):254-8. PMID: 18996907
294. Skytte AB, Cruger D, Gerster M, et al. Breast cancer after bilateral risk-reducing mastectomy. *Clin Genet.* 2011;79(5):431-7. PMID: 21199491
295. Hartmann JT, Von Vangerow A, Fels LM, et al. A randomized trial of amifostine in patients with high-dose VIC chemotherapy plus autologous blood stem cell transplanation. *Br J Cancer.* 2001;84(3):313-20. PMID: 11161394

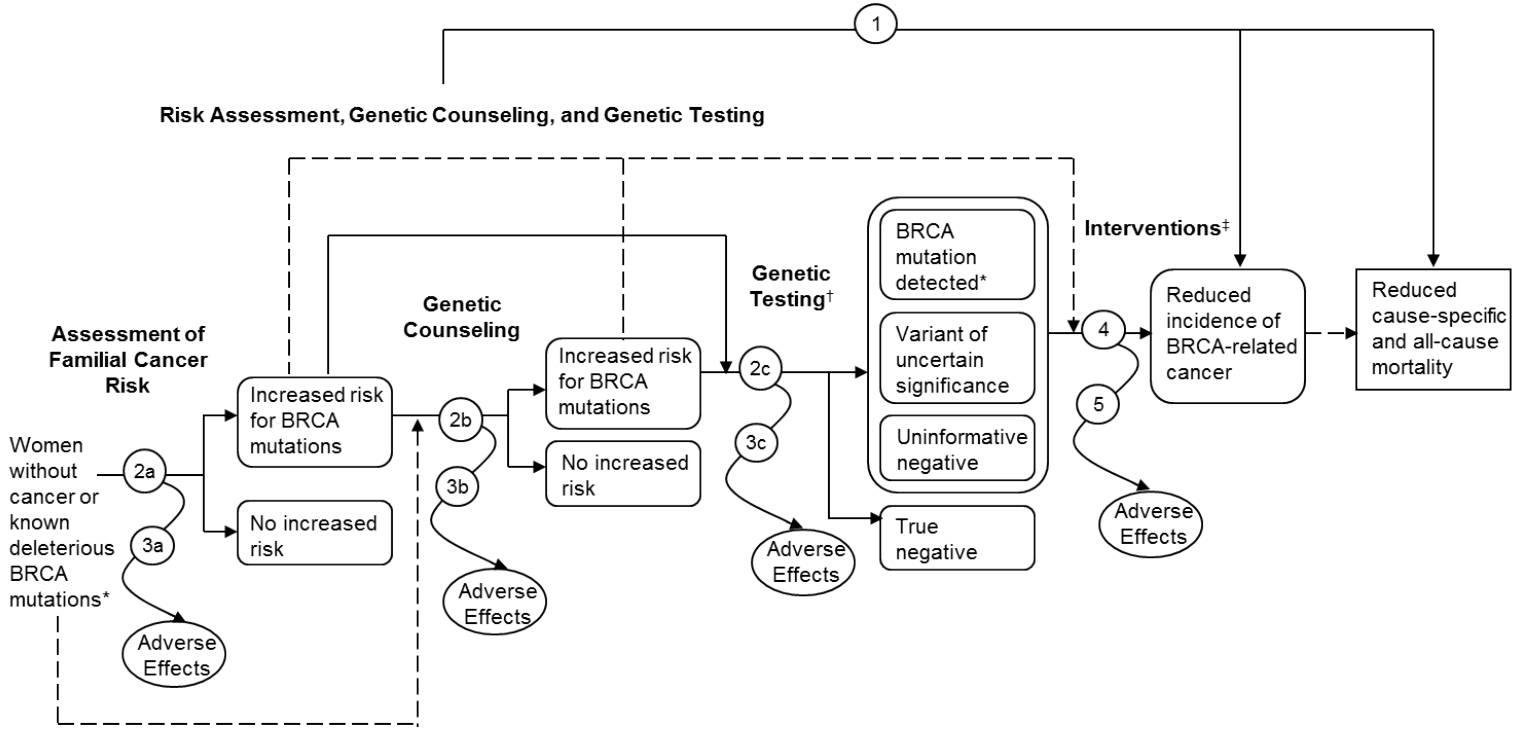
296. Olson JE, Sellers TA, Iturria SJ, et al. Bilateral oophorectomy and breast cancer risk reduction among women with a family history. *Cancer Epidemiol Biomarkers Prev.* 2004;28(5):357-60. PMID: 15542261
297. Kriege M, Brekelmans CT, Boetes C, et al. Differences between first and subsequent rounds of the MRISC breast cancer screening program for women with a familial or genetic predisposition. *Cancer*. 2006;106(11):2318-26. PMID: 16615112
298. Spiegel TN, Esplen MJ, Hill KA, et al. Psychological impact of recall on women with BRCA mutations undergoing MRI surveillance. *Breast*. 2011;20(5):424-30. PMID: 21612928
299. Barrett-Connor E, Mosca L, Collins P, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. *N Engl J Med.* 2006;355(2):125-37. PMID: 16837676
300. Contant CM, Menke-Pluijmers MB, Seynaeve C, et al. Clinical experience of prophylactic mastectomy followed by immediate breast reconstruction in women at hereditary risk of breast cancer (HB(O)C) or a proven BRCA1 and BRCA2 germ-line mutation. *Eur J Surg Oncol*. 2002;28(6):627-32. PMID: 12359199
301. Metcalfe KA, Esplen MJ, Goel V, et al. Psychosocial functioning in women who have undergone bilateral prophylactic mastectomy. *Psychooncology*. 2004;13(1):14-25. PMID: 14745742
302. Brandberg Y, Sandelin K, Erikson S, et al. Psychological reactions, quality of life, and body image after bilateral prophylactic mastectomy in women at high risk for breast cancer: a prospective 1-year follow-up study. *J Clin Oncol.* 2008;26(24):3943-9. PMID: 18711183
303. Gahm J, Wickman M, Brandberg Y. Bilateral prophylactic mastectomy in women with inherited risk of breast cancer—prevalence of pain and discomfort, impact on sexuality, quality of life and feelings of regret two years after surgery. *Breast*. 2010;19(6):462-9. PMID: 20605453
304. Brandberg Y, Arver B, Johansson H, et al. Less correspondence between expectations before and cosmetic results after risk-reducing mastectomy in women who are mutation carriers: a prospective study. *Eur J Surg Oncol*. 2012;38(1):38-43. PMID: 22032910
305. Wasteson E, Sandelin K, Brandberg Y, et al. High satisfaction rate ten years after bilateral prophylactic mastectomy: a longitudinal study. *Eur J Cancer Care*. 2011;20(4):508-13. PMID: 20597955
306. Finch A, Metcalfe KA, Chiang JK, et al. The impact of prophylactic salpingo-oophorectomy on menopausal symptoms and sexual function in women who carry a BRCA mutation. *Gynecol Oncol.* 2011;121(1):163-8. PMID: 21216453
307. Antoniou AC, Beesley J, McGuffog L, et al. Common breast cancer susceptibility alleles and the risk of breast cancer for BRCA1 and BRCA2 mutation carriers: implications for risk prediction. *Cancer Res*. 2010;70(23):9742-54. PMID: 21118973
308. Antoniou AC, Kartsonaki C, Sinilnikova OM, et al. Common alleles at 6q25.1 and 1p11.2 are associated with breast cancer risk for BRCA1 and BRCA2 mutation carriers. *Hum Mol Genet*. 2011;20(16):3304-21. PMID: 21593217
309. Milne RL, Antoniou AC. Genetic modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. *Ann Oncol*. 2011;22(Suppl 1):i11-7. PMID: 21285145

310. Osorio A, Milne RL, Alonso R, et al. Evaluation of the XRCC1 gene as a phenotypic modifier in BRCA1/2 mutation carriers. Results from the consortium of investigators of modifiers of BRCA1/BRCA2. *Br J Cancer.* 2011;104(8):1356-61. PMID: 21427728
311. Rebbeck TR, Mitra N, Domchek SM, et al. Modification of BRCA1-associated breast and ovarian cancer risk by BRCA1-interacting genes. *Cancer Res*. 2011;71(17):5792-805. PMID: 21799032
312. Fanale D, Amodeo V, Corsini LR, et al. Breast cancer genome-wide association studies: there is strength in numbers. *Oncogene*. 2012;31(17):2121-8. PMID: 21996731
313. Peng S, Lü B, Ruan W, et al. Genetic polymorphisms and breast cancer risk: evidence from meta-analyses, pooled analyses, and genome-wide association studies. *Breast Cancer Res Treat.* 2011;127(2):309-24. PMID: 21445572
314. Ripperger T, Gadzicki D, Meindl A, et al. Breast cancer susceptibility: current knowledge and implications for genetic counselling. *Eur J Hum Genet*. 2009;17(6):722-31. PMID: 19092773
315. van der Groep P, van der Wall E, van Diest PJ. Pathology of hereditary breast cancer. *Cell Oncol (Dordr)*. 2011;34(2):71-88. PMID: 21336636
316. Graeser MK, Engel C, Rhiem K, et al. Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. *J Clin Oncol.* 2009;27(35):5887-92. PMID: 19858402
317. Kirova YM, Stoppa-Lyonnet D, Savignoni A, et al. Risk of breast cancer recurrence and contralateral breast cancer in relation to BRCA1 and BRCA2 mutation status following breast-conserving surgery and radiotherapy. *Eur J Cancer.* 2005;41(15):2304-11. PMID: 16140006
318. Metcalfe K, Lynch HT, Ghadirian P, et al. Contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. *J Clin Oncol.* 2004;22(12):2328-35. PMID: 15197194
319. Weitzel JN, Robson M, Pasini B, et al. A comparison of bilateral breast cancers in BRCA carriers. *Cancer Epidemiol Biomarkers Prev.* 2005;14(6):1534-8. PMID: 15941968
320. Robertson L, Hanson H, Seal S, et al. BRCA1 testing should be offered to individuals with triple-negative breast cancer diagnosed below 50 years. *Br J Cancer.* 2012;106(6):1234-8. PMID: 22333603
321. Evans D, Gareth R, Lennard F, et al. Eligibility for magnetic resonance imaging screening in the United Kingdom: effect of strict selection criteria and anonymous DNA testing on breast cancer incidence in the MARIBS study. *Cancer Epidemiol Biomarkers Prev.* 2009;18(7):2123-31. PMID: 19567506
322. Statement of the American Society of Human Genetics on genetic testing for breast and ovarian cancer predisposition. *Am J Hum Genet.* 1994;55(5):i-iv. PMID: 7977337
323. American College of Obstetricians and Gynecologists; ACOG Committee on Practice Bulletins–Gynecology; ACOG Committee on Genetics; Society of Gynecologic Oncologists. ACOG Practice Bulletin No. 103: hereditary breast and ovarian cancer syndrome. *Obstet Gynecol*. 2009;113(4):957-66. PMID: 19305347
324. Balmaña J, Díez O, Rubio IT, et al. BRCA in breast cancer: ESMO clinical practice guidelines. *Ann Oncol*. 2011;22(Suppl 6):vi31-4. PMID: 21908500
325. Lancaster JM, Powell CB, Kauff ND, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol.* 2007;107(2):159-62. PMID: 17950381

326. Riley BD, Culver JO, Skrzynia C, et al. Essential elements of genetic cancer risk assessment, counseling, and testing: updated recommendations of the National Society of Genetic Counselors. *J Genet Couns*. 2012;21(2):151-61. PMID: 22134580
327. Robson ME, Storm CD, Weitzel J, et al. American Society of Clinical Oncology Policy Statement Update: genetic and genomic testing for cancer susceptibility. *J Clin Oncol.* 2010;28(5):893-901. PMID: 20065170
328. Cuzick J. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. *Lancet.* 2002;360(9336):817-24. PMID: 12243915
329. Vogel VG, Costantino JP, Wickerham DL, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. *JAMA.* 2006;295(23):2727-41. PMID: 16754727
330. Andresen EM, Catlin TK, Wyrwich KW, et al. Retest reliability of surveillance questions on health related quality of life. *J Epidemiol Community Health*. 2003;57(5):339-43. PMID: 12700216
331. Bados A, Gómez-Benito J, Balaguer G. The State-Trait Anxiety Inventory, trait version: does it really measure anxiety? *J Pers Assess*. 2010;92(6):560-7. PMID: 20954057
332. Bates GW, Trajstman SE, Jackson CA. Internal consistency, test-retest reliability and sex differences on the posttraumatic growth inventory in an Australian sample with trauma. *Psychol Rep*. 2004;94(3):793-4. PMID: 15217029
333. Baxter NN, Goodwin PJ, McLeod RS, et al. Reliability and validity of the body image after breast cancer questionnaire. *Breast J*. 2006;12(3):221-32. PMID: 16684320
334. Beck AT, Steer RA, Garbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin Psychol Rev*. 1988;8(1):77-100.
335. Bjelland I, Dahl AA, Haug TT, et al. The validity of the Hospital Anxiety and Depression Scale: an updated literature review. *J Psychosom Res*. 2002;52(2):69-77. PMID: 11832252
336. Lerman C, Trock B, Rimer BK, et al. Psychological side effects of breast cancer screening. *Health Psychol*. 1991;10(4):259-67. PMID: 1915212
337. Brehaut JC, O'Connor AM, Wood TJ, et al. Validation of a decision regret scale. *Med Decis Making*. 2003;23(4):281-92. PMID: 12926578
338. Carver CS, Scheier MF, Weintraub JK. Assessing coping strategies: a theoretically based approach. *J Pers Soc Psychol*. 1989;56(2):267-83. PMID: 2926629
339. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. *Psychol Med*. 1983;13(3):595-605. PMID: 6622612
340. Grandner MA, Kripke DF, Yoon IY, et al. Criterion validity of the Pittsburgh Sleep Quality Index: investigation in a non-clinical sample. *Sleep Biol Rhythms*. 2006;4(2):129-36. PMID: 22822303
341. Hopwood P, Fletcher I, Lee A, et al. A body image scale for use with cancer patients. *Eur J Cancer.* 2001;37(2):189-97. PMID: 11166145
342. Jackson C. The General Health Questionnaire. *Occup Med*. 2007;57(1):79.
343. Matalqah LM, Radaideh KM, Yusoff ZM, et al. Health-related quality of life using EQ-5D among breast cancer survivors in comparison with age-matched peers from the general population in the state of Penang, Malaysia. *J Pub Health*. 2011;19(5):475-80.

344. McHorney CA, Ware Jr JE, Lu JF, et al. The MOS 36-Item Short-Form Health Survey (SF-36), III: tests of data quality, scaling assumptions, and reliability across diverse patient groups. *Med Care*. 1994;32(1):40-66. PMID: 8277801
345. Parkerson GR, Broadhead WE, Tse CK. Original contributions. Validation of the Duke Social Support and Stress Scale. *Fam Med*. 1991;23(5):357-60. PMID: 1884930
346. Radtke JV, Terhorst L, Cohen SM. The Menopause-Specific Quality of Life Questionnaire: psychometric evaluation among breast cancer survivors. *Menopause*. 2011;18(3):289-95. PMID: 20881889
347. Schmitz N, Hartkamp N, Kiuse J, et al. The Symptom Check-List-90-R (SCL-90-R): a German validation study. *Qual Life Res*. 2000;9(2):185-93. PMID: 10983482
348. Schroevers MJ, Sanderman R, Van Sonderen E, et al. The evaluation of the Center for Epidemiologic Studies Depression (CES-D) scale: depressed and positive affect in cancer patients and healthy reference subjects. *Qual Life Res*. 2000;9(9):1015-29. PMID: 11332223
349. Shapiro DE, Rodrigue JR, Boggs SR, et al. Cluster analysis of the Medical Coping Modes Questionnaire: evidence for coping with cancer styles? *J Psychosom Res*. 1994;38(2):151-9. PMID: 8189404
350. Stanton AL, Kirk SB, Cameron CL, et al. Coping through emotional approach: scale construction and validation. *J Pers Soc Psychol*. 2000;78(6):1150-69. PMID: 10870915
351. Steed L. Further validity and reliability evidence for Beck Hopelessness Scale scores in a nonclinical sample. *Educ Psychol Meas*. 2001;61(2):303-16.
352. Stein KD, Jacobsen PB, Blanchard CM, et al. Further validation of the multidimensional Fatigue Symptom Inventory-Short Form. *J Pain Symptom Manage*. 2004;27(1):14-23. PMID: 14711465
353. Sullivan M, Karlsson J, Ware JE Jr. The Swedish SF-36 Health Survey, I: evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. *Soc Sci Med*. 1995;41(10):1349-58. PMID: 8560302
354. Thirlaway K, Fallowfield L, Cuzick J. The Sexual Activity Questionnaire: a measure of women's sexual functioning. *Qual Life Res*. 1996;5(1):81-90. PMID: 8901370
355. Wewers ME, Lowe NK. A critical review of visual analogue scales in the measurement of clinical phenomena. R*es Nurs Health*. 1990;13(4):227-36. PMID: 2197679
356. Zilberg NJ, Weiss DS, Horowitz MJ. Impact of Event Scale: a cross-validation study and some empirical evidence supporting a conceptual model of stress response syndromes. *J Consult Clin Psychol*. 1982;50(3):407-14. PMID: 7096742

Figure 1. Analytic Framework and Key Questions



Key Questions

1. Does risk assessment, genetic counseling, and genetic testing lead to reduced incidence of BRCA-related cancer and reduced cause-specific and all-cause mortality?
2a. What is the accuracy of methods to assess familial cancer risk for BRCA-related cancer when performed by a nongenetics specialist in a clinical setting?
2b. What are the benefits of genetic counseling in determining eligibility for genetic testing for BRCA-related cancer?
2c. What is the clinical validity of genetic testing for deleterious mutations in women with increased risk for BRCA-related cancer?
3. What are the potential adverse effects of a) risk assessment, b) genetic counseling, and c) genetic testing?
4. Do interventions reduce the incidence of BRCA-related cancer and mortality in women with increased risk?
5. What are the potential adverse effects of interventions to reduce risk for BRCA-related cancer?

* Clinically significant mutations of *BRCA1*, *BRCA2*, or related syndromes.
† Testing may be done on the unaffected woman, her relative with cancer, or relative with highest risk, as appropriate.
‡ Interventions include increased early detection through intensive screening (earlier and more frequent mammography, breast magnetic resonance imaging), risk-reducing medications (tamoxifen, raloxifene), and risk-reducing surgery (mastectomy, salpingo-oophorectomy).

Uninformative negative test = no known mutation in relatives, none detected in patient; True negative test = known mutation in relatives but none detected in patient.

Figure 2. Included Studies of Prevalence and Penetrance

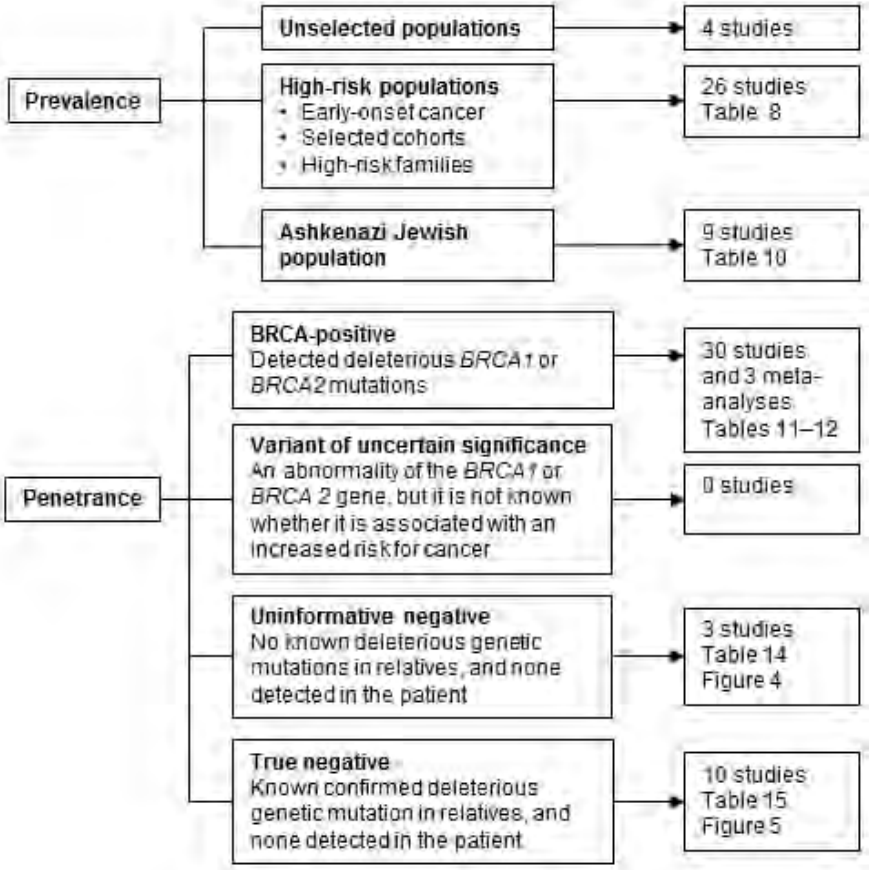
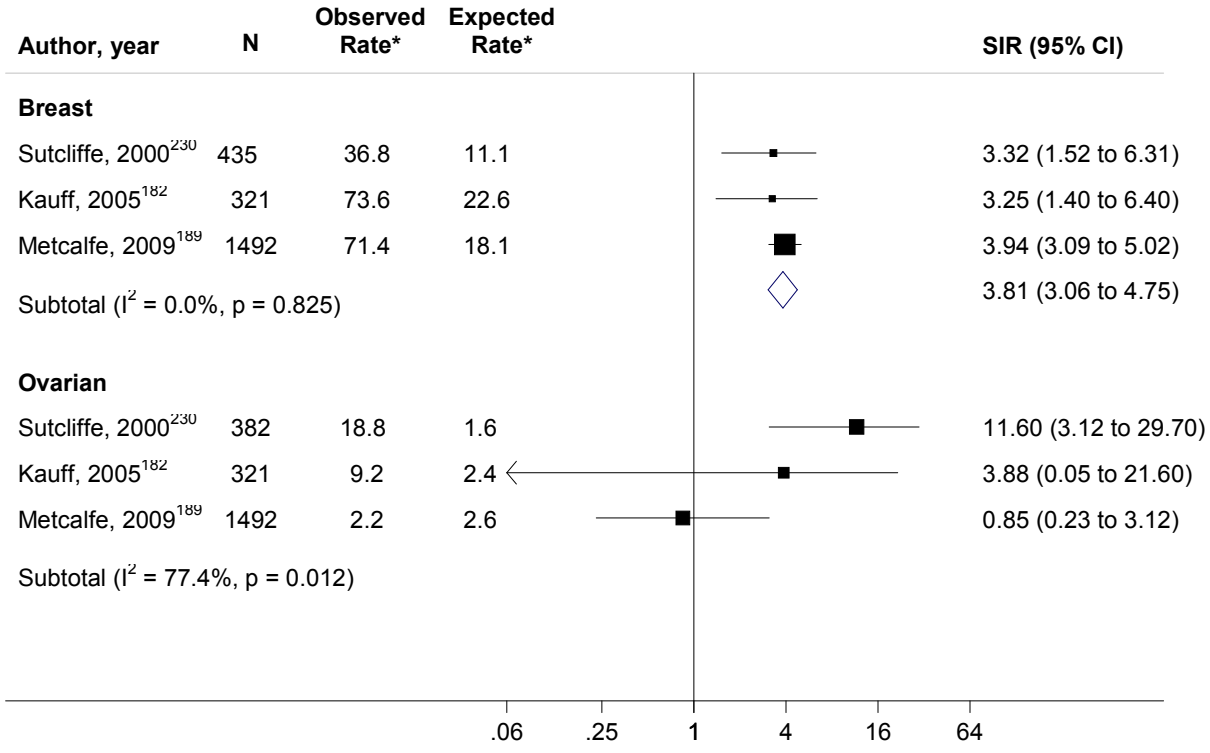


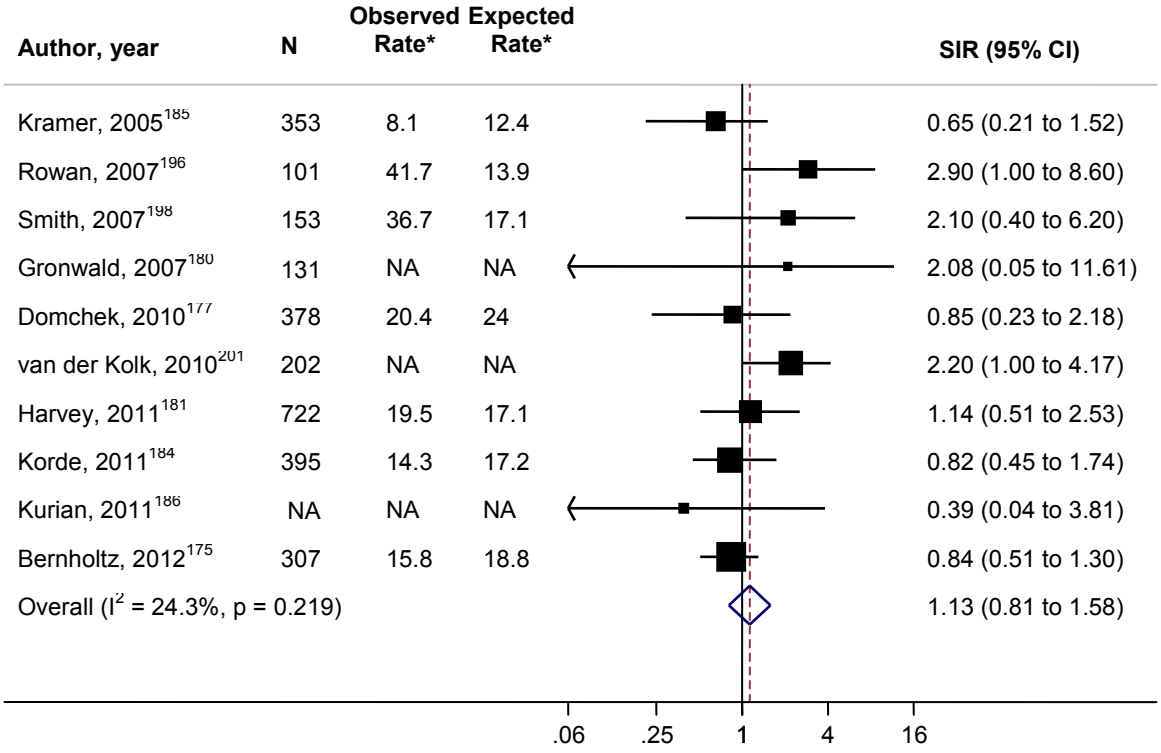
Figure 3. Meta-Analysis of Studies of Breast and Ovarian Cancer Incidence in Women With Uninformative Negative Results



*Per 10,000 person-years.

Abbreviations: CI = confidence interval; SIR = standardized incidence ratio.

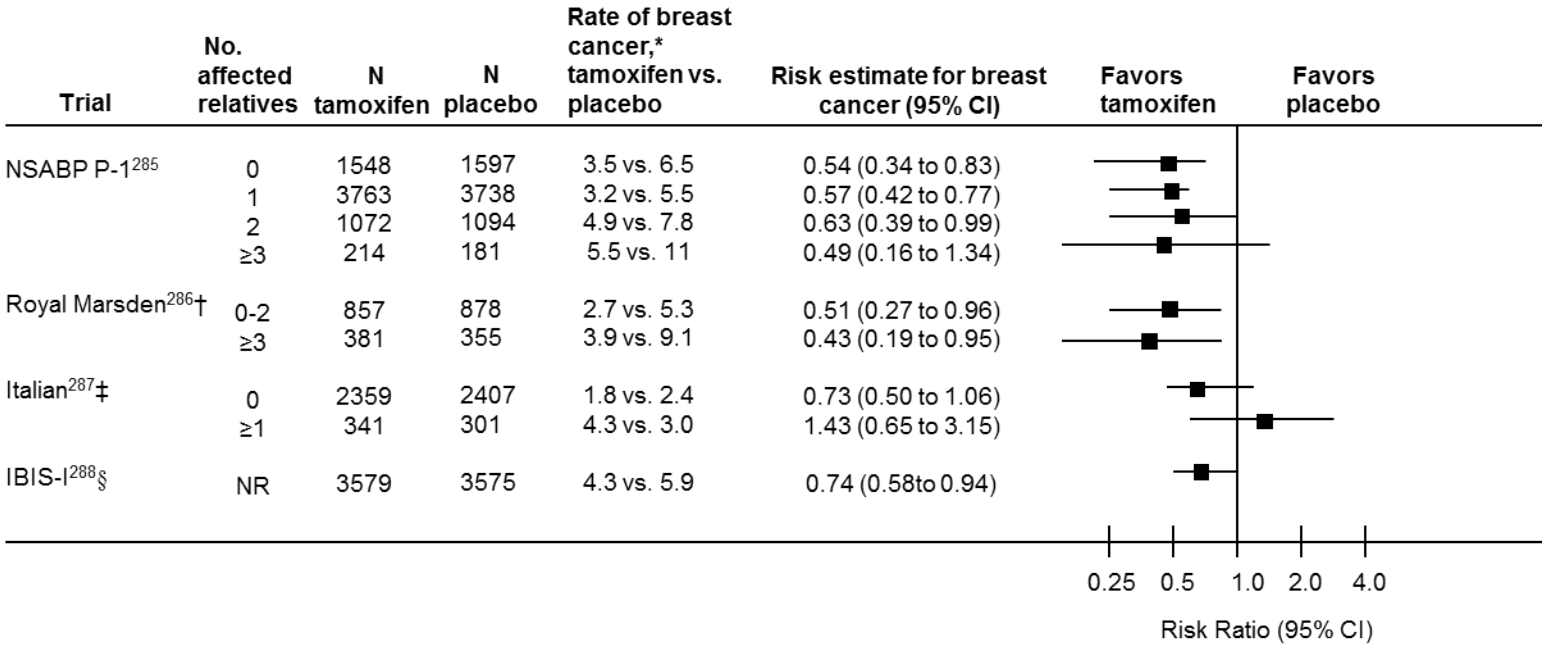
Figure 4. Meta-Analysis of Studies of Breast Cancer Incidence in Women With True Negative Results



*Per 10,000 person-years.

Abbreviations: CI = confidence interval; NA = not applicable; SIR = standardized incidence ratio.

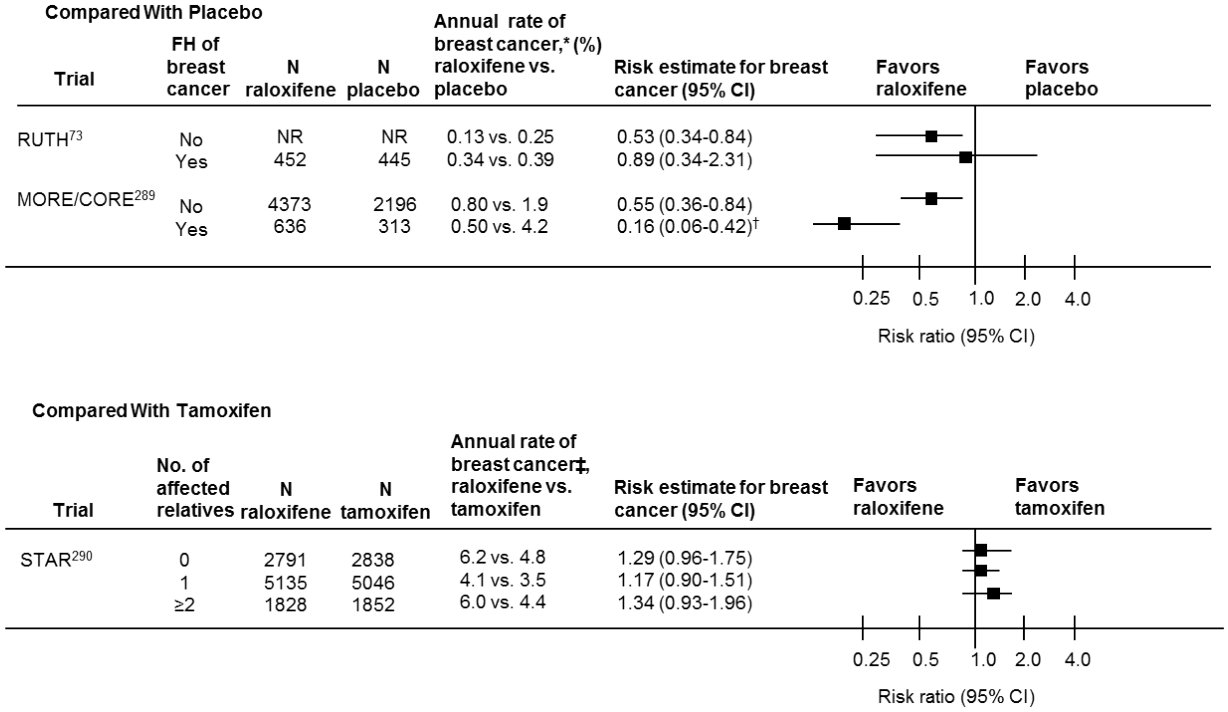
Figure 5. Invasive Breast Cancer Risk Reduction With Tamoxifen Use, by Family History



* Per 1,000 women-years.
† Analysis restricted to ER-positive tumors.
‡ Type of breast cancer not reported.
§ Results not presented by family history (97% of participants had some family history).

Abbreviations: CI = confidence interval; IBIS-I = International Breast Cancer Intervention Study; Italian = Italian Randomized Tamoxifen Prevention Trial; NSABP P-1 = National Surgical Adjuvant Breast and Bowel Project P-1 Trial.

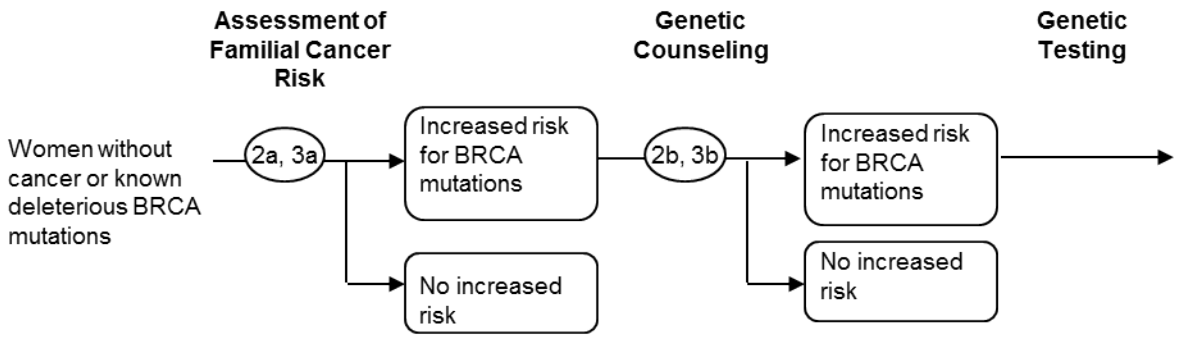
Figure 6. Invasive Breast Cancer Risk Reduction With Raloxifene Use, by Family History



* Per 10,000 women-years.
† Adjusted for age, estradiol level.
‡ Per 1,000 women-years.

Abbreviations: CI = confidence interval; CORE = Continuing Outcomes Relevant to Evista Trial; FH = family history; MORE = Multiple Outcomes for Raloxifene Evaluation Trial; NR = not reported; RUTH = Raloxifene Use for the Heart Trial; STAR = Study of Tamoxifen and Raloxifene Trial.

Figure 7. Summary of Key Questions 2a, 3a and 2b, 3b

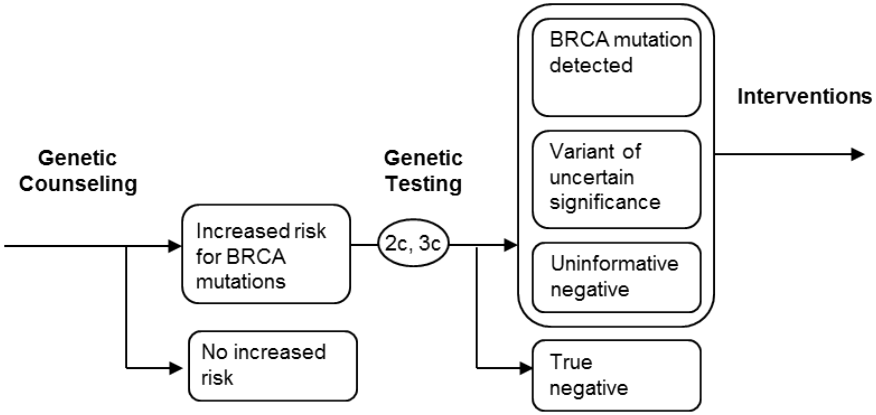


KQ 2a. Accuracy of Risk Assessment	
Risk Models	**Discriminatory Accuracy (c statistic)**
Referral criteria	No studies
General risk models	0.55–0.65 for breast cancer risk; models do predict mutation risk
Family history models	>0.80 for mutation risk
KQ 3a. Adverse Effects of Risk Assessment	
No studies	

KQs 2b, 3b. Benefits and Adverse Effects of Genetic Counseling			
	Number of Studies		
Measure	**Increase**	**Decrease**	**NS**
Breast cancer worry	0	8	9
Anxiety	0	5	8
Depression	0	1	6
Risk accuracy	15	2	5
Intention to test	1	4	0

NS = differences between counseled/noncounseled groups or before/after counseling are not statistically significant.

Figure 8. Summary of Key Questions 2c and 3c



KQ 2c. Clinical Validity of Genetic Testing

Population	Mutation Prevalence
Unselected	0.2%–0.3%
High-risk	
Breast or ovarian cancer onset ≤40 yrs	*BRCA1*: 4.3% *BRCA2*: 2.9% Combined: 6.0%
Breast cancer cohort	*BRCA1*: 1.8% *BRCA2*: 1.3%
Ovarian cancer cohort	*BRCA1*: 4.4% *BRCA2*: 5.6%
Family history of breast or ovarian cancer	*BRCA1*: 13.6% *BRCA2*: 7.9% Combined: 19.8%
Ashkenazi Jewish	
Unselected	*BRCA1*: 1% *BRCA2*: 1% Combined: 2.1%
Family history of breast or ovarian cancer	*BRCA1*: 6.4% *BRCA2*: 1.1% Combined: 10.2%

Test Result	Chance of Cancer (mutation penetrance)
Positive	% to age 70 or 75 yrs
1 person tested	*BRCA1*: 46% BC; 41% OC *BRCA2*: 50% BC; 17% OC
>1 person tested	*BRCA1*: 70% BC; 46% OC *BRCA2*: 71% BC; 23% OC
Ashkenazi Jewish	Combined: 34% BC; 21% OC
Variant of Unknown Significance	No studies
Negative	Standardized incidence rate
Uninformative	Breast: 3.81 (3.06–3.32) Ovarian: 0.85–11.6
True	Breast: 1.26 (0.79–2.01) Ovarian: 0–4.6

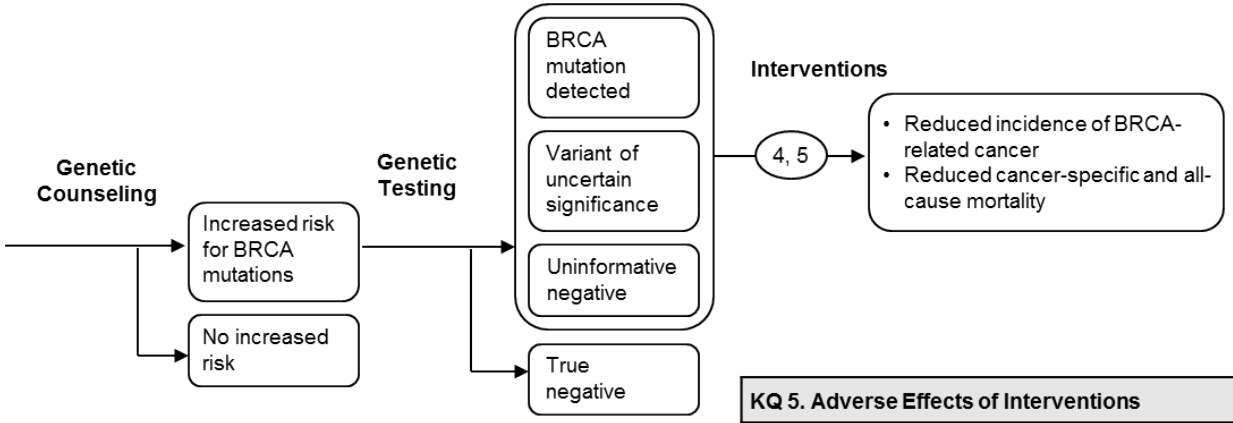
KQ 3c. Adverse Effects of Genetic Testing

Measure	Number of Studies		
	Increase	Decrease	NS
Breast cancer worry	4	1	0
Anxiety	5	3	4
Depression	1	1	4

NS = Differences between counseled/noncounseled groups or before/after counseling are not statistically significant.

Abbreviations: BC = breast cancer; OC = ovarian cancer.

Figure 9. Summary of Key Questions 4 and 5



KQ 4. Benefits of Interventions	
Intervention	**Risk Reduction**
Intensive Screening	No effectiveness studies
Risk-Reducing Medications	
Invasive breast cancer	30%–68%*
Ovarian cancer	No significant reduction
Mortality	No significant reduction
Risk-Reducing Surgery	
Breast cancer	RRM: 85%–100%; RRSO: 37%–100%†
Ovarian cancer	RRSO: 69%–100%
Mortality	RRM: 81%–100% breast cancer; RRSO: 55%–100% all-cause

KQ 5. Adverse Effects of Interventions	
Intervention	**Rates**
Intensive Screening (per episode or year)	
False-positive	4.6%–15% mammogram; 8.2%–14% MRI; 3.4% TVUS
Recall	3.9% mammogram; 11% MRI
Unneeded biopsy	27%–28% mammogram; 25%–43% MRI; 100% TVUS‡; 67% CA-125‡
Risk-Reducing Medication (over 5 years)	
Venous thrombosis	0.4%–0.7%
Endometrial cancer	Tamoxifen: 0.4%
Risk-Reducing Surgery	
Complications	RRM: 3%–59%; RRSO: no studies
Symptoms	RRM: 64%–87%; RRSO: no studies
Quality of life	RM: decrease anxiety; sexual RRSO: decrease sexual

* Risk reduction for all women; analysis by family history was similar.
† Includes studies of oophorectomy alone.
‡ Includes some women with symptoms.

Abbreviations: CA-125 = cancer antigen-125; MRI = magnetic resonance imaging; RRM = risk-reducing mastectomy; RRSO = risk-reducing salpingo-oophorectomy; TVUS = transvaginal ultrasound.

Table 1. Types of Clinical Testing for BRCA Mutations in the United States[96]

Test	Description	Approximate cost (U.S. $)*
Comprehensive testing	Gene sequencing of the entire length of both *BRCA1* and *BRCA2* and a five-site rearrangement panel of specific large-scale rearrangements.	>$3000
Single site testing	One specific gene mutation when the mutation in the family has already been identified.	$475
Multisite panel	Three specific gene changes common among Ashkenazi Jewish ancestry.	$575
BRCA Rearrangement Test	Large-scale rearrangements within the BRCA genes that would not have been detected through comprehensive testing.	$700

*Reflects costs prior to the recent U.S. Supreme Court decision against DNA patents.

Table 2. Recommendations of Other Groups

Organization, year	Recommendations
American Society of Clinical Oncology, 2010[327]	ASCO recommends genetic testing when: 1) there is personal or family history suggestive of genetic cancer susceptibility, 2) the test can be adequately interpreted, and 3) the results will aid in diagnosis or medical management of the patient or family members at hereditary risk of cancer. ASCO recommends genetic testing only when pre- and post-test counseling is included.
American Congress of Obstetricians and Gynecologists, 2009[323]	For patients who are likely to have hereditary breast and ovarian cancer syndrome, ACOG recommends further genetic risk assessment for women who have more than a 20%–25% chance of having an inherited predisposition to breast or ovarian cancer. ACOG also suggests genetic risk assessment may also be appropriate for patients with a 5%–10% chance of having hereditary risk. Recommended screening and prevention plans are based on individual risk factors and family history.
American Society of Human Genetics, 1994[322]	Testing should initially be offered and performed on an investigational basis by appropriately trained health care professionals who have a therapeutic relationship with the patient and are fully aware of the genetic, clinical, and psychological implications of testing, as well as of the limitations of existing test procedures. Linkage analysis is recommended for select high-risk families, if it will provide for more refined counseling than is currently available from family history alone. It is premature to offer population screening, until the risks associated with specific *BRCA1* mutations are determined.
National Comprehensive Cancer Network, 2012[50]	NCCN recommends risk assessment and counseling if the hereditary breast and/or ovarian cancer syndrome testing criteria are met. Genetic testing is recommended if criteria are met (see **Appendix A1**).
European Society for Medical Oncology, 2011[324]	In all cases in which a patient may be referred for BRCA testing, the ESMO Guidelines Working Group recommends informed consent and genetic counseling be completed first. Carriers should be encouraged to advise close family members to obtain genetic counseling.
National Society of Genetic Counselors, 2012[326]	Genetic testing should be offered to individuals with a personal or family history suggestive of an inherited cancer syndrome; when the test can be adequately interpreted; if testing will influence medical management of the patient or relatives; when potential benefits outweigh potential risks; if testing is voluntary; and when the individual seeking testing or their legal proxy can provide informed consent.
Society of Gynecologic Oncologists Education Committee, 2007[325]	The SGO Education Resource Panel for Hereditary Cancers believes that individuals with a personal risk of having an inherited predisposition to cancer of greater than approximately 20%–25% should undergo genetic risk assessment. It also believes that it is reasonable to offer genetic risk assessment to any individual with greater than approximately 5%–10% chance of having an inherited predisposition to cancer. Genetic testing for cancer predisposition requires informed consent that should include pretest education and counseling concerning the risks, benefits, and limitations of testing, including the implications of both positive and negative genetic test results.

Table 3. Risk Stratification Models

Model	Included variables						Calibration expected/observed cases (95% CI)*	Discriminatory accuracy c-statistic (95% CI)*
	Age, *y*	Menarche age, *y*	Age at birth of first child, *y*	First-degree relatives with breast cancer, *n*	Previous breast biopsy, *n*	Other factors		
Gail Model Variations								
Gail-2 5-year risk	<50; ≥50	≥14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	0; 1; ≥2	Bx: 0; 1; ≥2 AH: 0; ≥1	Not included	1.03 (0.88 to 1.21);[126] 0.94 (0.89 to 0.99);[132] 0.96 (0.84 to 1.17);[127] 0.79;[123] 1.12[121]	0.55 (0.51 to 0.60);[117] 0.60;[126] 0.58 (0.56 to 0.60);[119] 0.58;[132] 0.59 (0.54 to 0.63);[127] 0.60;[122] 0.61 (0.60 to 0.62)[135]
Gail-2 10-year risk	<50; ≥50	≥14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	0; 1; ≥2	Bx: 0; 1; ≥2 AH: 0; ≥1	Not included	0.69 (0.54 to 0.90)[133]	0.74 (0.67 to 0.80)[118]
African American Gail 5-year risk	<50; ≥50	≤13; >13	Not included	0; 1; ≥2	Bx: 0; 1; ≥2	African American race	1.08 (0.97 to 1.20)[129]	0.56 (0.54 to 0.58);[129] 0.56 (0.51 to 0.60)[117]
Models with Breast Density								
Chen 5-year risk	<50; ≥50	≥14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	0; 1; ≥2	Bx: 0; 1; ≥2	Breast density (%), BMI	Not reported	0.64[122]
BCSC† (pre-menopausal) 1-year risk	45–84 by 5-year groups	Not included	Not included	0; 1; ≥2; unknown	Bx: yes; no; unknown	Breast density (BIRADS)‡	1.00[119]	0.63 (0.60 to 0.66)[119]
BCSC† (post-menopausal) 1-year risk	45–84 by 5-year groups	Not included	<30; ≥30; none; unknown	0; 1; ≥2; unknown	Bx: 0; ≥1; unknown	Breast density (BIRADS), prior false-positive mammogram, BMI, menopause type, HT, race/ethnicity	1.01[119]	0.62 (0.62 to 0.63)[119]
BCSC 5-year risk	45–84 by 5-year groups	Not included	Not included	Yes; no	Bx: yes; no	Breast density (BIRADS), race/ethnicity	1.01 (0.99 to 1.03)[135]	0.66 (0.65 to 0.66)[135]
Other Models								
Rosner-Colditz†	<50; ≥50	14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	Yes; no	Not included	BMI, benign breast disease, menopause type, menopause age, HT use and duration, height, alcohol use, parity	1.00 (0.93 to 1.07)[133]	0.57 (0.55 to 0.59);[133] 0.64 (0.63 to 0.66) (ER+/PR+);[125] 0.61 (0.58 to 0.64) (ER-/PR-)[125]
Rosner-Colditz-2†	<50; ≥50	14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	Yes; no	AH: 0; ≥1	Benign breast disease presence or type	1.01 (0.94 to 1.09)[133]	0.63 (0.61 to 0.65);[133] 0.64 (type)[133]

Table 3. Risk Stratification Models

Model	Included variables						Calibration expected/observed cases (95% CI)*	Discriminatory accuracy c-statistic (95% CI)*
	Age, *y*	Menarche age, *y*	Age at birth of first child, *y*	First-degree relatives with breast cancer, *n*	Previous breast biopsy, *n*	Other factors		
Tyrer-Cuzick 10-year risk	<50; ≥50	≤12; >12	≤30; >30; none	1; 2; ≥2	Bx: 0; 1; ≥2 LCIS: 0; ≥1	BMI, height, menopause age, family history of ovarian/other cancer, age of cancer onset, bilateral or male breast cancer	1.09 (0.85 to 1.41)[118]	0.76 (0.70 to 0.82);[118] 0.54 (0.42 to 0.65)[120]
Italian-1§ 5-year risk	<50; ≥50	≥14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	0; 1; ≥2	Not included	Age of relative at diagnosis, diet score, alcohol use, BMI, HT, physical activity	1.04[121]	0.59 (vitamin);[121] 0.60 (diet)[121]
Italian-2† 20-year risk	<50; ≥50	14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	0; 1; ≥2	Bx: 0; 1; ≥2	Occupational and leisure physical activity, education, alcohol use, BMI	Not reported	0.62 (0.56 to 0.69) (age <50 years);[131] 0.57 (0.52 to 0.61) (age ≥50 y)[131]
Chlebowski 5-year risk	50–59; 60–69; 70–79	≥14; 12–13; ≤12	<20; 20–24; 25–29 or none; ≥30	0; ≥1	Bx: 0; 1; ≥2	BMI, menopause age, HT use and duration, race, alcohol use, parity, breastfeeding, smoking status, physical activity	Not reported	0.61 (0.59 to 0.63);[123] 0.62 (0.60 to 0.64) (ER+);[123] 0.53 (0.47 to 0.58) (ER-)[123]
Chlebowski-simplified 5-year risk	<50; ≥50	Not included	Not included	0; ≥1	Bx: 0; 1; ≥2	Not included	Not reported	0.58 (0.56 to 0.60) (ER+)[123]

* For invasive breast cancer, other outcomes are specifically indicated.
† Invasive and noninvasive breast cancer.
‡ BI-RADS categories include: 0 = unknown; 1 = entirely fat; 2 = scattered fibroglandular densities; 3 = heterogeneously dense; 4 = extremely dense.
§ Includes an Italian population and used incidence rates from the Italian Multicenter case-control study of Diet and Breast Cancer and from Italian cancer registries.

Abbreviations: AH = atypical hyperplasia; BCSC = Breast Cancer Surveillance Consortium; BI-RADS = Breast Imaging Reporting and Data System; BMI = body mass index; Bx = biopsy; CI = confidence interval; DCIS = ductal carcinoma in situ; ER- = estrogen receptor negative; ER+ = estrogen receptor positive; HT = hormone therapy; LCIS= lobular carcinoma in situ; PR- = progesterone receptor negative; PR+ = progesterone receptor positive.

Table 4. Familial Risk Stratification Models to Predict Individual Risk for Deleterious BRCA Mutations in Primary Care Settings

Model	Data collection and calculation	Relatives with breast and ovarian cancer	Other factors	Reference standard	Performance characteristics for predicting risk for BRCA mutations
Ontario Family History Assessment Tool (FHAT)[142,144-146]	Clinical scoring tool; referral threshold of 10 is equivalent to a 2-fold relative risk for breast or ovarian cancer	1st-, 2nd-, 3rd-degree	Age at diagnosis; bilateral breast cancer; breast and ovarian cancer in same person; male breast cancer; colon and prostate cancer	BRCAPRO; Claus	Sensitivity 91%–94%; specificity 15%–51%; PPV 31%; c statistic 0.68–0.83
Manchester Scoring System[56,139,141,144-146]	Clinical scoring tool; referral if ≥2 positive responses	1st-, 2nd-, 3rd-degree	Type of cancer (breast, ovarian, pancreatic, or prostate), affected family members, and age at diagnosis	BRCAPRO; Myriad II; BOADICEA; FHAT	Sensitivity 58%–93%; specificity 33%–71%; c statistic 0.75–0.80
Referral Screening Tool (RST)[140]	Clinical scoring tool; referral if ≥2 positive responses	1st-, 2nd-degree	Breast cancer at age ≤50 (self or relatives); ovarian cancer at any age (self or relatives); ≥2 breast cancer cases at age >50 on same side of family; male breast cancer; Jewish ancestry	BRCAPRO; Myriad II; BOADICEA; FHAT	Sensitivity 81%; specificity 92%; PPV 0.80; NPV 0.92; c statistic 0.87
Pedigree Assessment Tool (PAT)[143]	Clinical scoring tool; score ≥8 was optimal threshold	1st-, 2nd-, 3rd-degree	Breast cancer at age ≤50 or >50; ovarian cancer at any age; male breast cancer; Ashkenazi Jewish ancestry	Myriad II	Sensitivity 100%; specificity 93%; PPV 0.63; NPV 1.00; c statistic 0.96 (compared with Gail 5-year 0.39; Gail lifetime 0.59)
FHS-7[138]	Clinical scoring tool; one positive response was threshold	1st-degree with breast or ovarian cancer	Any relatives with breast cancer at age ≤50; bilateral breast cancer; breast and ovarian cancer in same person; male breast cancer; ≥2 relatives with breast and/or ovarian cancer; ≥2 relatives with breast and/or colon cancer	Claus; Gail; Tyrer-Cuzick; Penn II	Sensitivity 88%; specificity 56%; PPV 0.63; NPV 1.00; c statistic 0.96

Abbreviations: BOADICEA = Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; NPV = negative predictive value; PPV = positive predictive value.

Table 5. Studies of Genetic Counseling

Author, year	N	Provider of genetic counseling	Setting	Measures	Quality rating	Breast cancer worry		Anxiety		Depression		Risk perception		Intent to participate in testing	
						Increase	Decrease	Increase	Decrease	Increase	Decrease	More Accurate	Less Accurate	Increase	Decrease
Current report															
Bennett et al, 2008[150]	128	Genetic counselor	Cancer Genetics Service Center	DUKE-SSQ, HADS, IES, MCMQ,NSI	NA	0	X	0	0	0	0	NR	NR	NR	NR
Bennett et al, 2009[149]	128	Genetic counselor	Cancer Genetics Service Center	DUKE-SSQ, IES, MCMQ	NA	0	X	0	0	0	0	NR	NR	NR	NR
Bloom, 2006[151]	163	Master's level counselor	Telephone counseling	NSI	Poor	0	NR	NR	NR	NR	NR	NR	NR	NR	NR
Bowen et al, 2006[152]	221	Psychologist, genetic counselor	University	NSI, BSI	Fair	0	0	NR	NR	NR	NR	0	0	NR	NR
Brain et al, 2011[153]	263	Clinician	NR	CWS-R	NA	0	X*	NR	NR	NR	NR	X†	0	0	X†
Braithwaite et al, 2005[154]	72	Clinical nurse specialist	NR	NSI, STAI, HADS	Fair	0	X	NR	NR	NR	NR	NR	NR	NR	NR
Fry et al, 2003[155]	263	Genetics consultant & specialist breast surgeon vs. geneticist & genetics nurse specialist	Familial Breast Cancer Clinic	CWS	Fair	0	X‡	0	X§	NR	NR	X‖	0	NR	NR
Gurmankin et al, 2005[156]	125	Health care provider	University breast and ovarian cancer risk evaluation program	STAI, NSI	NA	0	X	NR	NR	NR	NR	X	0	NR	NR
Helmes et al, 2006[157]	340¶	Board certified genetic counselor	NR	NSI	Fair	NR	NR	NR	NR	NR	NR	X‡	0	NR	NR

Table 5. Studies of Genetic Counseling

Author, year	N	Provider of genetic counseling	Setting	Measures	Quality rating	Breast cancer worry		Anxiety		Depression		Risk perception		Intent to participate in testing	
						Increase	Decrease	Increase	Decrease	Increase	Decrease	More Accurate	Less Accurate	Increase	Decrease
Hopwood et al, 2004[158]	256	Genetic counselor	Cancer genetic service centers	NSI, GHQ, CWS	NA	0	X**	NR	NR	NR	NR	X**	0	0	X**
Kelly et al, 2008[159]	78	Genetic counselor	NR	NSI	NA	0	X	0	0	NR	NR	0	0	NR	NR
Matloff et al, 2006[160]	64¶	Certified genetic counselor	NR	NSI	Fair	NR	NR	NR	NR	NR	NR	0	X‡	NR	NR
Mikkelsen et al, 2007[161]	1971	Physicians	Clinical department	IES	Fair	NR	NR	NR	NR	NR	NR	X††	0	NR	NR
Mikkelsen et al, 2009[162]	1971	Physicians	Clinical department	HADS	Fair	NR	NR	NR	NR	NR	NR	0‡‡	0	NR	NR
Pieterse et al, 2011[163]	77¶	Clinical geneticists, residents in clinical genetics, genetic counselors	Department of medical genetics	VAS, NSI, PPC, STAI, IES	NA	0	X	0	0	0	0	NR	NR	NR	NR
Roshanai et al, 2009[164]	163	Specialist nurse	Cancer genetics clinic	SPIKES, HADS	Fair	NR	NR	0	X	NR	NR	X	0	NR	NR
Prior Report															
Bowen et al, 2002[57]	354	Genetic counselor or trained health counselor	NR	NSI	Fair	NR	NR	NR	NR	NR	NR	NR	NR	0	X
Bowen et al, 2004[62]	354	Genetic counselor or trained health counselor	NR	NSI	Fair	NR	NR	NR	NR	NR	NR	NR	NR	0	X
Brain et al, 2002[166]	740¶	Clinical geneticist and genetic nurse specialist	NR	STAI, NSI	Good	0	0	0	X	0	0	X	0	NR	NR

Table 5. Studies of Genetic Counseling

Author, year	N	Provider of genetic counseling	Setting	Measures	Quality rating	Breast cancer worry		Anxiety		Depression		Risk perception		Intent to participate in testing	
						Increase	Decrease	Increase	Decrease	Increase	Decrease	More Accurate	Less Accurate	Increase	Decrease
Burke et al, 2000[58]	356	Genetic counselor	Medical office	NSI	Fair	X	X	0	X	NR	NR	X	0	NR	NR
Cull et al, 1998[59,‖‖]	144[¶]	Geneticist and breast surgeon	Breast cancer family clinic	NSI, STAI, GHQ	Good	0	0	NR	NR	NR	NR	X	0	NR	X
Hopwood et al, 1998[167]	174	Family history clinics	Family history clinics	NSI, GHQ, PAS	Fair	NR	NR	0	0	0	0	X¶¶	X***	NR	NR
Lerman et al, 1996[168]	227	Genetic counselor	Cancer centers	IES	Fair	0	0	0	0	NR	NR	X	0	NR	NR
Lerman et al, 1999[60]	364	Oncology nurses or genetic counselor	Hospital cancer center	IES	Fair	0	0	NR	NR	NR	NR	X	0	NR	NR
Lobb et al, 2004[169]	193	Clinical geneticists, oncologist, genetic counselors	NR	NSI, IES, HADS	Good	0	0	NR	NR	NR	NR	NR	NR	X†††	0
Watson et al, 1998[171]	115	Clinical geneticist	Hospitals	GHQ-12, CWS, VAS	Good	NR	NR	0	0	0	0	0	0	NR	NR
Watson et al, 1999[170]	283	Clinical geneticists	Genetic counseling centers	NSI, GHQ, IES, STAI	Good	0	0	0	0	0	0	X‡‡‡	0	NR	NR

X=significant relationship; 0=studied, but not significant; NA=rating criteria not available; NR=not reported.
*Both interventions vs. control.
†Both treatment groups vs. control.
‡ Pre vs. post.
§Pre vs. post and A vs. B.
‖Counseling vs. GRACE.
¶Randomized.
**Both intervention groups.
††Time effect-change from pre to post.
‡‡Interventions vs. control.
§§At 2-week followup; NS by 8 months.
‖‖Study done in a country other than the United States (e.g. Scotland, Australia, or England).
¶¶Both treatment groups at treatment end.
***Video after counseling subjects at 1-month followup.
†††African American subjects only.
‡‡‡Risk provided as odds ratio.

Table 5. Studies of Genetic Counseling

Abbreviations: BSI = Brief Symptom Inventory; CWS = Cancer Worry Scale; GHQ = General Health Questionnaire; HADS = Hospital Anxiety and Depression Scale; IES = Impact of Event Scale; NR = not reported; NSI = nonstandard instrument; PAS = Psychiatric Assessment Schedule; PPC = Perceived Personal Control; SPIKES = Setting, Patient's perception, Invitation, Knowledge, Exploring/Empathy, Strategy/Summary; STAI = State-Trait Anxiety Inventory; VAS = Visual Analog Scale.

Table 6. Types of Genetic Counseling Provided in Included Studies

Author, year	Setting	Provider of genetic counseling	Components of genetic counseling
Current report			
Armstrong et al, 2005[148]	Not reported	Not reported	Genetic counseling not specified.
Bennett et al, 2008[150]	Cancer Genetics Service Center	Genetic counselor	Women with family history of breast/ovarian cancer referred by general practitioner or other medical specialists into the service. After assessment of information in family health questionnaire by genetic specialists, individual genetic risk of developing familial breast and ovarian cancer was calculated as a percentage of lifetime risk and stratified into high, moderate, and population risk levels. Women considered high risk for breast/ovarian cancer were offered counseling, genetic testing, and annual mammography; woman at moderate risk were offered annual mammography.
Bennett et al, 2009[149]	Cancer Genetics Service Center	Genetic counselor	See Bennett 2008.
Bloom et al, 2006[151]	Telephone counseling	Master's level counselor	Telephone counseling session included: establishment of rapport and determination of special concerns, emotional readiness, risk notification by providing modified Gail model lifetime risk estimate and discussing in terms of pretest self-assessment of risk, deescalation of tension regarding breast cancer checkup, evaluation of coping skills, reinforcement of problem solving and coping skills, information on health protective behaviors, early detection through American Cancer Society screening, and information on genetic testing when requested.
Bowen et al, 2006[152]	University	Psychologist, genetic counselor	**Group psychological counseling:** Psychologist led four 2-hour, weekly sessions of 5 to 6 women per group, with each session including a 20-min group cohesion activity followed by 1 of 4 major intervention components: risk assessment and perception, education, stress management, and problem solving and social support. **Individual genetic counseling:** Genetic counselor provided 1-hour counseling sessions and sessions covered several topics, including participant's family background, breast cancer risk assessment, *BRCA1* and *BRCA2* mutations in the Ashkenazi Jewish population, nongenetic risk factors for breast cancer, and breast screening.
Brain et al, 2011[153]	Not reported	Clinician	Women with a family history of breast cancer receive a specialist genetic assessment service. Control group received general risk level (low/population, moderate, or high) based on age, reproductive history, and minimal family history; intervention group received a specific percentage based on Claus model based on detailed family pedigree; genetic testing was available to women in intervention group at high risk (≥25% risk).
Braithwaite et al, 2005[154]	Not reported	Clinical nurse specialist	**Risk counseling:** Received pedigree with information from family history and assessed risk as low, moderate, or high based on GRACE guidelines; participants were mailed letters summarizing content afterward. **GRACE:** Completed pedigrees in GRACE and assessed their risk, learning their risk assessment and how to manage their risk; received a numerical estimate of lifetime risk, a visual display of cumulative risk with general population as comparator, and a qualitative description; clinical nurse specialists then offered to book mammography and arrange meetings with geneticists, where appropriate.

Table 6. Types of Genetic Counseling Provided in Included Studies

Author, year	Setting	Provider of genetic counseling	Components of genetic counseling
Fry et al, 2003[155]	Familial Breast Cancer Clinic	Genetics consultant and specialist breast surgeon; geneticist and genetics nurse specialist	**Standard (regional) service:** Self-report family history and baseline questionnaire completed by all women; genetics consultant and genetics nurse specialist assigned categorical risk via Cancer Research Campaign. Women at low risk received a letter; women at moderate or high risk were offered an appointment at familiar breast cancer clinic where a genetics consultant discussed risk status and breast surgeon discussed risk management. Where appropriate, clinical exams and mammography were included in the appointment. Patients' general practitioners received summary data, and patients received followup questionnaires 4 weeks and 6 months later. **Novel (community-based) service:** All women sent an appointment for a community-based clinic near their residence. Meetings run by genetics nurse specialist where family history collected and compared to published criteria (Cancer Research Campaign) to determine risk. Women at low risk offered information, reassurance, and discharged. Women at increased risk (moderate or high) were offered an appointment at a regional center with a geneticist and genetics nurse specialist, and asked to complete followup questionnaires at 4 weeks and 6 months.
Gurmankin et al, 2005[156]	University breast and ovarian cancer risk evaluation program	Health care provider	**Precounseling interview:** Assessed patient's breast cancer risk perception, BRCA mutation risk perception, worry about breast cancer, family history of cancer, breast cancer risk reduction behaviors, and demographic information. **Postcounseling interview:** Assessed patient's breast cancer risk, BRCA mutation risk, recall of actual risk information, and worry about breast cancer.
Helmes et al, 2006[157]	Not reported	Board certified genetic counselor	**In-person counseling:** Review of family history, discussion of breast cancer risk, and education about breast cancer genes; discussed genetic testing considerations, including implications of results, testing strategies, potential risks and benefits of test, costs and psychological effects of test; gave information packet with personal risk information comparing woman's risk with average woman's risk, personal computer-drawn 3-generation pedigree, brochures on self-breast exams, Pap test, and mammography; genetics visual aids, and list of community resources. **Telephone counseling:** Information packet was sent in the mail with instructions to open at the beginning of the telephone counseling, which was identical in content and structure to in-person counseling.
Hopwood et al, 2004[158]	Cancer genetic service centers	Genetic counselor	Genetic counseling prior to testing varied by participating center, but offered or recommended some of the following: risk estimation (based on molecular genetic analysis or more often on family history), genetic risk counseling, clinical exam, screening/surveillance for early tumor detection (mammography, endoscopy), information on preventive strategies (surgery, diet), family planning advice, and referral for psychological assessment/support.
Kelly et al, 2008[159]	Not reported	Genetic counselor	Review of family cancer history, personal risk factors for breast and ovarian cancer, mechanisms of cancer inheritance, meaning of a positive and negative test result, and risks and benefits associated with testing.
Matloff et al, 2006[160]	Not reported	Certified genetic counselor	Personalized letter summarizing patient data.
Mikkelsen et al, 2007[161]	University clinical departments	Physicians	Information on incidence of sporadic breast cancer, genetics, inheritance patterns, and estimated personal lifetime risk of inherited cancer.
Mikkelsen et al, 2009[162]	University clinical departments	Physicians	Information on incidence of sporadic breast cancer, genetics, inheritance patterns, and estimated personal lifetime risk of inherited cancer.

Table 6. Types of Genetic Counseling Provided in Included Studies

Author, year	Setting	Provider of genetic counseling	Components of genetic counseling
Pieterse et al, 2011[163]	Department of medical genetics	Clinical geneticists, residents in clinical genetics, genetic counselors	Session topics included family's occurrence of breast and other cancers, inheritance, and criteria on probability of inherited breast cancer, and the likelihood of hereditary breast cancer running in the family was estimated.
Roshanai et al, 2009[164]	University cancer genetic clinic	Specialist nurse	Included pedigree explanation, Buckman's Breaking Bad News model to inform at-risk relatives, pamphlet, videotape, copies of pedigree, and medical records.
Prior report			
Bowen et al, 2002[57]	Not reported	Genetic counselor or trained health counselor	**Individual genetic counseling**: Telephone contact with genetic counselor to review pedigree information and one 2-hour session following protocol based on standard genetic practice, with a letter sent to participant within 2 weeks summarizing the session. **Group psychosocial counseling:** Group of 4–6 participants met for four 2-hour sessions with trained health counselor, participants received risk assessment sheet, personalizing the group discussion to her own risk status, main topics: risk assessment, perception, screening, stress management and problem solving, social support.
Bowen et al, 2004[62]	Not reported	Genetic counselor or trained health counselor	**Individual genetic counseling**: Telephone contact with genetic counselor to review pedigree information and one 2-hour session following protocol based on standard genetic practice, with a letter sent to participant within 2 weeks summarizing the session. **Group psychosocial counseling:** Group of 4–6 participants met for four 2-hour sessions with trained health counselor, participants received risk assessment sheet, personalizing the group discussion to her own risk status, main topics: risk assessment, perception, screening, stress management and problem solving, social support.
Brain et al, 2002[166]	Not reported	Clinical geneticist and genetic nurse specialist	Breast cancer surveillance, option to enter UK Tamoxifen Prevention Trial, annual surgical followup with surveillance and advice, genetic risk assessment and counseling.
Burke et al, 2000[58]	Unclear	Genetic counselor	Adapted genetic counseling protocol for women with intermediate risk included precounseling telephone call gathering a complete family history, in-person genetic counseling session discussing breast cancer risk factors, focusing on issues relevant to the participant, reviewed pedigree information, communicated likelihood of mutation in participant's family, risk estimate sheet given to participant based on the Gail and Claus models and National Cancer Institute statistics for average risk, information about genetic testing, recommendations for breast cancer screening, and a followup letter summarizing the genetic counseling session.
Cull et al, 1998[59]	Breast cancer family clinic	Geneticist and breast surgeon	Individual meeting with geneticist to discuss individual risk and with breast surgeon to discuss risk management, participants either received a copy of the educational video about 10 days before the clinic consultation or took the video home after the postclinic assessment.
Hopwood et al, 1998[167]	Family history clinics	Unclear	Family history consultation, not otherwise described.
Lerman et al, 1996[168]	Comprehen-sive cancer centers	Genetic counselor	Discussion of individual factors contributing to elevated risk, presentation of individualized risk data, recommendations for annual mammography and clinical breast exams, and instruction in breast self-exam.

Table 6. Types of Genetic Counseling Provided in Included Studies

Author, year	Setting	Provider of genetic counseling	Components of genetic counseling
Lerman et al, 1999[60]	Hospital and cancer center	Oncology nurses or genetic counselor	**Education only:** Topics discussed included individual risk factors for breast and ovarian cancer and patterns of inheritance for breast and ovarian cancer susceptibility, subjects given qualitative estimates of risk of developing breast and ovarian cancer, and pedigrees reviewed, potential benefits, limitations, and risks of genetic testing for inherited breast and ovarian cancer susceptibility reviewed. **Education plus counseling:** Provided the same education and materials described above and subjects were guided through questions exploring personal issues related to cancer and genetic testing, discussed the emotional impact of having a family history of cancer, psychosocial implications of genetic testing for inherited breast and ovarian cancer susceptibility, anticipated reactions to positive and negative test result, and intentions to communicate test results to family members and friends.
Lobb et al, 2004[169]	Not reported	Clinical geneticists, oncologist, genetic counselors	Counselors provided counseling at their discretion and study was to assess the different aspects of counseling, which included information giving concerning: breast cancer genetics, genetic testing, family history and risk, prophylactic surgery, breast cancer prevention, screening and management; communication style including: facilitating patient involvement, facilitating understanding, patient centeredness and partnership building, and supportive and counseling communications.
Watson et al, 1998[171]	Hospitals	Clinical geneticist	Consultation provided information on pedigree based on risk calculation and information regarding management options based on risk level, with instructions offered on self-exam and clinical exam, with the intervention group also receiving an audiotape of the consultation to take home.
Watson et al, 1999[172]	Genetic counseling centers	Clinical geneticists	Not described.

Table 7. Standardized Measures Used to Assess Distress

Measure	Abbreviation	Description
Beck Depression Inventory[334]	BDI	A 21-question, multiple choice, self-report inventory for measuring the severity of depression. Scores of 0 to 9 indicate minimal depression, 10 to 18 mild depression, 19 to 29 moderate depression, and 30 to 63 severe depression.
Beck Hopelessness Scale[351]	BHS	A 20-item scale to quantify hopelessness, with scores ranging from 0 to 20 and a score above 9 indicating suicidal ideations.
Body Image after Breast Cancer[333]	BIBC	A 53-item questionnaire to assess the long-term impact of breast cancer on body image in 6 key areas: vulnerability, body stigma, limitations, body concerns, transparency, arm concerns.
Body Image Scale[341]	BIS	A 10-item questionnaire for assessing body image changes in patients with cancer.
Brief Symptom Inventory[339]	BSI	A 53-item self-reported psychological symptom scale.
Center for Epidemiologic Studies-Depression[348]	CES-D	Measures symptoms of depression on a 20-item scale with scores ranging from 0 to 60; scores above 15 indicating high levels of depressive symptoms.
Coping Orientation to Problems Experienced Scale[338]	COPE	Covers 14 coping strategies as potential responses to stressors.
Decision Regret Scale[337]	DRS	A 5-item questionnaire to measure dissatisfaction or misgiving after making a medical decision.
DUKE Social Support Questionnaire[345]	DUKE-SSQ	Used to measure access to and satisfaction with social support on 8 items with scores ranging from 1 to 5. Affective subscale (DUKE-SSQ-A) includes items 1, 2, and 8; confident subscale (DUKE-SSQ-C) includes items 3-7.
Emotional Approach Coping Scale[350]	None	A 52-item questionnaire to measure both problem solving (items 1-20) and emotion-based (items 21-32) coping strategies. An additional 4 questions pertain to alcohol and drug use.
EuroQoL-5 Dimensions[343]	EQ-5D	A short, self-reported questionnaire designed to evaluate an individual's state of overall health in 5 areas: mobility, self-care, usual activities, pain/discomfort, anxiety/depression.
General Health Questionnaire[342]	GHQ	A 60-item questionnaire to screen individuals for psychiatric disorders, scores are given as means and scores above 3 indicate disorders; a 30-item version of the same questionnaire uses a threshold of 6 to indicate general psychological distress.
Health-Related Quality of Life[330]	HR-QOL	A 14-item self-report questionnaire to assess an individual's quality of life based on healthy days (items 1-4), activity limitations (items 5-9), and symptoms (items 10-14).
Hospital Anxiety and Depression Scale[335]	HADS	A 14-item self-report scale for the detection of depression and anxiety in hospitalized patients. Scores range from 1 to 21, interpreted as normal (0 to 7), mild (8 to 10), moderate (11 to 14), and severe (15 to 21). Subscales for anxiety (HADS-A) and depression (HADS-D).
Impact of Events Scale[356]	IES	A 17-item questionnaire to measure an individual's level of distress in relation to a specific event or condition. Scores range from 0 to 75; scores 9 to 25 indicate moderate difficulties and above 26 indicate clinical adaptation difficulties. Several variations are also used: Impact of Events Scale-Revised (IES-R) 22-items (items A-V); Impact of Events Subscale-Intrusive Events (IES-I) (items A, B, C, F, I, N, P, T); Impact of Events Subscale-Avoidance (IES-A) (items E, G, H, K, L, M, Q, V); Impact of Events Subscale-Hyperarousal (IES-H) (items D, J, O, R, S, U).
Lerman Breast Cancer Worry Scale[336]	CWS or LCWS	A 3-item questionnaire to measure how frequently an individual worries about getting breast cancer and the impact of worrying on mood and performance of daily activities. A 6-item version of the same questionnaire has scores ranging from 6 to 24; higher scores mean greater levels of worry.
Medical Coping Modes Questionnaire[349]	MCMQ	A 19-item self-report questionnaire to quantify coping styles into 1 of 4 categories: confrontive, avoidant, resigned, nondominant.

Table 7. Standardized Measures Used to Assess Distress

Measure	Abbreviation	Description
Medical Outcomes Study 36-Item Short Form[344] Swedish Short Term-36 Health Survey[353]	SF-36 or MOS SF-36	A 36-question health questionnaire for measuring health and well-being in 8 core areas: physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, mental health. The Swedish Short Term-36 Health Survey is one of many variations.
Menopause-Specific Quality of Life Questionnaire[346]	MENQOL	A 29-item self-administered questionnaire to assess health-related quality of life postmenopause.
Multidimensional Fatigue Symptom Inventory-Short Form[352]	MFSI-SF	A 30-item questionnaire to measures perceived sleep disturbance.
Pittsburgh Sleep Quality Index[340]	PSQI	A measure of subjective sleep disturbance in clinical populations.
Post-Traumatic Growth Inventory[332]	PTGI	An instrument for assessing positive outcomes reported by persons who have experienced traumatic events.
Sexual Activity Questionnaire[354]	SAQ	A 3-section self-reported questionnaire to assess sexual functioning, including hormonal status, reasons for sexual inactivity, sexual functioning.
State-Trait Anxiety Inventory[331]	STAI	Measures an individual's current anxiety feelings. Scores range from 10 to 40. Scores above 22 indicate high anxiety.
Symptom Checklist-90[347]	SCL-90	A 90-question self-reported questionnaire to assess psychological status in the following categories somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism.
Visual Analogue Scale[355]	VAS	Any of a number of pain self-assessment tools where subjects indicate their level of pain in response to a continuous visual scale (no pain to worst pain ever experienced).

Table 8. Prevalence of *BRCA1* and *BRCA2* Mutations in High-Risk Populations

Author, year	Population	Gene	Inclusion criteria	Early-onset	Population-based	High-risk	Ashkenazi Jewish
Breast cancer							
Anglian BCSG, 2000[13]	U.K.	*BRCA1/2*	Population-based series of breast cancer from registry		X		NR
Newman et al, 1998[223]	Caucasian North Carolina	*BRCA1*	Women diagnosed as having first invasive breast cancer between 20 and 74 years		X		NR
Newman et al, 1998[223]	African American North Carolina	*BRCA1*	Women diagnosed as having first invasive breast cancer between 20 and 74 years		X		NR
Anton-Culver et al, 2000[204]	Caucasian Orange County, CA	*BRCA1*	Population-based sample of breast cancer cases		X		No
Anton-Culver et al, 2000[204]	Hispanic Orange County, CA	*BRCA1*	Population-based sample of breast cancer cases		X		No
Newman et al, 1998[223]	African American and Caucasian North Carolina	*BRCA1*	Women diagnosed as having first invasive breast cancer between 20 and 74 years; age 20-39 years	X			NR
Anton-Culver et al, 2000[204]	Total Orange County, CA	*BRCA1*	Population-based series of breast cancer cases; age <40 years	X			Yes/no
Anton-Culver et al, 2000[204]	Total Orange County, CA	*BRCA1*	Population-based series of breast cancer cases; age <40 years	X			Yes/no
Anglian BCSG, 2000[13]	U.K.	*BRCA1/2*	Population-based series of breast cancer from registry; age 35-44 years	X			NR
Anglian BCSG, 2000[13]	U.K.	*BRCA1/2*	Population-based series of breast cancer from registry; age <35 years	X			NR
FitzGerald et al, 1996[208]	Boston, MA	*BRCA1*	Breast cancer diagnosed <30 years	X			No
Peto et al, 1999[16]	U.K.	*BRCA1/2*	1) Women diagnosed with breast cancer <36 years 2) Women diagnosed with breast cancer 36-45 years	X			NR
Malone et al, 2000[220] Same population as Langston et al, 1996[217]	Washington	*BRCA1/2*	1) Women diagnosed with breast cancer <35 years 2) Women diagnosed with breast cancer <45 years with first-degree family history of breast cancer	X			NR
Eccles et al, 1998[207]	U.K.	*BRCA1*	1) Women diagnosed with breast cancer <40 years	X			NR
						X	NR
			2) Women with bilateral breast cancer diagnosed after 39 years 3) Women with a strong family history of breast/ovarian cancer			X	NR
Couch et al, 1997[46]	U.S.	*BRCA1*	1) Women with breast cancer who had a “familial risk factor” for breast cancer			X	No

Table 8. Prevalence of *BRCA1* and *BRCA2* Mutations in High-Risk Populations

Author, year	Population	Gene	Inclusion criteria	Early-onset	Population-based	High-risk	Ashkenazi Jewish
Tommasi et al, 2005[200]	Italy	*BRCA1*	Consecutive series of breast cancer patients plus a positive family history			X	NR
Ovarian cancer							
Janezic et al, 1999[216]	Orange County, CA	*BRCA1*	Population-based series of consecutive ovarian cancer cases		X		No
Stratton et al, 1997[228]	U.K.	*BRCA1*	Women diagnosed with ovarian cancer before age 70 years		X		NR
Anton-Culver et al, 2000[204]	Caucasian Orange County, CA	*BRCA1*	Population-based sample of ovarian cancer cases		X		No
Anton-Culver et al, 2000[204]	Hispanic Orange County, CA	*BRCA1*	Population-based sample of ovarian cancer cases		X		No
Risch et al, 2006[195] Same population as Risch, 2001[225]	Canada Hispanic	*BRCA1/2*	Population-based series of consecutive ovarian cancer		X		No
Risch et al, 2006[195] Same population as Risch, 2001[225]	Canada NonHispanic NonAshkenazi Jewish	*BRCA1/2*	Population-based series of consecutive ovarian cancer		X		No
Risch et al, 2006[195] Same population as Risch, 2001[225]	Total Canada	*BRCA1/2*	Population-based series of consecutive ovarian cancer, age <41 years	X			Yes/no
Gayther et al, 1999[212]	U.K. Familial Ovarian Cancer Registry	*BRCA1/2*	Families with ≥2 cases of ovarian cancer			X	No
Breast and ovarian cancer							
Beristain et al, 2007[174]	Spain	*BRCA1/2*	1) Early-onset breast cancer <40 years	X			NR
			2) Family history of breast and/or ovarian cancer			X	NR
Gayther et al, 1997[211]	UK	*BRCA2*	1) Families with multiple cases of breast cancer 2) Families with multiple cases of ovarian cancer			X	
Frank et al, 2002[47]	Myriad	*BRCA1/2*	Tested by Myriad for full gene			X	No
Weitzel et al, 2005[319]	Hispanic	*BRCA1/2*	Families presenting to the high-risk clinic for testing who were part of the Hereditary Cancer Registry. Had a calculated BRCA mutation probability >5% by any method.			X	No
Konecny et al, 2011[183]	Slovakia	*BRCA1/2*	Families presenting to high-risk clinic based on family history of breast and/or ovarian cancer			X	NR
Seymour et al, 2008[197]	Italy	*BRCA1/2*	Families presenting to high-risk clinic based on family history of breast and/or ovarian cancer			X	NR
Marroni et al, 2004[188]	Italy	*BRCA1/2*	High-risk families presenting for BRCA testing			X	NR
Nanda et al, 2005[193]	Asian	*BRCA1/2*	Families who presented for BRCA testing in high-risk clinics who had ≥2 cases of breast or ovarian			X	No

Table 8. Prevalence of *BRCA1* and *BRCA2* Mutations in High-Risk Populations

Author, year	Population	Gene	Inclusion criteria	Early-onset	Population-based	High-risk	Ashkenazi Jewish
			cancer among FDRs or SDRs				
Nanda et al, 2005[193]	Total NonAshkenazi Jewish	*BRCA1/2*	Families who presented for BRCA testing in high-risk clinics who had ≥2 cases of breast or ovarian cancer among FDRs or SDRs			X	No
Nanda et al, 2005[193]	Caucasian	*BRCA1/2*	Families who presented for BRCA testing in high-risk clinics who had ≥2 cases of breast or ovarian cancer among FDRs or SDRs			X	No
Nanda et al, 2005[193]	African American	*BRCA1/2*	Families who presented for BRCA testing in high-risk clinics who had ≥2 cases of breast or ovarian cancer among FDRs or SDRs			X	No
Nanda et al, 2005[193]	Hispanic	*BRCA1/2*	Families who presented for BRCA testing in high-risk clinics who had ≥2 cases of breast or ovarian cancer among FDRs or SDRs			X	No
Vaziri et al, 2001[202]	U.S.	*BRCA1/2*	Families in the Familial Cancer Registry who had ≥2 cases of breast or ovarian cancer among FDRs			X	NR
Neuhausen et al, 2009[194]	California, Ontario, Australia	*BRCA1/2*	Population-based case probands from cancer registries		X		No
Neuhausen et al, 2009[194]	Philadelphia, New York, Utah, California, Ontario Australia	*BRCA1/2*	Population-based case probands from cancer registries and high-risk families		X	X	No
Neuhausen et al, 2009[194]	Philadelphia, New York, Utah	*BRCA1/2*	Families who presented for BRCA testing in high-risk clinics with a family history of breast and/or ovarian cancer			X	No
Tamboom et al, 2010[199]	Estonia	*BRCA1/2*	Women diagnosed with breast cancer prior to age 45 years	X			NR
Tamboom et al, 2010[199]	Estonia	*BRCA1/2*	Families where the proband was diagnosed with breast or ovarian cancer and at least one relative was diagnosed with these cancers.			X	NR

Author, year	Women tested, *N*	*BRCA1* positive, *n*	*BRCA2* positive, *n*	*BRCA1* or *BRCA2* positive, *n*	*BRCA1* mutation frequency	*BRCA2* mutation frequency	*BRCA1* or *BRCA2* mutation frequency
Breast cancer							
Anglian BCSG, 2000[13]	1220	8	16	24	0.6%	1.3%	2.0%
Newman et al, 1998[223]	120	3			2.5%		
Newman et al, 1998[223]	88	0			0.0%		
Anton-Culver et al, 2000[204]	562	9			1.6%		
Anton-Culver et al, 2000[204]	42	0			0.0%		
Newman et al, 1998[223]	43	0			0.0%		

Table 8. Prevalence of *BRCA1* and *BRCA2* Mutations in High-Risk Populations

Author, year	Women tested, *N*	*BRCA1* positive, *n*	*BRCA2* positive, *n*	*BRCA1* or *BRCA2* positive, *n*	*BRCA1* mutation frequency	*BRCA2* mutation frequency	*BRCA1* or *BRCA2* mutation frequency
Anton-Culver et al, 2000[204]	41	2			4.8%		
Anton-Culver et al, 2000[204]	17	0			0.0%		
Anglian BCSG, 2000[13]	341	3	4	7	0.8%	1.2%	2.0%
Anglian BCSG, 2000[13]	57	2	4	6	3.5%	7.0%	11%
FitzGerald et al, 1996[208]	26	2			7.7%		
Peto et al, 1999[16]	254	9	6	15	3.5%	2.4%	5.9%
Peto et al, 1999[16]	363	7	8	15	1.9%	2.2%	4.1%
Malone et al, 2000[220]	203	12	7	19	5.9%	3.4%	9.3%
Same population as Langston et al, 1996[217]	235	16	11	27	7.1%	4.9%	12%
	155	10			6.5%		
Eccles et al, 1998[207]	45	0			0.0%		
Eccles et al, 1998[207]	30	8			27%		
Couch et al, 1997[46]	146	21			14%		
Tommasi et al, 2005[200]	100	7			7.0%		
Ovarian cancer							
Janezic et al, 1999[216]	104	2			1.9%		
Stratton et al, 1997[228]	374	13			3.5%		
Anton-Culver et al, 2000[204]	99	4			4.0%		
Anton-Culver et al, 2000[204]	12	0			0.0%		
Risch et al, 2006[195]	15	0	0	0	0.0%	0.0%	0.0%
Same population as Risch, 2001[225]	927	67	52	119	7.2%	5.6%	13%
	157	9	1	10	5.7%	0.6%	6.4%
Gayther et al, 1999[212]	112	40	8	48	36%	7.0%	43%
Breast and ovarian cancer							
Beristain et al, 2007[174]	72	0	0	0	0.0%	0.0%	0.0%
Beristain et al, 2007[174]	164	6	10	16	3.6%	6.1%	9.7%
Gayther et al, 1997[211]	290	64	25	89	22%	8.6%	31%
Frank et al, 2002[47]	6724			1055			16%
Weitzel et al, 2005[319]	110	25	9	34	23%	8.1%	31%
Konecny et al, 2011[183]	104		12			12%	
Konecny et al, 2011[183]	585	85			15%		
Seymour et al, 2008[197]	247			21			8.5%
Marroni et al, 2004[188]	560	80			14%		
Marroni et al, 2004[188]	464		53			11%	
Nanda et al, 2005[193]	2	0	0	0	0.0%	0.0%	0.0%
Nanda et al, 2005[193]	126	31	17	48	25%	13%	38%
Nanda et al, 2005[193]	78	24	12	36	31%	15%	46%
Nanda et al, 2005[193]	43	7	5	12	16%	12%	28%
Nanda et al, 2005[193]	3	0	0	0	0.0%	0.0%	0.0%
Vaziri et al, 2001[202]	104	18	2	20	17.30%	1.9%	19.2%
Neuhausen et al, 2009[194]	NR	NR	NR	NR	4.0%	3.7%	NR

Table 8. Prevalence of *BRCA1* and *BRCA2* Mutations in High-Risk Populations

Author, year	Women tested, *N*	*BRCA1* positive, *n*	*BRCA2* positive, *n*	*BRCA1* or *BRCA2* positive, *n*	*BRCA1* mutation frequency	*BRCA2* mutation frequency	*BRCA1* or *BRCA2* mutation frequency
Neuhausen et al, 2009[194]	4084	NR	193	NR	NR	4.7%	NR
Neuhausen et al, 2009[194]	4531	233	NR	NR	5.2%	NR	NR
Neuhausen et al, 2009[194]	NR	NR	NR	NR	9.9%	8.6%	NR
Tamboom et al, 2010[199]	64	4	0	4	6.3%	0.0%	6.3%
Tamboom et al, 2010[199]	47	6	1	7	12.8%	2.1%	14.9%

Abbreviations: FDR = first-degree relative; NR = not reported; SDR = second-degree relative.

Table 9. Summary of Meta-Analysis of Studies of Prevalence of *BRCA1* and *BRCA2* Mutations in High-Risk Populations

Population	Gene	Cancer type	Prevalence, % (95% CI)	I^2 (p-value)	Studies, *n* (ref)
Early-onset breast or ovarian cancer					
≤35 years	*BRCA1*	B	4.63 (2.47 to 8.52)	NA	5[13, 16, 208, 218, 220]
≤40 years	*BRCA1*	B	4.26 (2.61 to 6.87)	NA	10[13, 16, 174, 195, 204, 207, 208, 218, 220, 223]
≤40 years	*BRCA1*	O	5.17 (2.39 to 9.59)	NR	2[13, 195]
≤45 years	*BRCA1*	B	3.25 (1.72 to 6.06)	NA	11[13, 16, 174, 195, 199, 204, 207, 208, 218, 220, 223]
≤35 years	*BRCA2*	B	3.31 (1.17 to 9.00)	NA	3[13, 16, 220]
≤40 years	*BRCA2*	B	2.90 (1.35 to6.14)	NA	5[13, 16, 174, 195, 220]
≤40 years	*BRCA2*	O	0.64 (0.02 to 3.50)	NR	1[195]
≤45 years	*BRCA2*	B	2.31 (1.11 to 4.77)	NA	6[13, 16, 174, 195, 220]
≤35 years	*BRCA1* & *BRCA2*	B	7.78 (3.99 to 14.63)	NA	5[13, 16, 174, 195, 220]
≤40 years	*BRCA1* & *BRCA2*	B	5.98 (1.87 to17.47)	NA	3[13, 16, 220]
≤40 years	*BRCA1* & *BRCA2*	O	6.37 (3.10 to 11.40)	NR	1[195]
≤45 years	*BRCA1* & *BRCA2*	B	4.63 (1.91 to 10.80	NA	5[13, 16, 195,199, 220]
Selected high-risk cohorts					
	BRCA1	B	1.84 (0.72 to 4.63)	91% (0.190)	4[13, 194, 204, 223]
	BRCA2	B	1.31 (0.67 to 1.95)	NA	1[13]
	BRCA1	O	4.41 (2.47 to 7.74)	70% (0.006)	4[194, 204, 216, 228]
	BRCA2	O	5.61 (4.13 to 7.09)	NA	1[195]
High-risk families					
	BRCA1	B	13.58 (10.09 to 17.07)	86% (<0.001)	11[46, 174, 183, 188, 193, 194, 199, 200, 202, 207, 211]
	BRCA1	O	35.71 (26.92 to 44.51)	NA	1[212]
	BRCA2	B	7.90 (5.30 to 10.50)	73% (0.117)	8[174, 183, 188, 193,194, 199, 202, 211]
	BRCA2	O	7.14 (2.13 to 12.15)	NA	1[212]
	BRCA1 & *BRCA2*	B	19.78 (12.98 to 26.57)	94% (<0.001)	6[47, 174, 193, 197, 199, 211]
	BRCA1 & *BRCA2*	O	42.86 (33.79 to 51.92)	NA	1[212]
Ashkenazi Jewish					
	BRCA1 & *BRCA2*	NA	2.08 (1.28 to 2.88)	89% (<0.001)	4[20, 191, 214, 209]
	BRCA1	NA	1.01 (0.64 to 1.37)	74% (0.004)	5[20, 191, 209, 214, 229]
	BRCA2	NA	1.02 (0.72 to 1.33)	60% (0.028)	5[20, 191, 209, 214, 224]

Abbreviations: B = breast; CI = confidence interval; NA = not applicable; NR = not reported; O = ovarian.

Table 10. Prevalence of *BRCA1* and *BRCA2* Mutations in Ashkenazi Jewish Populations

Author, year	Population	Women tested, *N*	*BRCA1* positive, *n*	*BRCA2* positive, *n*	*BRCA1* or *BRCA2* positive, *n*	*BRCA1* mutation frequency	*BRCA2* mutation frequency	*BRCA1* or *BRCA2* mutation frequency
Fodor et al, 1998[209]	Population based (U.S.)	1715	20	18	38	1.2%	1.0%	2.2%
Hartge et al, 1999[214] Struewing et al, 1997[19]	Population based (U.S.)	3742	48	41	89	1.3%	1.1%	2.4%
Metcalfe et al, 2010[191]	Population based (Canada)	2080	10	12	22	0.5%	0.6%	1.1%
Oddoux et al, 1996[224]	Population based (U.S.)	1255		12			0.9%	
Roa et al, 1996[20]	Population based (U.S.)	2717	35			1.3%		
Roa et al, 1996[20]	Population based (U.S.)	2687		37			1.4%	
Roa et al, 1996[20]	Population based (Israel)	403	3			0.7%		
Roa et al, 1996[20]	Population based (Israel)	398		10			2.5%	
Struewing et al, 1995[229]	Population based (U.S.)	327	3			0.9%		
Struewing et al, 1995[229]	Population based (Israel)	369	3			0.8%		

Table 11. Penetrance of BRCA-Related Cancer in BRCA-Positive Women: Single Individual Tested

Author, year	Population or risk group	N	Breast cancer risk			Ovarian cancer risk		
			BRCA1 % (95% CI	*BRCA2* % (95% CI)	*BRCA1* and *BRCA2* % (95% CI)	*BRCA1* % (95% CI)	*BRCA2* % (95% CI)	*BRCA1* and *BRCA2* % (95% CI)
Risk to age 50 years								
Anglian Breast Cancer Study Group, 2000[13]	Cancer registry (U.K.)	8	32 (2 to 62)			11 (1 to74)		
Anglian Breast Cancer Study Group, 2000[13]	Cancer registry (U.K.)	16		18 (2 to 32)			3 (0 to 19)	
Chen et al, 2006[122]	High-risk (U.S.)	283	28 (24 to 34)			13 (9.7 to 17)		
Chen et al, 2006[122]	High-risk (U.S.)	143		23 (19 to29)			4 (2.2 to 6.2)	
Hopper et al, 1999[215]	Cancer registry <40 years (Australia)	18			10 (0 to 24)			
Marroni et al, 2004[188]	High-risk (Italy)	80	27 (20 to 34)			14 (7 to 22)		
Marroni et al, 2004[188]	High-risk (Italy)	53		26 (18 to 34)			3 (0 to 7)	
Risk to age 70 years								
Anglian Breast Cancer Study Group, 2000[13]	Cancer registry (U.K.)	8	47 (7 to 82)			36 (4 to 99)		
Anglian Breast Cancer Study Group, 2000[13]	Cancer registry (U.K.)	16		56 (5 to 80)			10 (1 to 55)	
Antoniou et al, 2002[15]	Cancer registry (U.K.)	Unclear	35.3	50.3		25.9	9.1	
Chen et al, 2006[122]	High-risk (U.S.)	283	46 (39 to 54)			39 (30 to 50)		
Chen et al, 2006[122]	High-risk (U.S.)	143		45 (36 to 51)			22 (14 to 32)	
Hopper et al, 1999[215]	Cancer registry <40 years (Australia)	18			36 (15 to 65)			
Lubinski et al, 2012[187]	Known mutation carriers (26 centers in Canada, U.S., and Poland)— U.S. Results	614			76 (NR)			
Lubinski et al, 2012[187]	Known mutation carriers (26 centers in Canada, U.S., and Poland)— Polish Results	863			57 (NR)			
Marroni et al, 2004[188]	High-risk (Italy)	80	39 (27 to 52)			43 (21 to 66)		
Marroni et al, 2004[188]	High-risk (Italy)	53		44 (29 to 58)			15 (4 to 26)	
Metcalfe et al, 2010[190]	Known mutation carriers (6 countries) 0 FDRs	3011	56	38		39		
Metcalfe et al, 2010[190]	Known mutation carriers (6 countries) 1 FDR	3011	57	46		55		
Metcalfe et al, 2010[190]	Known mutation carriers (6 countries) ≥2 FDRs	3011	72	85		68		

Table 11. Penetrance of BRCA-Related Cancer in BRCA-Positive Women: Single Individual Tested

Author, year	Population or risk group	N	Breast cancer risk			Ovarian cancer risk		
			BRCA1 % (95% CI	*BRCA2* % (95% CI)	*BRCA1* and *BRCA2* % (95% CI)	*BRCA1* % (95% CI)	*BRCA2* % (95% CI)	*BRCA1* and *BRCA2* % (95% CI)
Risk to age 80 years								
Risch et al, 2001[225]	Ovarian cancer registry (Canada)	39	39.1			19.4		
Risch et al, 2001[225]	Ovarian cancer registry (Canada)	21		11.9			6.1	
Risch et al, 2006[195]	Ovarian cancer registry (Canada)	75	90 (77 to 97)			24 (15 to 38)		
Risch et al, 2006[195]	Ovarian cancer registry (Canada)	54		41 (26 to 60)			8.4 (3.9 to 17)	

Abbreviations: BCLC= Breast Cancer Linkage Consortium; CI = confidence interval; FDR = first-degree relative; LoD = logarithm (base 10) of odds; NR = not reported.

Table 12. Penetrance of BRCA-Related Cancer in BRCA-Positive Women: Multiple Individuals Tested

Author, year	Population or risk group	N	Breast cancer risk			Ovarian cancer risk		
			BRCA1 % (95% CI)	*BRCA2* % (95% CI)	*BRCA1* and *BRCA2* % (95% CI)	*BRCA1* % (95% CI)	*BRCA2* % (95% CI)	*BRCA1* and *BRCA2* % (95% CI)
Risk to age 50 years								
Al-Mulla et al, 2009[172]	U.K. Exon 2	30	30					
Al-Mulla et al, 2009[172]	U.K. Exon 11	58	80					
Al-Mulla et al, 2009[172]	U.K. Other exons	28	85					
Al-Mulla et al, 2009[172]	U.K. Exon 13	20	92					
Antoniou et al, 2006[173]	French Canadian	25	20 (0 to 45)			1 (0 to 10)		
Antoniou et al, 2006[173]	French Canadian	27		21 (0 to 55)			0.4 (0 to 2)	
Evans et al, 2008[178]	U.K.	223	48 (SE, 0.023)			21 (SE, 0.02)		
Evans et al, 2008[178]	U.K.	162		42 (SE, 0.027)			4 (SE, 0.012)	
Ford et al, 1998[210]	High-risk (BCLC)	32		28 (9 to 44)			0.4 (0 to 1.1)	
Kramer et al, 2005[185]	U.S. Overall	23	0.44 (SE, 0.07)					
Kramer et al, 2005[185]	U.S. With ovaries	23	0.49 (SE, 0.09)					
Milne et al, 2008[192]	Spain	155	35 (15 to 47)			10 (0 to 25)		
Milne et al, 2008[192]	Spain	164		32 (17 to 44)			2 (0 to 9)	
Sutcliffe et al, 2000[230]	Ovarian cancer registry (U.K.) *BRCA 1/2* combined	319			700%			400%
van der Kolk et al, 2010[201]	Netherlands Positive index	111	51 (47 to 54)			21 (18 to 24)		
van der Kolk et al, 2010[201]	Netherlands Positive index	74		46 (41 to 51)			7 (4 to 9)	
Risk to age 70 years								
Antoniou et al, 2006[173]	French Canadian	25	72 (0 to 93)			38 (0 to 78)		
Antoniou et al, 2006[173]	French Canadian	27		75 (0 to 97)			49 (0 to 81)	
Brose et al, 2002[206]	U.S. Age-adjusted risk	147	73 (68 to 78)			41 (36 to 46)		
Evans et al, 2008[178]	U.K.	223	68 (SE, 0.033)			60 (SE, 0.037)		
Evans et al, 2008[178]	U.K.	162		75 (SE, 0.033)			30 (SE, 0.046)	
Ford et al, 1998[210]	High-risk (BCLC)	32		84 (43 to 95)			27 (0 to 47)	
Kramer et al, 2005[185]	U.S. Overall	23	0.76 (SE, 0.08)					
Kramer et al, 2005[185]	U.S. With ovaries	23	0.92 (SE, 0.08)					

Table 12. Penetrance of BRCA-Related Cancer in BRCA-Positive Women: Multiple Individuals Tested

Author, year	Population or risk group	*N*	Breast cancer risk			Ovarian cancer risk		
			BRCA1 **% (95% CI)**	***BRCA2*** **% (95% CI)**	***BRCA1*** **and** ***BRCA2*** **% (95% CI)**	***BRCA1*** **% (95% CI)**	***BRCA2*** **% (95% CI)**	***BRCA1*** **and** ***BRCA2*** **% (95% CI)**
Milne et al, 2008[192]	Spain	155	52 (26 to 69)			22 (0 to 40)		
Milne et al, 2008[192]	Spain	164		47 (29 to 60)			18 (0 to 35)	
Sutcliffe et al, 2000[230]	Ovarian cancer registry (U.K.) *BRCA 1/2* combined	319			11%			12%
van der Kolk et al, 2010[201]	Netherlands Negative index	111	60 (54 to 65)			52 (45 to 58)		
van der Kolk et al, 2010[201]	Netherlands Positive index	111	71 (67 to 76)			59 (53 to 64)		
van der Kolk et al, 2010[201]	Netherlands Negative index	74		78 (69 to 88)			13 (7 to19)	
van der Kolk et al, 2010[201]	Netherlands Positive index	74		87 (82 to 93)			34 (25 to 44)	

Abbreviations: BCLC = Breast Cancer Linkage Consortium; CI = confidence interval; SE = standard error.

Table 13. Summary of Meta-Analysis of Studies of Breast and Ovarian Cancer Penetrance in BRCA-Positive Women in High-Risk Populations

Outcome	Risk age, *y*	Multiple individuals tested			Single individual tested			All studies combined		
		Penetrance, % (95% CI)	I^2 (p-value)	Studies, *n* (ref)	Penetrance, % (95% CI)	I^2 (p-value)	Studies, *n* (ref)	Penetrance, % (95% CI)	I^2 (p-value)	Studies, *n* (ref)
BRCA1										
Breast cancer	50	47 (40 to 53)	60% (0.032)	6[172,173,178,185,192,201]	28 (24 to 32)	0% (0.94)	3[13,122,188]			NA
	70	70 (61 to 79)	83% (<0.001)	6[173,178,185,192,201,206]	46 (40 to 52)	0% (0.60)	5[13,15,122,188,191]			NA
Ovarian cancer	50	14 (3.8 to 23)	94% (<0.001)	4[173,178,192,201]	13 (10 to 16)	0% (0.99)	3[13,122,188]	14 (7 to 20)	89% (<0.001)	7[13,122,173,178,188,192,201]
	70	46 (35 to 57)	85% (<0.001)	5[173,178,192,201,206]	41 (32 to 49)	0% (0.81)	5[13,15,122,188,191]	45 (37 to 52)	65% (0.001)	10[13,15,122,173,178,188,191,192,201,206]
BRCA2										
Breast cancer	50	40 (33 to 46)	57% (0.056)	5[173,178,192,201,210]	23 (19 to 27)	0% (0.63)	3[13,122,188]			NA
	70	71 (59 to 83)	69% (0.012)	5[173,178,192,201,210]	50 (40 to 60)	33% (0.17)	5[13,15,122,188,191]			NA
Ovarian cancer	50	3 (1 to 4)	88% (<0.001)	5[173,178,192,201,210]	4 (2 to 5)	0% (0.88)	3[13,122,188]	3 (1 to 4)	84% (<0.001)	8[13,122,173,178,188,192,201,210]
	70	23 (12 to 34)	67% (0.016)	5[173,178,192,201,210]	17 (11 to 24)	0% (0.52)	4[13,15,122,188]	19 (13 to 25)	45% (0.068)	9[13,15,122,173,178,188,192,201,210]

Abbreviations: CI = confidence interval; NA = not applicable.

Table 14. Penetrance of BRCA-Related Cancer in Women With Uninformative Negative Results

Author, year	Population or risk group	Ascertainment	N	Risk to age, *y*	Breast cancer			Ovarian cancer		
					Cases observed, *n*	Cases expected, *n*	Relative risk (95% CI)	Cases observed, *n*	Cases expected, *n*	Relative risk (95% CI)
Kauff et al, 2005[182]	FDRs in high-risk families who test negative for BRCA	Families with breast cancer but no ovarian cancer	165 families 321 FDRs	85	8	2.46	3.25 (1.4 to 6.4)	1	0.26	3.88 (0.05 to 21.6)
Kauff et al, 2005[182]	SDRs in high-risk families who test negative for BRCA	Families with breast cancer but no ovarian cancer	165 families 262 SDRs	85	4	2.18	1.83 (0.49 to 4.69)	0	0.26	0 (NA to 14.3)
Kauff et al, 2005[182]	Probands in high-risk families who test negative for BRCA	Families with breast cancer but no ovarian cancer	165 families 165 probands	85	7	1.43	4.9 (1.96 to 10.11)	0	0.14	0 (NA to 25.6)
Metcalfe et al, 2009[189]	FDRs in high-risk families who test negative for BRCA	FDRs of breast cancer cases	365 families 1492 women	75	65	16.49	3.94 (3.09 to 5.02)	2	2.34	0.85 (0.23 to 3.12)
Sutcliffe et al, 2000[230]	FDRs and SDRs in high-risk families who test negative for BRCA	Families with ≥2 FDRs with ovarian cancer	56 families 382 relatives	85				4	0.35	11.6 (3.12 to 29.7)
Sutcliffe et al, 2000[230]	FDRs and SDRs in high-risk families who test negative for BRCA	Families with ≥2 FDRs with ovarian cancer	57 families 435 relatives	85	9	2.71	3.32 (1.52 to 6.31)			

Abbreviations: CI = confidence interval; FDR = first-degree relative; NA = not applicable; SDR = second-degree relative.

Table 15. Penetrance of BRCA-Related Cancer in Women With True Negative Results

Author, year	Population or risk group	N	Breast cancer			Ovarian cancer			Genotype	Prospective	Oophorectomy adjustment	Invasive only
			Cases observed, *n*	Cases expected, *n*	Relative risk (95% CI)	Cases observed, *n*	Cases expected, *n*	Relative risk (95% CI)				
Bernholtz, 2012[175]	True negatives Total	307	20	23.8	0.84 (0.51 to 1.30)				Known	Yes	Unknown	Unknown
Domchek, 2010[177]	True negatives FDRs or SDRs	378	2	3.8	0.52 (0.13 to 2.09)	0	0.4	NR	Known	Yes	No	Yes
Domchek, 2010[177]	True negatives FDRs or SDRs	378	2	0.9	2.3 (0.57 to 9.19)				Known	Yes	No	No
Gronwald, 2007[180]	True negatives FDRs	131	2.5	1.2	2 (not given)				54% known; remainder probabilistically assigned	No	No	Unknown
Harvey, 2011[181]	True negatives Total	722	6		1.14 (0.51 to 2.53)				Known	Yes	Yes	Yes
Harvey, 2011[181]	True negatives FDRs and SDRs	442			1.29 (0.58 to 2.88)				Known	Yes	Yes	Yes
Harvey, 2011[181]	True negatives*	424			0.48 (0.12 to 1.93)				Known	Yes	Yes	Yes
Korde, 2011[184]	True negatives FDRs	102			0.66 (0.13 to 1.94)				Known or inferred	Yes	Yes	No
Korde, 2011[184]	True negatives FDRs	102			1.33 (0.49 to 2.91)				Known or inferred	Yes	Yes	No
Korde, 2011[184]	True negatives SDRs	182			0.97 (0.35 to 2.11)				Known or inferred	Yes	Yes	No
Korde, 2011[184]	True negatives TDRs	111			0.69 (0.01 to 3.83)				Known or inferred	Yes	Yes	No
Korde, 2011[184]	True negatives Total	395	10	12	0.75 (0.34 to 1.41)				Known or inferred	Yes	Yes	No
Korde, 2011[184]	True negatives Total	395	10	12	0.82 (0.39 to 1.51)				Known or inferred	Yes	Yes	No
Korde, 2011[184]	True negatives Total	395	10		0.95 (0.45 to 1.74)				Known or inferred	Yes	Yes	No
Kramer, 2005[185]	True negatives Total	353	5		0.65 (0.21 to1.52)				Known or inferred	Yes	Yes	Unknown
Kurian, 2011[186]	True negatives FDRs	NR			0.39 (0.04 to 3.81)				Untested were probabilistically assigned	Unknown	Unknown	No
Rowan, 2007[196]	True negatives FDRs or SDRs	101	3	1	2.9 (1.0 to 8.6)	0	1.7	NR	Known	Yes	Unknown	Yes
Smith, 2007[198]	True negatives Total	258	28	5.3	5.3 (3.5 to 7.7)	4	0.9	4.6 (1.2 to 11.7)	Known	No	No	No
Smith, 2007[198]	True negatives FDRs	184	18	3.6	5 (2.9 to 7.8)				Known	No	No	No

Table 15. Penetrance of BRCA-Related Cancer in Women With True Negative Results

Author, year	Population or risk group	N	Breast cancer			Ovarian cancer			Genotype	Prospective	Oophorectomy adjustment	Invasive only
			Cases observed, *n*	Cases expected, *n*	Relative risk (95% CI)	Cases observed, *n*	Cases expected, *n*	Relative risk (95% CI)				
Smith, 2007[198]	True negatives FDRs	166	13	3.2	4 (2.1 to 6.9)				Known	No	No	No
Smith, 2007[198]	True negatives FDRs	153	3	1.4	2.1 (0.4 to 6.2)				Known	Yes	No	No
van der Kolk, 2010[201]	True negatives FDRs	128	5	2.5	2 (0.7 to 4.7)	0	0.3	0 (0 to 12)	Known	Yes	Yes	No
van der Kolk, 2010[201]	True negatives FDRs	74	4	1.6	2.5 (0.7 to 6.3)	0	0.2	0 (0 to 20.4)	Known	Yes	Yes	No

*No family history in the nonmutation carrying parental line.

Abbreviations: FDR = first-degree relative; NR = not reported; SDR = second-degree relative; TDR = third-degree relative.

Table 16. Studies of Distress After Genetic Testing

Author, year, quality rating	N, study design	Mutation status	Genetic counseling	Comparison	Measures of distress	Breast cancer worry	Anxiety	Depression
Current report								
Arver et al, 2004[235] NA	63; pre-post	Positive or negative	Genetically trained oncologist and oncology nurse	A) Pretest B) 2 months post results C) 1 year post results	HADS, SF-36	NR	X decrease C & B vs. A	0
Dagan and Shochat, 2009[236] Fair	73; case-control	Positive or negative	Unknown	A) Carriers (n=17) B) Noncarriers (n=20) C) Age-matched controls (n=36)	HR-QOL, CRW, BSI	X higher A & B vs. C	0	0
Ertmanski et al, 2009[237] NA	56; pre-post	Positive	Unknown	A) Pretest B) 1 month post results C) 1 year post results	STAI, IES	NR	0	NR
Foster et al, 2007[238] Fair	154; prospective cohort	Positive or negative	Unknown	A) Carriers (n=53) B) Noncarriers (n=101)	GHQ, CWS-R	X decrease over time for A & B	X increase over time for A & B	NR
Geirdal et al, 2005[240] Good	10,244; prospective cohort	Positive or unknown	Unknown	A) Positive (n=68) B) Not tested but FBOC (n=176) C) Not tested, age-matched controls (n=10,000)	HADS, GHQ, BHS, IES	NR	X higher B vs. A	X higher B vs. A
Geirdal and Dahl, 2008[239] Good	242; prospective cohort	Positive or unknown	Unknown	A) Positive (n=68) B) Not tested, but FBOC (n=174)	HADS, COPE	NR	X higher B vs. A	NR
Kinney et al, 2005[243] Poor	52; prospective cohort	Positive or negative	Certified genetic professional	A) Carriers (n=NR) B) Noncarriers (n=NR)	STAI, IES, CES-D	NR	X decrease B only over time	NR
Low et al, 2008[244] Fair	47; prospective cohort	Positive, true negative, or uncertain (grouped with true negative)	Genetic counselor	A) Positive (n=7) B) True negative + uncertain (n=40)	IES-R, COPE, PTGI	NR	X higher A vs. B	NR
Metcalfe et al, 2012[249] NA	17; pre-post	Positive	Unknown	A) Pretest B) 1 year post results C) 2 years post results	IES	X increase B vs. A & C	NR	NR
Reichelt et al, 2004[245] Good	209; prospective cohort	Positive, negative, or unknown	Medical geneticist or experienced genetic counselor	A) Carriers (n=141) B) Noncarriers (68)	HADS, GHQ, BHS, IES	NR	0	0
Reichelt et al, 2008[246] NA	181; pre-post	Positive or true negative	Genetic counselor	A) Pretest B) 6 weeks post results C) 18 months post results	HADS, IES	NR	0	0

Table 16. Studies of Distress After Genetic Testing

Author, year, quality rating	N, study design	Mutation status	Genetic counseling	Comparison	Measures of distress	Breast cancer worry	Anxiety	Depression
van Dijk et al, 2006[248] Good	132; prospective cohort	Positive, true negative, or uninformative	Unknown	A) Positive (n=22) B) True negative (n=41) C) Uninformative (n=69)	IES, NSI	X higher A vs. B & C	X higher A vs. B & C	NR
Prior report								
Meiser et al, 2002[250] Good	143; prospective cohort	Positive or negative	Unknown	A) Carriers (n=30) B) Noncarriers (n=59) C) Not tested (n=51)	BDI, IES, MBSS, STAI, NSI	X higher A vs. C	X lower B vs. A & C	X lower B vs. A & C

X = statistically significant; 0 = studied but not significant.

Abbreviations: BDI = Beck Depression Inventory; BHS = Beck Hopelessness Scale; BSI = Brief Symptom Inventory; CES-D = Center for Epidemiologic Studies-Depression Scale; COPE = Emotional Approach Coping Scale; CRW = Cancer-Related Worry Scale; CWS-R = Cancer Worry Scale-Revised; FBOC = familial breast and/or ovarian cancer; GHQ = General Health Questionnaire; HADS = Hospital Anxiety and Depression Scale; HR-QOL = Health Related-Quality of Life; IES = Impact of Events Scale; IES-R = Impact of Events Scale-Revised; MBSS = Miller Behavioral Style Scale; NA = not applicable; NR = not reported; NSI = not standardized instrument; PTGI = Post-Traumatic Growth Inventory; SF-36 = Swedish SF-36 Health Survey; STAI = State-Trait Anxiety Inventory.

Table 17. Studies of Test Characteristics of Mammography vs. MRI for Breast Cancer Screening*

Author, year	Risk categories, *n*	Inclusion criteria	Mean age at entry, y (range)	Screening interval	Followup, mo	Mammography vs. MRI		
							Sensitivity, %	Specificity, %
Cortesi et al, 2006[273]	Mutation carrier: 48 High: 674 Intermediate: 257 Slight increase: 346	BRCA carrier Positive FH Male breast cancer Suspected positive FH	42 (20-75) 42 (15-75) 43 (19-67) 40 (18-75)	Varied by risk category and age	Median, 55	Mutation carrier†	50 vs. 100	NR
Leach et al, 2005[274] MARIBS study	*BRCA1*: 39 *BRCA2*: 86 High: 424	*BRCA1* carrier/relative *BRCA2* carrier/relative FH positive/other mutation/syndrome	Median, 40 (31-55)	Annual	Variable, ≥2 scans per woman	*BRCA1* *BRCA2* All women	23 vs. 92‡; C=92 50 vs. 58; C=92 40 vs. 77‡; C=94	92 vs. 79‡; C=74 94 vs. 82‡; C=78 93 vs. 81‡; C=77
Le-Petross et al, 2011[276]	*BRCA1*: 37 *BRCA2*: 36	*BRCA1* carrier/relative *BRCA2* carrier/relative	Median 44 (23-75)	Bi-annual, alternating	Median, 24	*BRCA1/2*	Unable to report§ vs. 92	82 vs. 87
Rijnsburger et al, 2010[278] Dutch MRISC study	*BRCA1*: 422 *BRCA2*: 172 High: 1069 Moderate: 489 Other: 5	*BRCA1* carrier *BRCA2* carrier 30%-50% lifetime risk for BC‖ (high-risk) 15%-30% lifetime risk for BC‖ (moderate-risk) Other mutation carrier	*BRCA1*: 39 *BRCA2*: 40 High risk: 41 Moderate risk: 40	Annual	48	*BRCA1* *BRCA2* High Moderate	25 vs. 67‡ 62 vs. 69 46 vs. 77 47 vs. 67	95 vs. 91 94 vs. 92 95 vs. 89 95 vs. 90

*Includes women from families with known mutations or breast cancer.
†MRI was not used to screen other risk categories.
‡p<0.05.
§All screen-detected cancers were detected by MRI only; mammography was not performed after detection with MRI to calculate sensitivity.
‖Based on modified Claus tables.

Abbreviations: BC = breast cancer; C = mammography plus MRI; FH = family history; MARIBS = Magnetic Resonance Imaging Breast Screening; MRI = magnetic resonance imaging; MRISC = Magnetic Resonance Imaging Screening Study; NA = not applicable; NR = not reported.

Table 18. Results of Trials of Risk-Reducing Medications: Cancer and Mortality Benefits[115,116]

	Raloxifene vs. tamoxifen		Tamoxifen vs. placebo			Raloxifene vs. placebo		
Outcome	Risk ratio (95% CI)	Events reduced/ increased, *n* (95% CI)*	Risk ratio (95% CI) (Trials, *n*)†	Placebo rate (SE)‡	Events reduced/ increased, *n* (95% CI)*	Risk ratio (95% CI) (Trials, *n*)†	Placebo rate (SE)‡	Events reduced/ increased, *n* (95% CI)*
Invasive breast cancer	1.24 (1.05 to 1.47)§	5 (1 to 9) fewer tamoxifen	0.70 (0.59 to 0.82) (4)	4.70 (1.02)	7 (4 to 12) fewer tamoxifen	0.44 (0.27 to 0.71) (2)	3.19 (0.59)	9 (4 to 4) fewer raloxifene
ER+ invasive breast cancer	0.93 (0.72 to 1.24)‖		0.58 (0.42 to 0.79) (4)	3.67 (0.78)	8 (3 to 13) fewer tamoxifen	0.33 (0.18 to 0.61) (2)	2.45 (0.42)	8 (4 to 12) fewer raloxifene
ER- invasive breast cancer	1.15 (0.75 to 1.77)‖		1.19 (0.92 to 1.55) (4)			1.25 (0.67 to 2.31) (2)		
Noninvasive breast cancer	1.22 (0.95 to 1.59)§		0.85 (0.54 to 1.35)¶ (4)			1.47 (0.75 to 2.91) (2)		
All-cause mortality	0.84 (0.70 to 1.02)§		1.07 (0.90 to 1.27) (4)			0.84 (0.64 to 1.10)** (2)		

*Numbers of events reduced for benefits or increased for harms compared with placebo or other comparator per 1,000 women, assuming 5 years of use.
†If meta-analysis.
‡Per 1,000 women. Estimated from a meta-analysis of rates from the placebo groups from the same trials included in the risk ratios.
§Updated results from the Study of Tamoxifen and Raloxifene (STAR), 2010.
‖Initial results from STAR, 2006.
¶ Risk ratio for noninvasive breast cancer was significantly reduced in the 2005 National Surgical Adjuvant Breast and Bowel Project P-1 (60 vs. 93 events; RR, 0.63 [95% CI, 0.45-0.89]).
** Updated meta-analysis.

Abbreviations: CI = confidence interval; ER- = estrogen receptor negative; ER+ = estrogen receptor positive; SE = standard error.

Table 19. Studies of Risk-Reducing Surgery

Author, year, quality rating	Inclusion criteria	Risk factors Enrolled, *n*	Mean age at surgery, y	Breast cancer incidence Risk estimate (95% CI)	Ovarian cancer incidence Risk estimate (95% CI)	Mortality Risk estimate (95% CI)	Mean followup,* y
Mastectomy							
Surgery vs. no surgery							
Domchek et al, 2010[292] Fair	*BRCA 1/2* carrier No history of salpingo-oophorectomy	*BRCA1* positive n=415†	37	0/43 vs. 19/372 HR NA	NR	NR	2.7
		BRCA2 positive n=245‡	39	0/32 vs. 15/213 HR NA	NR	NR	2.5
Skytte et al, 2011[294] Good	*BRCA1/2* carrier No history of mastectomy or salpingo-oophorectomy	*BRCA1* positive n=201 *BRCA2* positive n=10	NR	3/96 vs. 16/211 HR, 0.39 (0.12 to 1.36)	NR	NR	NR§
Surgery group (observed vs. expected)‖							
Evans et al, 2009[293]¶ NA	Lifetime risk of breast cancer >25%	High-risk *BRCA1/2* positive** n=202	NR	0/307 vs. 21.3 HR NA	NR	NR	7.5
Salpingo-oophorectomy or oophorectomy							
Surgery vs. no surgery							
Domchek et al, 2010[292]¶ Fair	*BRCA1/2* carrier No history of salpingo-oophorectomy	*BRCA1* positive n=1003†	42	14% (32/236) vs. 20% (129/633) HR, 0.63 (0.41 to 0.96)	2% (6/342) vs. 7% (49/661) HR, 0.31 (0.12 to 0.82)	All cause: 2% (8/327) vs. 7% (43/608) HR, 0.52 (0.24 to 1.14)	5.6
		BRCA2 positive n=554‡	46	7% (7/100) vs. 23% (94/401) HR, 0.36 (0.16 to 0.82)	0/123 vs. 14/431 HR NA	All cause: 0/120 vs. 17/403 HR NA	5.8
Kramer et al, 2005[185]†† Fair	*BRCA1*-positive family‡‡; no history of bilateral mastectomy	*BRCA1* positive n=98	NR	18% (6/33) vs. 42% (27/65) HR, 0.38 (0.15 to 0.97)	NR	NR	16.5
		BRCA1 negative n=353	NR	3% (1/34) vs. 1% (4/319) HR NR	NR	NR	16.5
		Undetermined mutation status n=222	NR	0% (0/18) vs. 2.5% (5/204) HR NA	NR	NR	16.5
Surgery group (observed vs. expected)§§							
Olson et al, 2004[296]†† NA	Women with bilateral oophorectomy	High-risk‖ ‖ Surgery <60 years n=55	<60	3/55 vs. 5.4 RR, 0.56 (0.11 to 1.33)	NR	NR	NA
		Surgery <50 years n=41	<50	1/41 vs. 3.9 RR, 0.26 (0.001 to 0.99)	NR	NR	NA
		Moderate risk¶¶ Surgery <60 years n=193	<60	9/193 vs. 10.9 RR, 0.83 (0.38 to 1.44)	NR	NR	NA
		Surgery <50 years n=130	<50	5/130 vs. 7.7 RR, 0.65 (0.21 to 1.32)	NR	NR	NA

Table 19. Studies of Risk-Reducing Surgery

Author, year, quality rating	Inclusion criteria	Risk factors Enrolled, *n*	Mean age at surgery, y	Breast cancer incidence Risk estimate (95% CI)	Ovarian cancer incidence Risk estimate (95% CI)	Mortality Risk estimate (95% CI)	Mean followup,* y
Prior report							
Mastectomy							
Hartmann et al, 1999[290] Hartmann et al, 2001[291] NA	Family history of breast cancer	High risk n=214	42	3/214 vs. 37 expected***; risk reduction, 92% (77% to 98%)	n=2	Breast cancer: 2/214 vs.10 expected***; risk reduction, 81% (31% to 98%)	14 (median)
		Moderate risk n=425		4/425 vs. 37 expected‡‡; risk reduction, 89.5% (p<0.001)	n=0	Breast cancer: 0/425 vs. 10 expected‡‡; risk reduction, 100% (70% to 100%)	
		BRCA1 or *BRCA2* positive††† n=18	41	0/18 vs. 6.1/18 expected‡‡‡; risk reduction, 100% (51% to 100%) 0/18 vs. 4.5/18 expected§§§; risk reduction, 100% (33% to 100%)	NR	NR	13.4 (median)
Oophorectomy (surgery vs. no surgery)							
Struewing et al,1995[229] Poor	Families with ≥3 cases of ovarian cancer or ≥2 cases of ovarian cancer and ≥1 cases of breast cancer <age 50	First-degree relatives of breast or ovarian cancer cases n=390 N =12 families	NR	3/44 vs. 14/346 Risk estimate: NR	2/44 vs. 8/346‖ ‖ ‖ Risk estimate: NR	NR	NR¶¶¶

*Based on followup to censoring date.
†*BRCA1* carriers evaluated in group including those with and without surgery.
‡*BRCA2* carriers evaluated in group including those with and without surgery.
§Total at-risk time in surgery group was 378.7 years vs. 934.6 years in the no surgery group.
‖Expected incidence based on life tables.
¶Study included women with prior breast cancer; only data on women with no prior breast cancer included in evidence review.
**Total number of women with *BRCA1/2* mutation, regardless of breast cancer history; study did not provide the number of women with a mutation and no prior history of breast cancer.
†† Oophorectomy performed.
‡‡ Families testing positive for *BRCA1* mutation; families had multiple breast and ovarian cancer cases prior to testing.
§§ Expected incidence based on Gail model.
‖ ‖ One first-degree relative with breast cancer before age 50 years or one first-degree relative with ovarian cancer at any age and at least one other first- or second-degree relative with either diagnosis at any age.
¶¶One first-degree relative with breast cancer at any age.
***Based on control group of sisters.
†††Subgroup of high-risk group.
‡‡‡Based on high-penetrance model.
§§§Based on low-penetrance model.
‖ ‖ ‖Incidence includes postoophorectomy ovarian carcinomatosis.
¶¶¶Followup for ovarian cancer incidence was 1665 person-years for no surgery group, 460 person-years for surgery group; followup for breast cancer incidence was 1587 person-years for no surgery group, 484 person-years for surgery group.

Abbreviations: CI = confidence interval; HR = hazard ratio; NA = not applicable; NR = not reported; RR = relative risk.

Table 20. Harms of Intensive Screening for Breast Cancer Using Mammography vs. MRI in High-Risk Women

Author, year	N (*BRCA1/2*) # of cancer cases	Age at entry, y	Screening interval Followup, y	False-positive rate	False-negative, n	Recall rates	Unneeded* additional exams or imaging Unneeded* biopsy
Kriege et al, 2004[277] Kriege et al, 2006[297] Dutch MRISC study	1909 (14/4) 39 *BRCA1* 45 *BRCA2*	Mean, 40	Annual, same-day Mean, 2.7	n=39 cancers First imaging round (prior mammography): 5.5% vs. 14%; p<0.001 Subsequent imaging rounds: 4.6% vs. 8.2%; p<0.001	n=39 cancers First imaging round (prior mammography): 12 vs. 1 Subsequent imaging rounds: 12 vs. 4	NR	n=45 cancers Exams†: 207 vs. 420 Biopsy: 28% (7/25‡) vs. 43% (24/56‡)
Leach et al, 2005[274] MARIBS study	649 (13/6) 33	Median, 40	Annual, same-day Variable followup, ≥2 scans	NR	NR	279 recalls overall 3.9% vs. 11% per woman-year Combined tests: 13% per woman-year 245/279 recalls for benign findings 8.5 recalls per cancer detected	All study arms§ Ultrasound: 38% (93/245) Core biopsy: 13% (32/245) FNA: 19% (47/245) Surgery: 3% (7/245) 0.21 benign biopsies per cancer detected
Le-Petross et al, 2011[276]	73 (51/49) 13	Median, 44	Biannual, alternating mammography with MRI Median, 2	15% (11/73) vs. 11% (8/73)	NR	NR	Imaging: 73% (8/11) vs. 50% (4/8) Biopsy: 27% (3/11) vs. 25% (2/8) Imaging plus biopsy: 0% vs. 25% (2/8)

*Women who were diagnosed as cancer free.
†Additional investigation included ultrasound ± fine needle biopsy or repeat mammography or repeat MRI.
‡Women with BIRADS ≥3 on mammography or MRI.
§Results not reported by imaging arm.

Abbreviations: BIRADS = Breast Imaging Reporting and Data System; FNA = fine needle aspiration; MARIBS = Magnetic Resonance Imaging Breast Screening; MRI = magnetic resonance imaging; MRISC = Magnetic Resonance Imaging Screening Study; NA = not applicable; NR = not reported.

Table 21. Distress Due to Intensive Screening for Breast Cancer in Women Who Are Mutation Carriers

Author, year, quality rating	N, study design	Mutation status	Comparison	Measures of distress	Anxiety	Depression	Sexual activity	Body image	General QOL
Rijnsburger et al, 2004[275] Fair	288; prospective cohort and pre-post	35 *BRCA1/2* mutation positive	A) CBE (n=287) B) CBE + mammography (n=134) C) CBE + MRI (n=109)	SF-36, EQ-5D, VAS, SCL-90	0	NR	NR	NR	0
Spiegel et al, 2011[298] NA	55; pre-post	*BRCA1:* 30/55 (54.5%) *BRCA2:* 25/55 (45.5%)	A) Recall examinations (n=18) B) No recall examinations (n=37)	HADS, WIS	X increase A vs. B*	0	NR	NR	0

X = statistically significant difference; 0 = studied but not significant.
*At 4 to 6 weeks after screening only, returned to baseline levels by 6 months.

Abbreviations: CBE = clinical breast examination; EQ-5D = EuroQoL-5 Dimensions; HADS = Hospital Anxiety and Depression Scale; MRI = magnetic resonance imaging; NA = not applicable; NR = not reported; QOL = quality of life; SCL-90 = Symptom Checklist-90; SF-36 = Short-Form 36-Item Health Survey; VAS = Visual Analogue Scale; WIS = Breast Cancer Worry Interference Scale.

Table 22. Results of Trials of Risk-Reducing Medications: Adverse Effects[115,116]

	Raloxifene vs. tamoxifen		Tamoxifen vs. placebo			Raloxifene vs. placebo		
Outcome	Risk ratio (95% CI)	Events reduced/ increased, *n* (95% CI)*	Risk ratio (95% CI) (Trials, *n*)†	Placebo rate (SE)‡	Events reduced/ increased, *n* (95% CI)*	Risk ratio (95% CI) (Trials, *n*)†	Placebo rate (SE)‡	Events reduced/ increased, *n* (95% CI)*
Thromboembolic events§	0.75 (0.60 to 0.93)‖	4 (1 to 7) more tamoxifen	1.93 (1.41 to 2.64) (4)	0.91 (0.19)	4 (2 to 9) more tamoxifen	1.60 (1.15 to 2.23) (2)	2.34 (0.25)	7 (2 to 15) more raloxifene
Deep vein thrombosis	0.72 (0.54 to 0.95)‖	3 (1 to 5) more tamoxifen	1.45 (0.89 to 2.37) (2)			1.91 (0.87 to 4.23) (2)		
Pulmonary embolus	0.80 (0.57 to 1.11)‖		2.69 (1.12 to 6.47) (2)	0.19 (0.07)	2 (0.1 to 6) more tamoxifen	2.19 (0.97 to 4.97) (2)		
Coronary heart disease events	1.10 (0.85 to 1.43)¶		1.00 (0.79 to 1.27) (4)			0.95 (0.84 to 1.06) (2)		
Stroke	0.96 (0.64 to1.43)¶		1.36 (0.89 to 2.08) (4)			0.96 (0.67 to 1.38) (2)		
Endometrial cancer	0.55 (0.36 to 0.83)‖	5 (2 to 9) more tamoxifen	2.13 (1.36 to 3.32) (3)	0.75 (0.15)	4 (1 to 10) more tamoxifen	1.11 (0.65 to 1.89)** (3)		
Cataracts	0.80 (0.72 to 0.95)‖	15 (8 to 22) more tamoxifen	1.25 (0.93 to 1.67)†† (3)			0.93 (0.84 to 1.04) (2)		

*Numbers of events increased for harms compared with placebo or other comparator per 1000 women, assuming 5 years of use.
†If meta-analysis.
‡Per 1000 women. Estimated from a meta-analysis of rates from the placebo groups from the same trials included in the risk ratios.
§ Includes deep vein thrombosis and pulmonary embolus.
‖ Updated results from the Study of Tamoxifen and Raloxifene (STAR), 2010.
¶Initial results from STAR, 2006.
** Updated meta-analysis.
††The risk ratio for cataracts was significantly increased in the NSABP P-1, 1998 (574 vs. 507 events; RR, 1.14 [95% CI, 1.01 to 1.29]).

Abbreviations: CI = confidence interval; NR = not reported; SE = standard error.

Table 23. Distress Due to Risk-Reducing Surgery

Author, year	N, study design	Mutation status	Comparison	Measures of distress	Anxiety	Depression	Sexual activity	Body image	General QOL
Mastectomy									
Brandberg et al, 2008[302] Brandberg et al, 2012[304]	90; pre-post	37/90 (41.1%) *BRCA1* 13/90 (14.4%) *BRCA2* 2/90 (2.2%) unknown mutation	A) Before surgery (n=81) B) 6 months after (n=71) C) 1 year after (n=65)	NSI, SAQ, BIS, HADS, SF-36	X decrease B & C vs. A	0	X* decrease C vs. A & B	0	NR
Gahm et al, 2010[303]	1784; case-series	NR	A) Surgery (n=59) B) Control (n=1725)	NSI, SF-36, DRS	NR	NR	NR	NR	0
Metcalfe et al, 2004[301]	60; case-series	21.7% *BRCA1/2*	A) Age <50 years (n=46) B) Age ≥50 years (n=14)	BSI, BIBC, IES, SAQ	0	NR	0	NR	NR
Salpingo-oophorectomy									
Finch et al, 2011[306]	67; pre-post	*BRCA1* or *BRCA2*	A) Before surgery B) After surgery	MENQOL, SAQ	NR	NR	X decrease B vs. A	NR	NR

X = statistically significant difference; 0 = studied but not significant.
*For pleasure subscale of SAQ only.

Abbreviations: BIBC=Body Image after Breast Cancer; BIS = Body Image Scale; BSI=Brief Symptom Inventory; DRS=Decision Regret Scale; HADS=Hospital Anxiety and Depression Scale; IES=Impact of Events Scale; MENQOL=Menopause-Specific Quality of Life-Intervention; NSI=not standard instrument; NR = not reported; QOL=quality of life; SAQ=Sexual Activity Questionnaire; SF-36 = Short-Form 36-Item Health Survey.

Table 24. Summary of Evidence

Studies, *n*	Design	Limitations	Consistency	Applicability	Overall quality	Findings
Key Question 1. Does risk assessment, genetic counseling, and genetic testing lead to reduced incidence of BRCA-related cancer and reduced cause-specific and all cause mortality?						
No studies	NA	NA	NA	NA	NA	NA
Key Question 2a. What is the accuracy of methods to assess familial cancer risk for BRCA-related cancer when performed by a nongenetics specialist in a clinical setting? Key Question 3a. What are the potential adverse effects of risk assessment?						
Systematic review of 13 general risk models; 10 studies of 5 familial risk models; no studies of the accuracy of referral criteria or adverse effects of risk assessment.	Diagnostic accuracy; cohort; case-control	Reference standards and study designs varied across studies; risk was based on self-reported information.	Consistent	High	Good	General risk models that predict risk for breast cancer, such as the Gail, are modest predictors for individuals (c-statistic, 0.55 to 0.65). Familial risk models (FHAT, Manchester, RST, PAT, and FHS-7) predict risk for BRCA mutations, are intended to guide referrals to genetic counseling, and have high accuracy (c-statistic, >0.80).
Key Question 2b, 3b. What are the benefits and potential adverse effects of genetic counseling for determining eligibility for genetic testing for BRCA-related cancer?						
16 studies of distress, accuracy of risk perception, and intention for genetic testing.	RCT, cohort, case-control, before-after	Noncomparable comparison groups; small studies; outcome measures varied.	Consistent	High	Fair	Counseling decreased cancer worry, anxiety, and depression; increased the accuracy of risk perception; and decreased intention for mutation testing.
Key Question 2c. What is the clinical validity of genetic testing for deleterious mutations in women with increased risk for BRCA-related cancer?						
32 new and 38 earlier studies provided data for meta-analysis estimates to determine the likelihood of BRCA mutations in women in specific risk populations (prevalence) and their chances of developing breast or ovarian cancer based on results of genetic testing (penetrance).	Cohort, cross-sectional, descriptive studies	Studies are heterogeneous; laboratory techniques differed; no studies outside high-risk populations; bias in estimates; no studies in women with variants of uncertain significance.	Consistent	Moderate	Fair	Prevalence is 0.2%-0.3% in general populations: 3% women with breast cancer, 6% women with breast cancer onset age ≤40, 10% women with ovarian cancer, and 20% high-risk families; for Ashkenazi Jewish women, 2% in unselected populations and 10% high-risk families. Positive test results indicate risks for breast cancer to age 70 of 46%-70% for *BRCA1* and 50%-71% for *BRCA2*; for ovarian cancer, 41%-46% for *BRCA1* and 17%-23% for *BRCA2*; in Ashkenazi Jewish women, 34% for breast cancer and 21% for ovarian cancer. Uninformative negative test results are associated with increased risk for breast cancer (SIR, 3.81 [95% CI, 3.06 to 4.75]), while true negative results are not (SIR, 1.13 [95% CI, 0.81 to 1.58]); estimates for ovarian cancer were highly heterogeneous.
Key Question 3c. What are the potential adverse effects of genetic testing?						
13 studies of distress measures and risk perception	Cohort; case-control; before-after	No studies of other outcomes; high loss to followup; comparison groups and measures varied.	Mixed	High	Fair	Breast cancer worry and anxiety increased for women with positive results and decreased for others, although results differed across studies. Risk perception improved after receiving test results.

Table 24. Summary of Evidence

Studies, *n*	Design	Limitations	Consistency	Applicability	Overall quality	Findings
Key Question 4. Do interventions reduce the incidence of BRCA-related cancer and mortality in women with increased risk?						
Intensive screening: no effectiveness studies	NA	NA	NA	NA	NA	NA
Risk-reducing medications: systematic review; 6 placebo-controlled trials (4 tamoxifen, 2 raloxifene) and 1 head-to-head trial (STAR)	RCT	No results for BRCA mutation carriers; trials are heterogeneous and data are lacking on doses, duration, and timing of use.	Consistent	Moderate	Good	Tamoxifen and raloxifene reduced invasive breast cancer by 30%-68% compared with placebo; reduction was greater for women with family history of breast cancer, but confidence intervals were overlapping. Reduction was significant for ER+ but not ER- cancer. Noninvasive breast cancer and mortality were not significantly reduced.
Risk-reducing surgery: 4 studies of mastectomy and 3 of oophorectomy or salpingo-oophorectomy	Cohort	Comparison groups varied.	Consistent	High	Fair	For high-risk women and mutation carriers, mastectomy reduced breast cancer 85%-100% and breast cancer mortality 81%-100%; salpingo-oophorectomy reduced breast cancer 37%-100%, ovarian cancer 69%-100%, and all-cause mortality 55%-100%.
Key Question 5. What are the potential adverse effects of interventions to reduce risk for BRCA-related cancer?						
Intensive screening: 3 studies of physical harms of breast cancer screening and 2 studies of anxiety; 1 study of physical harms of ovarian cancer screening	Cohort	No RCTs; screening intervals and false-positive calculations varied between studies; some studies lacked within-cohort comparison groups.	Consistent	High	Poor	False-positive rates, unnecessary imaging, and unneeded surgeries were higher for women undergoing intensive screening for breast and ovarian cancer. Most women experienced no anxiety after screening with MRI, mammography, or clinical breast examination, although women recalled had transient anxiety.
Risk-reducing medications: no studies provided results by mutation status; 1 systematic review; 6 placebo-controlled trials (4 tamoxifen, 2 raloxifene) and 1 head-to-head trial	RCT	No results for BRCA mutation carriers; trials are heterogeneous and data on long-term effects are incomplete.	Consistent	High	Good	Tamoxifen and raloxifene increased thromboembolic events compared with placebo. Tamoxifen increased endometrial cancer and cataracts compared with raloxifene. Both caused undesirable side effects for some women.
Risk-reducing surgery: 5 studies of complications, physical effects, or distress	Case-series; before-after studies	Lack of studies; small numbers of participants; no comparison groups.	NA	Low	Poor	Some women experienced physical complications of surgery, had postsurgical symptoms, or changes in body image. Some women had improved anxiety.

Abbreviations: BC = breast cancer; FHAT = Family History Assessment Tool; MRI = magnetic resonance imaging; NA = not applicable; OC = ovarian cancer; PAT = Pedigree Assessment Tool; RCT = randomized, controlled trial; RST = Referral Screening Tool; SIR = standardized incidence rate; STAR = Study of Tamoxifen and Raloxifene.

Appendix A1. Referral Criteria, Adapted From National Comprehensive Cancer Network Guidelines[50]

Table 1. Criteria for Further Genetic Risk Evaluation

a) Unaffected individual and a family history of ≥1 of these:	≥2 breast primaries, either in 1 individual or 2 different individuals from the same side of family (maternal or paternal)
	≥1 ovarian cancer primary from the same side of the family (maternal or paternal)
	First- or second-degree relative with breast cancer age ≤45 years
	A combination of breast cancer with ≥1 of the following: thyroid cancer, sarcoma, adrenocortical carcinoma, endometrial cancer, pancreatic cancer, brain tumor, diffuse gastric cancer, dermatologic manifestations and/or marocephaly, or leukemia/lymphoma on the same side of the family (especially if early-onset)
	A known mutation in a breast cancer susceptibility gene within the family
	Male breast cancer
b) Individuals at increased risk, may have modified inclusion (e.g., Ashkenazi Jewish with above at any age)	

- One or more of these criteria is suggestive of hereditary breast/ovarian cancer (HBOC) syndrome that warrants further personalized risk assessment, genetic counseling, and management. The maternal and paternal sides should be considered independently. Other malignancies reported in some HBOC families include prostate and melanoma.
- Individuals with limited family history, such as less than 2 first- or second-degree female relatives or female relatives surviving beyond age 45 years in either lineage, may have an underestimated probability of familial mutation.
- For the purposes of these guidelines, invasive and ductal carcinoma in situ breast cancer should be included.
- Close blood relatives include first-, second-, and third-degree relatives.
- For the purposes of these guidelines, fallopian tube and primary peritoneal cancer are included. Ovarian/fallopian tube/primary peritoneal cancer are component tumors of hereditary nonpolyposis colorectal cancer/Lynch syndrome; be attentive for clinical evidence of this syndrome.
- Two breast primaries include bilateral (contralateral) disease or 2 or more clearly separate ipsilateral primary tumors either synchronously or asynchronously.

Table 2. Criteria for Genetic Testing for HBOC Syndrome

a) Individual from a family with a known deleterious *BRCA1* or *BRCA2* mutation	
b) Personal history of breast cancer and ≥1 of these:	Diagnosed at age ≤45 years
	Diagnosed at age ≥50 years with ≥1 close blood relatives with breast cancer at age 50 years and/or ≥1 close blood relatives with epithelial ovarian cancer at any age
	2 breast primaries when first breast cancer diagnosis occurred at age ≤50 years
	Diagnosed at age ≤60 years with a triple negative breast cancer
	Diagnosed at age ≤50 years with a limited family history
	Diagnosed at any age, with ≥2 close blood relatives with breast and/or epithelial ovarian cancer at any age
	Diagnosed at any age with ≥2 close blood relatives with pancreatic cancer at any age
	Close male blood relative with breast cancer
	Individual of ethnicity associated with higher mutation frequency (e.g., Ashkenazi Jewish)
	Personal history of epithelial ovarian cancer
	Personal history of male breast cancer
	Personal history of pancreatic cancer at any age with ≥2 close blood relatives with breast and/or ovarian cancer and/or pancreatic cancer at any age
c) No personal history of breast cancer, but ≥1 of these:	First- or second-degree blood relative meeting any of the above criteria
	Third-degree blood relative with breast cancer and/or ovarian cancer with ≥2 close blood relatives with breast cancer (≥1 with breast cancer at age ≤50 years) and/or ovarian cancer

- Testing of unaffected family members should only be considered when no affected family member is available, and then the unaffected family member with the highest probability of mutation should be tested. Significant limitations of interpreting test results should be discussed.
- Testing for Ashkenazi Jewish founder-specific mutation(s) should be performed first. Full sequencing may be considered if ancestry also includes nonAshkenazi Jewish relatives or other HBOC criteria are met. Founder mutations exist in other populations.

Appendix A1. Referral Criteria, Adapted From National Comprehensive Cancer Network Guidelines[50]

- Individuals with limited family history, such as less than 2 first- or second-degree female relatives or female relatives surviving beyond age 45 years in either lineage, may have an underestimated probability of familial mutation.
- For the purposes of these guidelines, invasive and ductal carcinoma in situ breast cancer should be included.
- Close blood relatives include first-, second-, and third-degree relatives.
- For the purposes of these guidelines, fallopian tube and primary peritoneal cancer are included. Ovarian/fallopian tube/primary peritoneal cancer are component tumors of hereditary nonpolyposis colorectal cancer/Lynch syndrome; be attentive for clinical evidence of this syndrome.
- Two breast primaries include bilateral (contralateral) disease or 2 or more clearly separate ipsilateral primary tumors either synchronously or asynchronously.

Appendix A2. Definitions of Terms Used in Systematic Review

Term or Phrase	Definition
BRCA-related cancer	Predominantly breast, ovarian, fallopian tube, and peritoneal
Genetic counseling	A service delivered by a qualified health professional that provides a comprehensive evaluation of familial risk for inherited disorders using kindred analysis and other methods, patient education, discussion of the benefits and harms of genetic testing, interpretation of results after testing, and discussion of management options
True negative test	Known confirmed deleterious genetic mutation in relatives, and none detected in the patient
Uninformative negative test	No known deleterious genetic mutations in relatives, and none detected in the patient
Variant of uncertain significance	An abnormality of the *BRCA1* or *BRCA2* gene, but it is not known whether it is associated with an increased risk for cancer
Analytic validity*	Technical test performance measured by analytic sensitivity and specificity, reliability, and assay robustness
Clinical validity*	The test's ability to accurately and reliably predict the future disorder measured by clinical sensitivity and specificity, and predictive values of positive and negative tests that take into account the disorder prevalence
Clinical utility*	Balance of benefits and harms when the test is used to influence patient management. For risk assessment, clinical utility is determined by improved health outcomes based on prevention or early detection strategies

*Defined by the Centers for Disease Control and Prevention Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group for tests of risk assessment/susceptibility (*Genet Med.* 2009;11:3-14).

Ethical, legal, and social implications of genetic testing

Database: Ovid MEDLINE(R) without Revisions <2004to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/ or exp ovarian neoplasms/ (140349)
2 exp Mass Screening/ or gene.mp. or genes.mp. or genetic$.mp. or brca$.mp. (1445145)
3 exp LEGISLATION/ (75)
4 exp JURISPRUDENCE/ (74415)
5 lj.fs. (120944)
6 3 or 4 or 5 (161388)
7 exp bioethical issues/ or exp bioethics/ or ethic$.mp. or bioethic$.mp. (67517)
8 exp human rights/ (62937)
9 6 or 7 or 8 (229177)
10 1 and 2 and 9 (529)
11 limit 10 to (human and english language) (471)

Genetic testing

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Preventive Medicine/ (5575)
2 exp Family Practice/ (30023)
3 exp Primary Health Care/ (46956)
4 exp Physicians, Family/ (8506)
5 1 or 2 or 3 or 4 (83722)
6 exp Breast Neoplasms/ or exp ovarian cancer/ (140349)
7 exp Genetic Predisposition to Disease/ (64428)
8 exp Genetic Screening/ (18587)
9 6 and (7 or 8) (5051)
10 exp Breast Neoplasms/ge or exp ovarian cancer/ge (26159)
11 9 or 10 (26498)
12 5 and 11 (107)

Genetic counseling

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
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1 exp Genetic Counseling/ or Genetic counseling.mp. or genetic counselling.mp. (9041)
2 decision making.mp. or exp Decision Making/ (101487)
3 exp RISK/ (521470)
4 risk$.mp. (946578)
5 exp Breast Neoplasms/ or breast neoplasm$.mp. or Breast cancer$.mp. or exp ovarian neoplasms/ or ovarian cancer$.mp. or ovarian neoplasm$.mp. (159780)
6 1 and (2 or 3 or 4) and 5 (845)

Prediction of disease occurrence

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/mo, pc, ep, eh or exp ovarian neoplasms/mo, pc, ep, eh (26852)
2 exp GENES, BRCA1/ or exp BRCA1 PROTEIN/ or brca1.mp. (7633)
3 exp GENES, BRCA2/ or exp BRCA2 PROTEIN/ or brca2.mp. (4955)
4 2 or 3 (8589)
5 exp Breast Neoplasms/ge or exp ovarian neoplasms/ge (26159)
6 (sensitivity and specificity).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (248591)
7 exp "Sensitivity and Specificity"/ (297204)
8 risk$.mp. or exp RISK/ (972965)
9 5 and (6 or 7 or 8) (8244)
10 1 and 4 and 9 (1154)

Harms of risk assessment and testing

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/ or exp ovarian neoplasms/ (140349)
2 exp genetic screening/ae or exp genetic services/ae or exp genetic counseling/ae or exp genetic screening/px or exp genetic services/px or genetic counseling/px (1216)
3 exp Breast Neoplasms/ge or exp ovarian neoplasms/ge (26159)
4 exp stress, psychological/ (45845)
5 ((psycholog$ or emotion$ or mental$) adj3 (stress$ or strain$ or burden$ or toll)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (46774)

6 exp anxiety/ or anxious$.mp. or anxiet$.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (74486)
7 4 or 5 or 6 (117272)
8 (1 and 2) or (3 and 7) (519)

General interventions

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/nu, pc, dh, rt, dt, rh, su, th, tr or exp ovarian Neoplasms/nu, pc, dh, rt, dt, rh, su, th, tr (64932)
2 exp Treatment Outcome/ or treatment outcome$.mp. (481071)
3 exp "Outcome Assessment (Health Care)"/ or outcome assessment$.mp. (506781)
4 1 or 2 or 3 (568295)
5 exp Breast Neoplasms/mo, ep, eh or exp ovarian Neoplasms/mo, ep, eh (21305)
6 exp Breast Neoplasms/ or exp ovarian neoplasms/ (140349)
7 exp MORTALITY/ or mortal$.mp. or mortality.fs. (447369)
8 exp INCIDENCE/ or incidence$.mp. or epidemiology.fs. or ethnology.fs. (866897)
9 7 or 8 (1173771)
10 6 and 9 (32386)
11 5 or 10 (32386)
12 exp RISK/ (521470)
13 risk$.mp. (946578)
14 exp Genetic Predisposition to Disease/ or genetic predisposition to disease$.mp. (64440)
15 pedigree.mp. or exp PEDIGREE/ (35569)
16 12 or 13 or 14 or 15 (1034441)
17 exp Breast Neoplasms/ge or exp ovarian neoplasms/ge (26159)
18 exp GENES, BRCA1/ or exp BRCA1 PROTEIN/ or brca1.mp. (7633)
19 exp GENES, BRCA2/ or exp BRCA2 PROTEIN/ or brca2.mp. (4955)
20 17 or 18 or 19 (29475)
21 4 and 11 and 16 and 20 (769)

Harms of interventions

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/dt, su or exp ovarian neoplasms/dt, su (44424)

2 exp Breast Neoplasms/pc or exp ovarian neoplasms/pc (7801)
3 chemoprevention.mp. or exp CHEMOPREVENTION/ (14341)
4 primary prevention.mp. or exp Primary Prevention/ (52812)
5 2 or 3 or 4 (73750)
6 postoperative complications.mp. or exp Postoperative Complications/ (194521)
7 intraoperative complications.mp. or exp Intraoperative Complications/ (23574)
8 ae.xs. or ct.fs. (11782)
9 exp stress, psychological/ (45845)
10 ((psycholog$ or emotion$ or mental$) adj3 (stress$ or strain$ or burden$ or toll)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (46774)
11 ((psycholog$ or emotion$ or mental$) adj3 (stress$ or strain$ or burden$ or fear$ or toll)).mp. (47508)
12 exp anxiety/ or anxiet$.mp. or anxious$.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (74486)
13 9 or 10 or 11 or 12 (117833)
14 6 or 7 or 8 or 13 (337084)
15 1 and 5 and 14 (49)

BRCA studies

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp case control studies/ (417789)
2 brca$.mp. (8951)
3 1 and 2 (663)
4 exp breast neoplasms/ (113859)
5 exp ovarian neoplasms/ (30269)
6 4 or 5 (140349)
7 3 and 6 (578)

Prediction models

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---

1 (gail adj model$).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (120)
2 (claus adj model$).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (23)
3 1 or 2 (135)
4 exp Models, Statistical/ (183287)
5 exp risk/ (521470)
6 exp Breast Neoplasms/ge [Genetics] (21383)
7 4 and 5 and 6 (487)
8 3 or 7 (613)
9 limit 8 to humans (613)
10 limit 9 to abstracts (584)
11 limit 9 to english (601)
12 10 or 11 (613)

Prophylactic surgery interventions

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/pc [Prevention & Control] (7136)
2 exp Ovarian Neoplasms/pc [Prevention & Control] (1016)
3 (mastectom$ or oophoectom$ or ovariectom$).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (27586)
4 1 or 2 (7801)
5 3 and 4 (872)
6 (family adj5 histor$).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (26320)
7 exp Genetic Predisposition to Disease/ (64428)
8 brca.mp. (1378)
9 (brca1 or brca2).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (8589)
10 6 or 7 or 8 or 9 (93492)
11 5 and 10 (488)
12 limit 11 to human (488)

13 limit 12 to english language (446)
14 limit 12 to abstracts (380)
15 13 or 14 (479)

Tamoxifen and raloxifene

Database: Ovid MEDLINE(R) without Revisions <2004 to 2012>
Search Strategy:
---
1 exp Breast Neoplasms/pc [Prevention & Control] (7136)
2 exp Ovarian Neoplasms/pc [Prevention & Control] (1016)
3 1 or 2 (7801)
4 (family adj5 histor$).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (26320)
5 exp Genetic Predisposition to Disease/ (64428)
6 brca.mp. (1378)
7 (brca1 or brca2).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (8589)
8 4 or 5 or 6 or 7 (93492)
9 exp Selective Estrogen Receptor Modulators/ (12837)
10 (serm or serms or tamoxifen or raloxifene).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (14849)
11 9 or 10 (16490)
12 3 and 8 and 11 (153)
13 exp Contraceptives, Oral/ (13048)
14 3 and 8 and 13 (54)
15 12 or 14 (195)
16 limit 15 to humans (195)
17 limit 16 to abstracts (166)
18 limit 16 to english (176)
19 17 or 18 (191)

Appendix B2. Inclusion and Exclusion Criteria

	Include	Exclude
Population	Asymptomatic adult (age 18 years or older) women with a family history of breast and/or ovarian cancer	Men, children, women with prior history of breast and/or ovarian cancer, no family history of breast and/or ovarian cancer
Interventions	Risk assessment, genetic counseling, and genetic testing for deleterious *BRCA1* or *BRCA2* mutations, interventions primarily aimed at reducing the risk of BRCA-related cancer in women with deleterious mutations: intensive screening (e.g., earlier and more frequent mammography, breast magnetic resonance imaging), use of medications (e.g., tamoxifen, raloxifene), and risk-reducing surgery (e.g., mastectomy, oophorectomy)	Surveillance, referral practices, testing for polymorphisms
Outcomes	Invasive breast cancer, invasive ovarian cancer, other BRCA-related cancer (fallopian tube, peritoneal), mortality (all cause, cancer-specific). Harms include inaccurate risk assessment; inappropriate testing; false-positive and false-negative results; adverse impact on the patient's relationships with family; false reassurance; incomplete testing; misinterpretation of the test result; anxiety; cancer worry; immediate and long-term harms associated with breast imaging, risk-reducing medications, and risk-reducing surgery; and ethical, legal, and social implications	Increased detection, predictors of adherence, uptake of screening or risk-reducing interventions
Study types and designs	Randomized, controlled trials; prospective and retrospective cohort studies; case-control studies; cross-sectional studies (for harms); systematic reviews; and meta-analyses	Case reports

Randomized, Controlled Trials (RCTs) and Cohort Studies

Criteria:

- Initial assembly of comparable groups: RCTs—adequate randomization, including concealment and whether potential confounders were distributed equally among groups; cohort studies—consideration of potential confounders with either restriction or measurement for adjustment in the analysis; consideration of inception cohorts
- Maintenance of comparable groups (includes attrition, cross-overs, adherence, contamination)
- Important differential loss to follow-up or overall high loss to follow-up
- Measurements: equal, reliable, and valid (includes masking of outcome assessment)
- Clear definition of interventions
- Important outcomes considered
- Analysis: adjustment for potential confounders for cohort studies, or intention-to-treat analysis for RCTs; for cluster RCTs, correction for correlation coefficient

Definition of ratings based on above criteria:

Good: Meets all criteria: Comparable groups are assembled initially and maintained throughout the study (follow-up at least 80 percent); reliable and valid measurement instruments are used and applied equally to the groups; interventions are spelled out clearly; important outcomes are considered; and appropriate attention to confounders in analysis.

Fair: Studies will be graded "fair" if any or all of the following problems occur, without the important limitations noted in the "poor" category below: Generally comparable groups are assembled initially but some question remains whether some (although not major) differences occurred in follow-up; measurement instruments are acceptable (although not the best) and generally applied equally; some but not all important outcomes are considered; and some but not all potential confounders are accounted for.

Poor: Studies will be graded "poor" if any of the following major limitations exists: Groups assembled initially are not close to being comparable or maintained throughout the study; unreliable or invalid measurement instruments are used or not applied at all equally among groups (including not masking outcome assessment); and key confounders are given little or no attention.

Case Control Studies

Criteria:

- Accurate ascertainment of cases
- Nonbiased selection of cases/controls with exclusion criteria applied equally to both
- Response rate
- Diagnostic testing procedures applied equally to each group
- Measurement of exposure accurate and applied equally to each group
- Appropriate attention to potential confounding variable

***Definition of ratings based on criteria above*:**

Good: Appropriate ascertainment of cases and nonbiased selection of case and control participants; exclusion criteria applied equally to cases and controls; response rate equal to or greater than

80 percent; diagnostic procedures and measurements accurate and applied equally to cases and controls; and appropriate attention to confounding variables.

Fair: Recent, relevant, without major apparent selection or diagnostic work-up bias but with response rate less than 80 percent or attention to some but not all important confounding variables.

Poor: Major selection or diagnostic work-up biases, response rates less than 50 percent, or inattention to confounding variables.

Systematic Reviews

Criteria:

- Search dates reported?
- Search methods reported?
- Comprehensive search?
- Inclusion criteria reported?
- Selection bias avoided?
- Validity criteria reported?
- Validity assessed appropriately?
- Methods used to combine studies reported?
- Findings combined appropriately?
- Conclusions supported by data?

Definitions of ratings based on above criteria:

Good: Meets all criteria: reports comprehensive and reproducible search methods and results; reports pre-defined criteria to select studies and reports reasons for excluding potentially relevant studies; adequately evaluates quality of included studies and incorporates assessments of quality when synthesizing data; reports methods for synthesizing data and uses appropriate methods to combine data qualitatively or quantitatively; conclusions supported by the evidence reviewed.

Fair: Studies will be graded fair if they fail to meet one or more of the above criteria, but the limitations are not judged as being major.

Poor: Studies will be graded poor if they have a major limitation in one or more of the above criteria.

Source: Harris et al, 2001[100]

Expert reviewers

Bruce Nedrow Calogne, M.D., M.P.H., President and CEO, Colorado Trust; Chair, Centers for Disease Control and Prevention Evaluating Genomic Applications for Practice and Prevention (EGAPP) Workgroup; Associate Professor of Family Medicine, Department of Family Medicine, University of Colorado Denver School of Medicine (UCD) and Associate Professor of Preventive Medicine and Biometrics, UCD Colorado School of Public Health

Kelly Metcalfe, R.N., Ph.D., Associate Professor, Lawrence S. Bloomberg Faculty of Nursing, University of Toronto

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Mark Robson, M.D., Clinical Director, Clinical Genetics Service, Memorial Sloan Kettering Cancer Center

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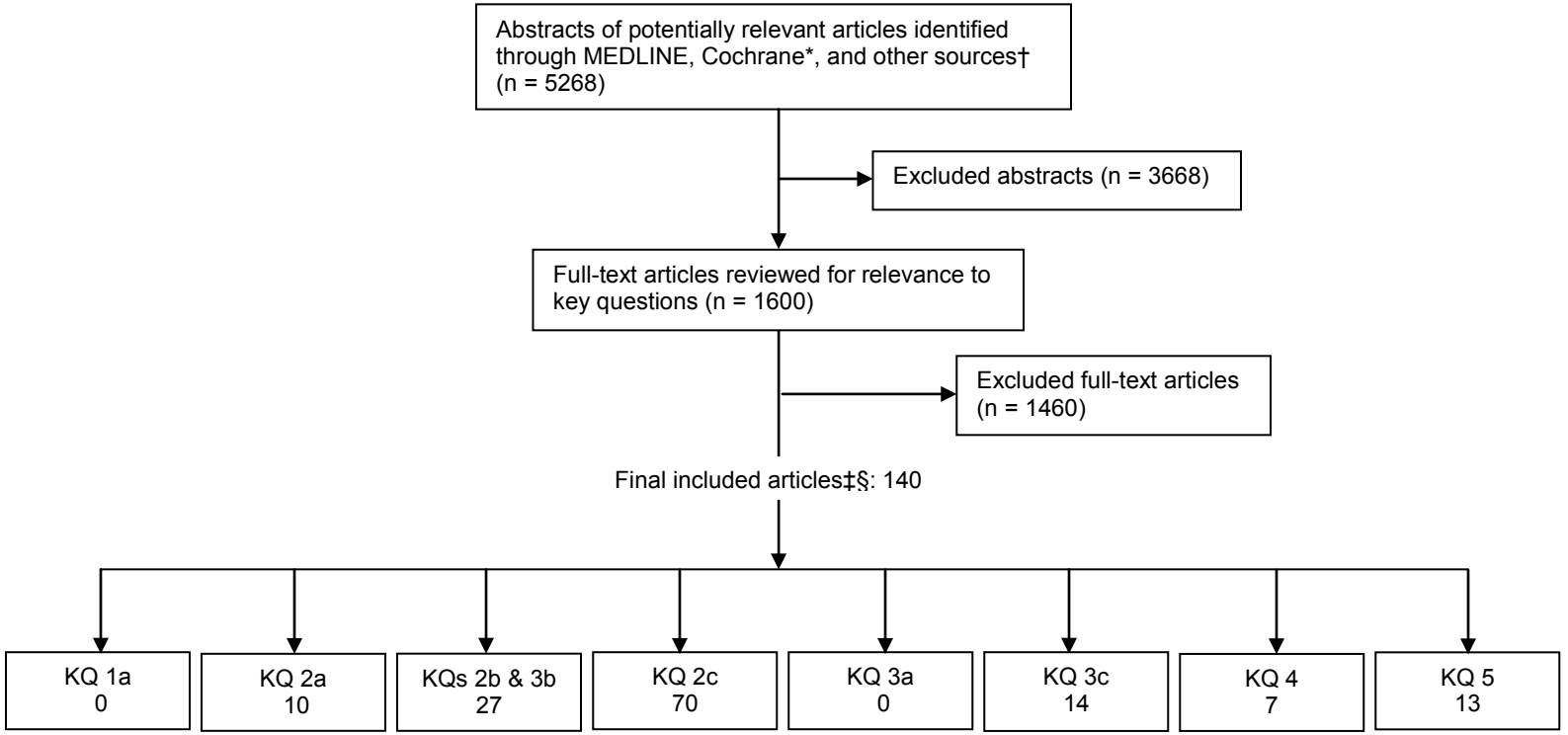
Joseph Chin, M.D., Office of Clinical Standards and Quality, Centers for Medicare and Medicaid Services

Mark H. Greene, M.D., Chief, Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health

Katherine Kolor, Ph.D., Office of Public Health Genomics, Centers for Disease Control and Prevention

Jacqueline Miller, M.D., Office of Public Health Genomics, Centers for Disease Control and Prevention

Appendix B5. Literature Flow Diagram



*Cochrane databases include the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews.
†Identified from reference lists, hand searching, and suggestions by experts.
‡Studies that provided data and contributed to the body of evidence were considered "included."
§Studies may contribute data to more than one key question.

Abbreviation: KQ = key question.

Appendix B6. Excluded Studies List

Key to exclusion codes

2	Background
3	Wrong population
4	Wrong intervention
5	Wrong publication type
6	Conducted prior to 2004
7	Foreign language study, otherwise included
8	Wrong outcome

Myriad Genetic Laboratories, Inc. http://www.myriadtests.com/index.php. Accessed 25 Oct, 2011
Exclusion code: 2

Ad Hoc Committee on Genetic Counseling of the American Society of Human Genetics. *Am J Hum Genet.* 1975;27:240-242, [PMID: 1124768]
Exclusion code: 2

Tarasoff v. Regents of the University of California 551 P.2d 334, Supreme Court of California 1976
Exclusion code: 5

Statement of the American Society of Human Genetics on genetic testing for breast and ovarian cancer predisposition. *Am J Hum Genet.* 1994;55(5):i-iv, [PMID: 7977337]
Exclusion code: 2

FL recognizes duty to warn patient of transmissibility of genetic disease to child - Pate v. Threlkel, 661 So.2d 278 (Fla. 1995), rehearing denied (Oct 10, 1995), Supreme Court of Florida 1995)
Exclusion code: 5

Statement of the American Society of Clinical Oncology: Genetic Testing for Cancer Susceptibility. *J. Clin. Oncol.* 1996;14(5):1730-1736, [PMID: 8622094]
Exclusion code: 2

Safer v. Estate of Pack, 677 A.2d 1188-NJ: Appellate Div. 1996, Superior Court of New Jersey, Appellate Division 1996
Exclusion code: 5

Tenuto v. Lederle Laboratories, Div. of American Cyanamid Co., 90 N.Y.2d 606, 687 N.E.2d 1300, 665 N.Y.S.2d 17, The Court of Appeals of the State of New York 1997
Exclusion code: 5

Molloy v. Meier C9 02 1821 C2 02 1837 C9 02 1821 C2 02 1837 C2 02 1837, Court Appeals of Minnesota 2003
Exclusion code: 5

Genetic susceptibility to breast and ovarian cancer: Assessment, counseling and testing guidelines: New York State Department of Health;2004
Exclusion code: 2

Gene patent fight. *New Sci.* 2005;186(2505):7, [PMID: 16178103]
Exclusion code: 5

Society of Gynecologic Oncologists Clinical Practice Committee Statement on Prophylactic Salpingo-oophorectomy. *Gynecol. Oncol.* 2005;98(2):179-181, [PMID: 15979696]
Exclusion code: 5

Summaries for patients. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: U.S. Preventive Services Task Force recommendations. *Ann. Intern. Med.* 2005;143(5):I47, [PMID: 16144889]
Exclusion code: 5

National Institutes of Health State-of-the-Science Conference statement: management of menopause-related symptoms. *Ann. Intern. Med.* 2005;142(12 Pt 1):1003-1013, [PMID: 15968015]

Exclusion code: 5

State by state comparison of insurance regulations. *J. Natl. Cancer Inst.* 2006;98(15):1034, [PMID: 16882939]
Exclusion code: 5

First do no harm. *Lancet Oncol.* 2009;10(10):927, [PMID: 19796744]
Exclusion code: 5

Study of Tamoxifen and Raloxifene (STAR) for the Prevention of Breast Cancer in Postmenopausal Women. 2011; http://www.cancer.gov/clinicaltrials/search/view?cdrid=651291&version=healthprofessional. Accessed 25 Oct, 2011
Exclusion code: 2

Hereditary breast and ovarian cancer: risks and challenges. Proceedings of a meeting. September 10-12, 2009. Bari, Italy. *Ann. Oncol.* 2011;22 Suppl 1:i5-68, [PMID: 21438196]
Exclusion code: 5

Aalfs CM, Mollema ED, Oort FJ, de Haes JCJM, Leschot NJ, Smets EMA. Genetic counseling for familial conditions during pregnancy: An analysis of patient characteristics. *Clin. Genet.* 2004;66(2):112-121, [PMID: 15253761]
Exclusion code: 3

Abeliovich D, Kaduri L, Lerer I, et al. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. *Am. J. Hum. Genet.* 1997;60(3):505-514, [PMID: 9042909]
Exclusion code: 2

Abrahams E, Silver M. The case for personalized medicine. *J Diabetes Sci Technol.* 2009;3(4):680-684, [PMID: 20144313]
Exclusion code: 5

Acheson LS, Wang C, Zyzanski SJ, et al. Family history and perceptions about risk and prevention for chronic diseases in primary care: a report from the family healthware impact trial. *Genet Med.* 2010;12(4):212-218, [PMID: 20216073]
Exclusion code: 3

Acheson LS, Zyzanski SJ, Stange KC, Deptowicz A, Wiesner GL. Validation of a self-administered, computerized tool for collecting and displaying the family history of cancer. *J. Clin. Oncol.* 2006;24(34):5395-5402, [PMID: 17088568]
Exclusion code: 8

Ackermann S, Lux MP, Fasching PA, et al. Acceptance for preventive genetic testing and prophylactic surgery in women with a family history of breast and gynaecological cancers. *Eur. J. Cancer Prev.* 2006;15(6):474-479, [PMID: 17106324]
Exclusion code: 8

Acog. ACOG Practice Bulletin No. 89. Elective and risk-reducing salpingo-oophorectomy. *Obstet. Gynecol.* 2008;111(1):231-241, [PMID: 18165419]
Exclusion code: 5

Adams-Campbell LL, Makambi KH, Frederick WAI, Gaskins M, Dewitty RL, McCaskill-Stevens W. Breast cancer risk assessments comparing Gail and CARE models in African-American women. *Breast J.* 2009;15 Suppl 1:S72-75, [PMID: 19775333]
Exclusion code: 8

Ader T, Susswein LR, Callanan NP, Evans JP. Attitudes and practice of genetic counselors regarding anonymous testing for BRCA1/2. *J Genet Couns.* 2009;18(6):606-617, [PMID: 19798553]
Exclusion code: 8

Agnantis NJ, Paraskevaidis E, Roukos D. Preventing breast, ovarian cancer in BRCA carriers: rational of prophylactic surgery and promises of surveillance. *Ann. Surg. Oncol.* 2004;11(12):1030-1034, [PMID: 15545500]
Exclusion code: 5

Agnusdei D. Clinical efficacy of raloxifene in postmenopausal women. *Eur. J. Obstet.*

Gynecol. Reprod. Biol. 1999;85(1):43-46, [PMID: 10428320]
Exclusion code: 5

Akbari MR, Zhang S, Fan I, et al. Clinical impact of unclassified variants of the BRCA1 and BRCA2 genes. *J. Med. Genet.* 2011;48(11):783-786, [PMID: 21965345]
Exclusion code: 2

Alarcon F, Bourgain C, Gauthier-Villars M, Plante-Bordeneuve V, Stoppa-Lyonnet D, Bonaiti-Pellie C. PEL: an unbiased method for estimating age-dependent genetic disease risk from pedigree data unselected for family history. *Genet. Epidemiol.* 2009;33(5):379-385, [PMID: 19089844]
Exclusion code: 5

Albada A, Ausems MGEM, Otten R, Bensing JM, van Dulmen S. Use and evaluation of an individually tailored website for counselees prior to breast cancer genetic counseling. *J. Cancer Educ.* 2011;26(4):670-681, [PMID: 21533850]
Exclusion code: 4

Albada A, van Dulmen S, Ausems MGEM, Bensing JM. A pre-visit website with question prompt sheet for counselees facilitates communication in the first consultation for breast cancer genetic counseling: findings from a randomized controlled trial. *Genet Med.* 2012;14(5):535-542, [PMID: 22241101]
Exclusion code: 3

Albada A, van Dulmen S, Lindhout D, Bensing JM, Ausems MGEM. A pre-visit tailored website enhances counselees' realistic expectations and knowledge and fulfils information needs for breast cancer genetic counselling. *Fam Cancer.* 2012;11(1):85-95, [PMID: 21901499]
Exclusion code: 3

Albada A, van Dulmen S, Otten R, Bensing JM, Ausems MGEM. Development of E-info gene(ca): a website providing computer-tailored information and question prompt prior to breast cancer genetic counseling. *J Genet Couns.* 2009;18(4):326-338, [PMID: 19440661]
Exclusion code: 8

Allain DC. Genetic counseling and testing for common hereditary breast cancer syndromes: a paper from the 2007 William Beaumont hospital symposium on molecular pathology. *J Mol Diagn.* 2008;10(5):383-395, [PMID: 18687797]
Exclusion code: 5

Allain DC, Sweet K, Agnese DM. Management options after prophylactic surgeries in women with BRCA mutations: a review. *Cancer Control.* 2007;14(4):330-337, [PMID: 17914333]
Exclusion code: 5

Altschuler A, Somkin CP. Women's decision making about whether or not to use breast cancer chemoprevention. *Women Health.* 2005;41(2):81-95, [PMID: 16219589]
Exclusion code: 8

American Civil Liberties Union. ACLU and PUBPAT Ask Supreme Court to Rule that Patents on Breast Cancer Genes Are Invalid. 2012; http://www.aclu.org/womens-rights/aclu-and-pubpat-ask-supreme-court-rule-patents-breast-cancer-genes-are-invalid. Accessed 24 Oct, 2012
Exclusion code: 2

American College of Medical Genetics Professional Practice and Guidelines Committee. Genetic susceptibility to breast and ovarian cancer: Assessment, counseling, and testing guidelines executive summary. 1999; http://www.health.ny.gov/diseases/cancer/obcancer/contents.htm. Accessed 16 Oct 2012
Exclusion code: 2

American College of Surgeons. Cancer Program Standards 2012. http://www.facs.org/cancer/coc/programstandards2012.html. Accessed 26 Feb 2013
Exclusion code: 2

American Congress of Obstetricians and Gynecologists, ACOG Committee on Practice Bulletins--Gynecology, ACOG Committee on Genetics, Society of Gynecologic Oncologists. ACOG Practice Bulletin No. 103: Hereditary breast and ovarian cancer syndrome. *Obstet.*

Gynecol. 2009;113(4):957-966, [PMID: 19305347]
Exclusion code: 5

Amir E, Evans DG, Shenton A, et al. Evaluation of breast cancer risk assessment packages in the family history evaluation and screening programme. *J. Med. Genet.* 2003;40(11):807-814, [PMID: 14627668]
Exclusion code: 2

Amir E, Freedman O. Underestimation of risk by Gail model extends beyond women with atypical hyperplasia. *J. Clin. Oncol.* 2009;27(9):1526; author reply 1527, [PMID: 19204192]
Exclusion code: 5

Amir E, Freedman OC, Seruga B, Evans DG. Assessing women at high risk of breast cancer: a review of risk assessment models. *J. Natl. Cancer Inst.* 2010;102(10):680-691, [PMID: 20427433]
Exclusion code: 2

Andersen M, Drescher CW, Zheng Y, et al. Changes in cancer worry associated with participation in ovarian cancer screening. *Psychooncology.* 2007;16(9):814-820, [PMID: 17225260]
Exclusion code: 3

Anderson E, Berg J, Black R, et al. Prospective surveillance of women with a family history of breast cancer: auditing the risk threshold. *Br. J. Cancer.* 2008;98(4):840-844, [PMID: 30000894]
Exclusion code: 3

Anderson G, Jun M, Choi K. Breast cancer screening for Korean women must consider traditional risks as well as two genetic risk factors: genetic polymorphisms and inheritable gene mutations. *Cancer Nurs.* 2007;30(3):213-222, [PMID: 17510585]
Exclusion code: 5

Andersson I, Janzon L. Reduced breast cancer mortality in women under age 50: updated results from the Malmo Mammographic Screening Program. *J Natl Cancer Inst Monogr.* 1997;22:63-67, [PMID: 9709278]
Exclusion code: 3

Ando N, Iwamitsu Y, Kuranami M, et al. Concerns about inherited risk of breast cancer prior to diagnosis in Japanese patients with breast complaints. *Fam Cancer.* 2011;10(4):681-689, [PMID: 21701918]
Exclusion code: 4

Andolf E, Jorgensen C, Astedt B. Ultrasound examination for detection of ovarian carcinoma in risk groups. *Obstet. Gynecol.* 1990;75(1):106-109, [PMID: 2404221]
Exclusion code: 8

Andresen EM, Catlin TK, Wyrwich KW, Jackson-Thompson J. Retest reliability of surveillance questions on health related quality of life. *J. Epidemiol. Community Health.* 2003;57(5):339-343, [PMID: 12700216]
Exclusion code: 2

Andrews L, Meiser B, Apicella C, Tucker K. Psychological impact of genetic testing for breast cancer susceptibility in women of Ashkenazi Jewish background: a prospective study. *Genet Test.* 2004;8(3):240-247, [PMID: 15727246]
Exclusion code: 3

Andrieu N, Goldgar DE, Easton DF, et al. Pregnancies, breast-feeding, and breast cancer risk in the International BRCA1/2 Carrier Cohort Study (IBCCS). *J. Natl. Cancer Inst.* 2006;98(8):535-544, [PMID: 16622123]
Exclusion code: 8

Andrykowski MA, Boerner LM, Salsman JM, Pavlik E. Psychological Response to Test Results in an Ovarian Cancer Screening Program: A Prospective, Longitudinal Study. *Health Psychol.* 2004;23(6):622-630, [PMID: 15546230]
Exclusion code: 3

Ang P, Lim IHK, Lee TC, et al. BRCA1 and BRCA2 mutations in an Asian clinic-based population detected using a comprehensive strategy. *Cancer Epidemiol. Biomarkers Prev.* 2007;16(11):2276-2284, [PMID: 18006916]
Exclusion code: 3

Annunziata MA, Muzzatti B, Narciso D, et al. Mood state profile and coping strategies after BRCA-1/2 genetic test disclosure: a retrospective study in Italy. *Support. Care Cancer.* 2011;19(6):733-735, [PMID: 21267604]
Exclusion code: 3

Anonymous. Ovary removal reduces cancer risk for BRCA1/2 carriers. *Health News.* 2006;12(10):6-7, [PMID: 17162794]
Exclusion code: 5

Antill Y, Reynolds J, Young M-A, et al. Risk-reducing surgery in women with familial susceptibility for breast and/or ovarian cancer. *Eur. J. Cancer.* 2006;42(5):621-628, [PMID: 16434187]
Exclusion code: 3

Antill YC, Reynolds J, Young MA, et al. Screening behavior in women at increased familial risk for breast cancer. *Fam Cancer.* 2006;5(4):359-368, [PMID: 16817030]
Exclusion code: 4

Antoniou A, Cunningham A, Peto J. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br J Ca.* 2008;98(8):1457-1466, [PMID: 18349832]
Exclusion code: 2

Antoniou AC, Beesley J, McGuffog L, et al. Common breast cancer susceptibility alleles and the risk of breast cancer for BRCA1 and BRCA2 mutation carriers: Implications for risk prediction. *Cancer Res.* 2010;70(23):9742-9754, [PMID: 21118973]
Exclusion code: 2

Antoniou AC, Gayther SA, Stratton JF, Ponder BAJ, Easton DF. Risk models for familial ovarian and breast cancer. *Genet. Epidemiol.* 2000;18(2):173-190, [PMID: 10642429]
Exclusion code: 2

Antoniou AC, Goldgar DE, Andrieu N, et al. A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. *Genet. Epidemiol.* 2005;29(1):1-11, [PMID: 15880399]
Exclusion code: 5

Antoniou AC, Kartsonaki C, Sinilnikova OM, et al. Common alleles at 6q25.1 and 1p11.2 are associated with breast cancer risk for BRCA1 and BRCA2 mutation carriers. *Hum. Mol. Genet.* 2011;20(16):3304-3321, [PMID: 21593217]
Exclusion code: 2

Antoniou AC, Pharoah PD, McMullan G, et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. *Br. J. Cancer.* 2002;86(1):76-83, [PMID: 11857015]
Exclusion code: 2

Antoniou AC, Pharoah PPD, Smith P, Easton DF. The BOADICEA model of genetic susceptibility to breast and ovarian cancer. *Br. J. Cancer.* 2004;91(8):1580-1590, [PMID: 15381934]
Exclusion code: 2

Antoniou AC, Rookus M, Andrieu N, et al. Reproductive and hormonal factors, and ovarian cancer risk for BRCA1 and BRCA2 mutation carriers: Results from the International BRCA1/2 carrier cohort study. *Cancer Epidemiol. Biomarkers Prev.* 2009;18(2):601-610, [PMID: 19190154]
Exclusion code: 8

Appleton S, Watson M, Rush R, et al. A randomised controlled trial of a psychoeducational intervention for women at increased risk of breast cancer. *Br. J. Cancer.* 2004;90(1):41-47, [PMID: 14710204]
Exclusion code: 6

Arai M, Utsunomiya J, Miki Y. Familial breast and ovarian cancers. *Int J Clin Oncol.* 2004;9(4):270-282, [PMID: 15375703]
Exclusion code: 5

Arason A, Jonasdottir A, Barkardottir RB, et al. A population study of mutations and LOH at breast cancer gene loci in tumours from sister pairs: two recurrent mutations seem to account

for all BRCA1/BRCA2 linked breast cancer in Iceland. *J. Med. Genet.* 1998;35(6):446-449, [PMID: 9643283]
Exclusion code: 2

Ardern-Jones A, Kenen R, Eeles R. Too much, too soon? Patients and health professionals' views concerning the impact of genetic testing at the time of breast cancer diagnosis in women under the age of 40. *Eur. J Cancer Care.* 2005;14(3):272-281, [PMID: 15952973]
Exclusion code: 3

Armakolas A, Ladopoulou A, Konstantopoulou I, et al. BRCA2 gene mutations in Greek patients with familial breast cancer. *Hum. Mutat.* 2002;19(1):81-82, [PMID: 11754111]
Exclusion code: 3

Armel SR, Hitchman K, Millar K, et al. The use of family history questionnaires: An examination of genetic risk estimates and genetic testing eligibility in the non-responder population. *J Genet Couns.* 2011;20(4):355-364, [PMID: 21448763]
Exclusion code: 8
Armel SR, McCuaig J, Finch A, et al. The effectiveness of family history questionnaires in cancer genetic counseling. *J Genet Couns.* 2009;18(4):366-378, [PMID: 19459037]
Exclusion code: 3

Armstrong K, Schwartz JS, Randall T, Rubin SC, Weber B. Hormone replacement therapy and life expectancy after prophylactic oophorectomy in women with BRCA1/2 mutations: A decision analysis. *J. Clin. Oncol.* 2004;22(6):1045-1054, [PMID: 14981106]
Exclusion code: 6

Armstrong K, Weber B, Ubel PA, Peters N, Holmes J, Schwartz JS. Individualized survival curves improve satisfaction with cancer risk management decisions in women with BRCA1/2 mutations. *J. Clin. Oncol.* 2005;23(36):9319-9328, [PMID: 16361631]
Exclusion code: 8

Arnold LD, Colditz GA. Hysterectomy With oophorectomy: implications for clinical decision making. *Arch. Intern. Med.* 2011;171(8):768-769, [PMID: 21518945]
Exclusion code: 5

Artioli G, Borgato L, Cappetta A, et al. Overall survival in BRCA-associated ovarian cancer: case-control study of an Italian series. *Eur. J. Gynaecol. Oncol.* 2010;31(6):658-661, [PMID: 21319511]
Exclusion code: 3

Arver B, Borg Å, Lindblom A. First BRCA1 and BRCA2 gene testing implemented in the health care system of Stockholm. *Genet Test.* 2001;5(1):1-8, [PMID: 11336395]
Exclusion code: 6

Arver B, Isaksson K, Atterhem H, et al. Bilateral prophylactic mastectomy in Swedish women at high risk of breast cancer: a national survey. *Ann. Surg.* 2011;253(6):1147-1154, [PMID: 21587115]
Exclusion code: 3

Ashikari RH, Ashikari AY, Kelemen PR, Salzberg CA. Subcutaneous mastectomy and immediate reconstruction for prevention of breast cancer for high-risk patients. *Breast Cancer.* 2008;15(3):185-191, [PMID: 18575951]
Exclusion code: 5

Atkins D, Chang SM, Gartlehner G, et al. Assessing applicability when comparing medical interventions: AHRQ and the Effective Health Care Program. *J. Clin. Epidemiol.* 2011;64(11):1198-1207, [PMID: 21463926]
Exclusion code: 4

Augustine KM, Bogan TL. Operating a comprehensive high-risk breast cancer management program in a community hospital setting. *AWHONN Lifelines.* 2004;8(5):434-440, [PMID: 15560622]
Exclusion code: 5

Auranen A, Song H, Waterfall C, et al. Polymorphisms in DNA repair genes and epithelial ovarian cancer risk. *Int. J. Cancer.* 2005;117(4):611-618, [PMID: 15924337]
Exclusion code: 3

Appendix B6. Excluded Studies List

Avard D, Bridge P, Bucci LM, et al. Partnering in oncogenetic research - The INHERIT BRCAs experience: opportunities and challenges. *Fam Cancer.* 2006;5(1):3-13, [PMID: 16528603]
Exclusion code: 5

Aziz S, Kuperstein G, Rosen B, et al. A genetic epidemiological study of carcinoma of the fallopian tube. *Gynecol. Oncol.* 2001;80:341-345, [PMID: 11263928]
Exclusion code: 2

Bacha OM, Gregoire J, Grondin K, et al. Effectiveness of risk-reducing salpingo-oophorectomy in preventing ovarian cancer in a high-risk French Canadian population. *Int J Gynecol Cancer.* 2012;22(6):974-978, [PMID: 22740003]
Exclusion code: 3

Bados A, Gómez-Benito J, Balaguer G. The state-trait anxiety inventory, trait version: Does it really measure anxiety? *J. Pers. Assess.* 2010;92(6):560-567, [PMID: 20954057]
Exclusion code: 2

Baer HJ, Brawarsky P, Murray MF, Haas JS. Familial risk of cancer and knowledge and use of genetic testing. *J. Gen. Intern. Med.* 2010;25(7):717-724, [PMID: 20361271]
Exclusion code: 3

Baildam AD. Best surgical prophylaxis--risk-reducing mastectomy for women at high personal risk of breast cancer. *Breast.* 2006;15 Suppl 2:S21-25, [PMID: 17382857]
Exclusion code: 5

Baile WF, Buckman R, Lenzi R, Glober G, Beale EA, Kudelka AP. SPIKES - A six-step protocol for delivering bad news: Application to the patient with cancer. *Oncologist.* 2000;5(4):302-311, [PMID: 10964998]
Exclusion code: 2

Bakos AD, Hutson SP, Loud JT, Peters JA, Giusti RM, Greene MH. BRCA mutation-negative women from hereditary breast and ovarian cancer families: a qualitative study of the BRCA-negative experience. *Health Expect.* 2008;11(3):220-231, [PMID: 18816319]
Exclusion code: 2

Ballinger LL. Hereditary gynecologic cancers: risk assessment, counseling, testing and management. *Obstet. Gynecol. Clin. North Am.* 2012;39(2):165-181, [PMID: 22640709]
Exclusion code: 5

Balmana J, Diez O, Rubio I, Castiglione M, Group EGW. BRCA in breast cancer: ESMO Clinical Practice Guidelines. *Ann. Oncol.* 2010;21 Suppl 5:v20-22, [PMID: 20555082]
Exclusion code: 2

Balmaña J, Díez O, Rubio IT, Cardoso F. BRCA in breast cancer: ESMO clinical practice guidelines. *Ann. Oncol.* 2011;22(SUPPL. 6):vi31-vi34, [PMID: 21908500]
Exclusion code: 2

Balmana J, Sanz J, Bonfill X, et al. Genetic counseling program in familial breast cancer: analysis of its effectiveness, cost and cost-effectiveness ratio. *Int. J. Cancer.* 2004;112(4):647-652, [PMID: 15382046]
Exclusion code: 8

Bancroft EK, Locke I, Ardern-Jones A, et al. The carrier clinic: an evaluation of a novel clinic dedicated to the follow-up of BRCA1 and BRCA2 carriers--implications for oncogenetics practice. *J. Med. Genet.* 2010;47(7):486-491, [PMID: 20472659]
Exclusion code: 3

Bandera CA, Muto MG, Schorge JO, Berkowitz RS, Rubin SC, Mok SC. BRCA1 Gene Mutations in Women With Papillary Serous Carcinoma of the Peritoneum *Obstet. Gynecol.* 1998;92(4):596-600, [PMID: 9764635]
Exclusion code: 2

Banegas MP, Gail MH, LaCroix A, et al. Evaluating breast cancer risk projections for Hispanic women. *Breast Cancer Res. Treat.* 2012;132(1):347-353, [PMID: 22147080]
Exclusion code: 8

Barks P, Goldgar C. Hereditary breast and ovarian cancer. *JAAPA.* 2012;25(3):63-65, [PMID: 22514962]

Exclusion code: 4

Barlow WE, White E, Ballard-Barbash R, et al. Prospective breast cancer risk prediction model for women undergoing screening mammography. *J. Natl. Cancer Inst.* 2006;98(17):1204-1214, [PMID: 16954473]
Exclusion code: 8

Barlow-Stewart K, Taylor SD, Treloar SA, Stranger M, Otlowski M. Verification of consumers' experiences and perceptions of genetic discrimination and its impact on utilization of genetic testing. *Genet Med.* 2009;11(3):193-201, [PMID: 19287242]
Exclusion code: 3

Barnes DR, Lee A, Investigators E, kConFab I, Easton DF, Antoniou AC. Evaluation of association methods for analysing modifiers of disease risk in carriers of high-risk mutations. *Genet. Epidemiol.* 2012;36(3):274-291, [PMID: 22714938]
Exclusion code: 5

Barnes MN, Chhieng DF, Dreher M, et al. Feasibility of performing chemoprevention trials in women at elevated risk of ovarian carcinoma: initial examination of celecoxib as a chemopreventive agent. *Gynecol. Oncol.* 2005;98(3):376-382, [PMID: 15993933]
Exclusion code: 8

Barrett-Connor E, Mosca L, Collins P, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. *N. Engl. J. Med.* 2006;355(2):125-137, [PMID: 16837676]
Exclusion code: 2

Barrett-Connor E, Wenger NK, Grady D, et al. Hormone and nonhormone therapy for the maintenance of postmenopausal health: the need for randomized controlled trials of estrogen and raloxifene. *J Womens Health.* 1998;7(7):839-847, [PMID: 9785310]
Exclusion code: 2

Barsevick AM, Montgomery SV, Ruth K, et al. Intention to communicate BRCA1/BRCA2 genetic test results to the family. *Journal of Family Psychology.* 2008;22(2):303-312, [PMID: 18410217]
Exclusion code: 2

Barton M, CN W, IL L, EL H, SJ R, JG E. Complications following bilateral prophylactic mastectomy. *J Natl Cancer Inst Monogr.* 2005;35:61-66, [PMID: 16287887]
Exclusion code: 3

Basham VM, Lipscombe JM, Ward JM, et al. BRCA1 and BRCA2 mutations in a population-based study of male breast cancer. *Breast Cancer Res.* 2002;4(1), [PMID: 11879560]
Exclusion code: 3

Bates GW, Trajstman SEA, Jackson CA. Internal consistency, test-retest reliability and sex differences on the posttraumatic growth inventory in an Australian sample with trauma. *Psychol. Rep.* 2004;94(3 I):793-794, [PMID: 15217029]
Exclusion code: 2

Batista LI, Lu KH, Beahm EK, Arun BK, Bodurka DC, Meric-Bernstam F. Coordinated prophylactic surgical management for women with hereditary breast-ovarian cancer syndrome. *BMC Cancer.* 2008;8:101, [PMID: 18410690]
Exclusion code: 3

Baty BJ, Dudley WN, Musters A, Kinney AY. Uncertainty in BRCA1 cancer susceptibility testing. *Am J Med Genet C Semin Med Genet.* 2006;142C(4):241-250, [PMID: 17068806]
Exclusion code: 3

Baum M. Ramifications of screening for breast cancer: consent for screening. *BMJ.* 2006;332(7543):728, [PMID: 16565139]
Exclusion code: 5

Baumanis L, Evans JP, Callanan N, Susswein LR. Telephoned BRCA1/2 genetic test results: prevalence, practice, and patient satisfaction. *J Genet Couns.* 2009;18(5):447-463, [PMID: 19462222]
Exclusion code: 8

Baxter NN, Goodwin PJ, McLeod RS, Dion R, Devins G, Bombardier C. Reliability and

validity of the body image after breast cancer questionnaire. *Breast J.* 2006;12(3):221-232, [PMID: 16684320]
Exclusion code: 2

Beauchamp TL, Childress JF. *Principles of Biomedical Ethics*. 4 ed. New York: Oxford University Press; 1994.
Exclusion code: 5

Beck AT, Steer RA, Garbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin. Psychol. Rev.* 1988;8(1):77-100.
Exclusion code: 2

Becker S, Cazares LH, Watson P, et al. Surfaced-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) differentiation of serum protein profiles of BRCA-1 and sporadic breast cancer. *Ann. Surg. Oncol.* 2004;11(10):907-914, [PMID: 15383419]
Exclusion code: 8

Beckmann MW, Bani MR, Fasching PA, Strick R, Lux MP. Risk and risk assessment for breast cancer: molecular and clinical aspects. *Maturitas.* 2007;57(1):56-60, [PMID: 17386982]
Exclusion code: 5

Begg CB. On the use of familial aggregation in population-based case probands for calculating penetrance. *J. Natl. Cancer Inst.* 2002;94(16):1221-1226, [PMID: 12189225]
Exclusion code: 2

Begg CB, Haile RW, Borg A, et al. Variation of breast cancer risk among BRCA1/2 carriers. *JAMA.* 2008;299(2):194-201, [PMID: 18182601]
Exclusion code: 3

Begum S, Grunfeld EA, Ho-Asjoe M, Farhadi J. An exploration of patient decision-making for autologous breast reconstructive surgery following a mastectomy. *Patient Educ. Couns.* 2011;84(1):105-110, [PMID: 20688457]
Exclusion code: 8

Belkić KL, Cohen M, Márquez M, et al. Screening of high-risk groups for breast and ovarian cancer in Europe: A focus on the Jewish population. *Oncology Reviews.* 2010;4(4):233-267.
Exclusion code: 5

Screening for ovarian cancer: a systematic review (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-31998008413.
Exclusion code: 5

Bellcross CA. *Evaluation of a cancer genetics referral screening tool*, Bellcross, Cecelia A : U Wisconsin - Madison, US; 2008
Exclusion code: 2

Bellcross CA, Kolor K, Goddard KAB, Coates RJ, Reyes M, Khoury MJ. Awareness and utilization of BRCA1/2 testing among U.S. primary care physicians. *Am. J. Prev. Med.* 2011;40(1):61-66, [PMID: 21146769]
Exclusion code: 2

Benjamin C, Flynn M, Hallett C, Ellis I, Booth K. The use of the life course paradigm and life course charts to explore referral for family history of breast cancer. *Int. J. Nurs. Stud.* 2008;45(1):95-109, [PMID: 17157849]
Exclusion code: 5

Bennett IC, Muller J, Cockburn L, et al. Outcomes of multimodality breast screening for women at increased risk of familial breast cancer. *World J. Surg.* 2010;34(5):979-986, [PMID: 20108093]
Exclusion code: 4

Bennett P, Parsons E, Brain K, Hood K, reTrace Study T. Long-term cohort study of women at intermediate risk of familial breast cancer: experiences of living at risk. *Psychooncology.* 2010;19(4):390-398, [PMID: 19514016]
Exclusion code: 3

Bennett P, Phelps C, Brain K, Hood K, Gray J. A randomized controlled trial of a brief self-help coping intervention designed to reduce distress when awaiting genetic risk information. *J.*

Psychosom. Res. 2007;63(1):59-64, [PMID: 17586338]
Exclusion code: 3

Bennett P, Wilkinson C, Turner J, et al. The impact of breast cancer genetic risk assessment on intentions to perform cancer surveillance behaviors. *J Genet Couns.* 2007;16(5):617-623, [PMID: 17549613]
Exclusion code: 8

Benshushan A, Rojansky N, Chaviv M, et al. Climacteric symptoms in women undergoing risk-reducing bilateral salpingo-oophorectomy. *Climacteric.* 2009;12(5):404-409, [PMID: 19479488]
Exclusion code: 8

Beran TM, Stanton AL, Kwan L, et al. The trajectory of psychological impact in BRCA1/2 genetic testing: does time heal? *Ann. Behav. Med.* 2008;36(2):107-116, [PMID: 18787910]
Exclusion code: 3

Berek JS, Chalas E, Edelson M, et al. Prophylactic and risk-reducing bilateral salpingo-oophorectomy: recommendations based on risk of ovarian cancer. *Obstet. Gynecol.* 2010;116(3):733-743, [PMID: 20733460]
Exclusion code: 5

Berg WA, Blume JD, Adams AM, et al. Reasons women at elevated risk of breast cancer refuse breast MR imaging screening: ACRIN 6666. *Radiology.* 2010;254(1):79-87, [PMID: 20032143]
Exclusion code: 8

Berlin L, Hall FM. More mammography muddle: emotions, politics, science, costs, and polarization. *Radiology.* 2010;255(2):311-316, [PMID: 20413746]
Exclusion code: 5

Berliner JL, Fay AM, Practice Issues Subcommittee of the National Society of Genetic Counselors' Familial Cancer Risk Counseling Special Interest G. Risk assessment and genetic counseling for hereditary breast and ovarian cancer: recommendations of the National Society of Genetic Counselors. *J Genet Couns.* 2007;16(3):241-260, [PMID: 17508274]
Exclusion code: 2

Effectiveness of prophylactic surgery, chemoprevention, and intensive surveillance in women carriers of BRCA 1/2 gene mutations (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006001132.
Exclusion code: 5

Bermejo-Perez MJ, Marquez-Calderon S, Llanos-Mendez A. Effectiveness of preventive interventions in BRCA1/2 gene mutation carriers: a systematic review. *Int. J. Cancer.* 2007;121(2):225-231, [PMID: 17471565]
Exclusion code: 6

Bermejo-Pérez MJ, Márquez-Calderón S, Llanos-Méndez A. Cancer surveillance based on imaging techniques in carriers of BRCA1/2 gene mutations: A systematic review. *Br. J. Radiol.* 2008;81(963):172-179, [PMID: 18208856]
Exclusion code: 6

Bernholtz S, Laitman Y, Kaufman B, Paluch Shimon S, Friedman E. Cancer risk in Jewish BRCA1 and BRCA2 mutation carriers: effects of oral contraceptive use and parental origin of mutation. *Breast Cancer Res. Treat.* 2011;129(2):557-563, [PMID: 21499684]
Exclusion code: 8

Berrington de Gonzalez A, Berg CD, Visvanathan K, Robson M. Estimated risk of radiation-induced breast cancer from mammographic screening for young BRCA mutation carriers. *J. Natl. Cancer Inst.* 2009;101(3):205-209, [PMID: 19176458]
Exclusion code: 8

Berry D, Parmigiani G, Sanchez J, Schildkraut J, Winer E. Probability of carrying a mutation of breast-ovarian cancer gene BRCA1 based on family history. *J. Natl. Cancer Inst.* 1997;89(3):227-238, [PMID: 9017003]
Exclusion code: 2

Besic N, Cernivc B, De Grève J, et al. BRCA2 gene mutations in Slovenian male breast cancer patients. *Genet Test.* 2008;12(2):203-209, [PMID: 18439106]
Exclusion code: 3

Biglia N, Defabiani E, Ponzone R, Mariani L, Marenco D, Sismondi P. Management of risk of breast carcinoma in postmenopausal women. *Endocr Relat Cancer.* 2004;11(1):69-83, [PMID: 15027886]
Exclusion code: 5

Biglia N, Mariani L, Ponzone R, Sismondi P. Oral contraceptives, salpingo-oophorectomy and hormone replacement therapy in BRCA1-2 mutation carriers. *Maturitas.* 2008;60(2):71-77, [PMID: 18472232]
Exclusion code: 5

Bilimoria MM, Jordan VC. The duration of adjuvant tamoxifen therapy. *Cancer Treat. Res.* 1998;94:181-193, [PMID: 9587688]
Exclusion code: 5

Bingham R. Hereditary breast and ovarian cancer: research on how women respond to genetic testing. *Nurs Womens Health.* 2012;16(4):319-324, [PMID: 22900808]
Exclusion code: 5

Bird S. Breast screening and failure to diagnose breast cancer. *Aust. Fam. Physician.* 2009;38(4):237-238, [PMID: 19350074]
Exclusion code: 5

Bish A, Sutton S, Jacobs C, Levene S, Ramirez A, Hodgson S. No news is (not necessarily) good news: Impact of preliminary results for BRCA1 mutation searches. *Genet Med.* 2002;4(5):353-358, [PMID: 12394348]
Exclusion code: 3

Biswas S, Berry DA. Determining joint carrier probabilities of cancer-causing genes using Markov chain Monte Carlo methods. *Genet. Epidemiol.* 2005;29(2):141-154, [PMID: 16025444]
Exclusion code: 8

Biswas S, Tankhiwale N, Blackford A, et al. Assessing the added value of breast tumor markers in genetic risk prediction model BRCAPRO. *Breast Cancer Res. Treat.* 2012;133(1):347-355, [PMID: 22270937]
Exclusion code: 4

Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale: An updated literature review. *J. Psychosom. Res.* 2002;52(2):69-77, [PMID: 11832252]
Exclusion code: 2

Bjorge T, Lie AK, Hovig E, et al. BRCA1 mutations in ovarian cancer and borderline tumours in Norway: a nested case-control study. *Br. J. Cancer.* 2004;91(10):1829-1834, [PMID: 15477862]
Exclusion code: 3

Bjorvatn C, Eide GE, Hanestad BR, et al. Risk perception, worry and satisfaction related to genetic counseling for hereditary cancer. *J Genet Couns.* 2007;16(2):211-222, [PMID: 17279329]
Exclusion code: 3

Blecharz P, Szatkowski W, Klimek M, Urbanski K. [The prevalence of BRCA1 mutations among families at high-risk of breast and ovarian cancer in province of Malopolska between 2004-2009]. *Przegl. Lek.* 2009;66(12):1046-1048, [PMID: 20514903]
Exclusion code: 7

Bleret V, Cusumano P, Dezfoulian B, et al. [Bilateral prophylactic mastectomy for women at very high risk for breast cancer: beyond the technique]. *Rev. Med. Liege.* 2011;66(5-6):250-253, [PMID: 21826956]
Exclusion code: 7

Bleret V, Desreux J, Cusumano P, et al. [Primary and secondary prevention for women at high risk for breast cancer]. *Rev. Med. Liege.* 2011;66(5-6):245-249, [PMID: 21826955]
Exclusion code: 7

Bober SL, Hoke LA, Duda RB, Regan MM, Tung NM. Decision-making about tamoxifen in women at high risk for breast cancer: clinical

and psychological factors. *J. Clin. Oncol.* 2004;22(24):4951-4957, [PMID: 15598980]
Exclusion code: 8

Bober SL, Hoke LA, Duda RB, Tung NM. Recommendation recall and satisfaction after attending breast/ovarian cancer risk counseling. *J Genet Couns.* 2007;16(6):755-762, [PMID: 17674165]
Exclusion code: 3

Boccardo F, Rubagotti A, Amoroso D, et al. Sequential tamoxifen and aminoglutethimide versus tamoxifen alone in the adjuvant treatment of postmenopausal breast cancer patients: results of an Italian cooperative study. *J. Clin. Oncol.* 2001;19(22):4209-4215, [PMID: 11709564]
Exclusion code: 3

Bodmer D, Ligtenberg MJL, van der Hout AH, et al. Optimal selection for BRCA1 and BRCA2 mutation testing using a combination of 'easy to apply' probability models. *Br. J. Cancer.* 2006;95(6):757-762, [PMID: 16909138]
Exclusion code: 3

Boenink M. Unambiguous test results or individual independence? The role of clients and families in predictive BRCA-testing in the Netherlands compared to the USA. *Soc. Sci. Med.* 2011;72(11):1793-1801, [PMID: 20650557]
Exclusion code: 8

Bolton K, Chenevix-Trench G, Goh C. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. *JAMA.* 2012;307(4):382-390, [PMID: 22274685]
Exclusion code: 2

Bonadona V, Sinilnikova OM, Chopin S, et al. Contribution of BRCA1 and BRCA2 germ-line mutations to the incidence of breast cancer in young women: results from a prospective population-based study in France. *Genes. Chromosomes Cancer.* 2005;43(4):404-413, [PMID: 15887246]
Exclusion code: 3

Bondy ML, Newman LA. Assessing breast cancer risk: evolution of the Gail Model. *J. Natl. Cancer Inst.* 2006;98(17):1172-1173, [PMID: 16954464]
Exclusion code: 5

Bordeleau LJ, Lipa JE, Neligan PC. Management of the BRCA mutation carrier or high-risk patient. *Clin. Plast. Surg.* 2007;34(1):15-27; abstract v, [PMID: 17307068]
Exclusion code: 5

Borgen PI, Hill AD, Tran KN, al. e. Patient regrets after bilateral prophylactic mastectomy. *Ann. Surg. Oncol.* 1998;5(7):603-606, [PMID: 9831108]
Exclusion code: 5

Borry P, Fryns JP, Schotsmans P, Dierickx K. Attitudes towards carrier testing in minors: A systematic review. *Genet. Couns.* 2005;16(4):341-352, [PMID: 16440876]
Exclusion code: 3

Borry P, Stultiens L, Nys H, Dierickx K. Attitudes towards predictive genetic testing in minors for familial breast cancer: a systematic review. *Crit. Rev. Oncol. Hematol.* 2007;64(3):173-181, [PMID: 17553690]
Exclusion code: 3

Bosetti C, Negri E, Trichopoulos D, et al. Long-term effects of oral contraceptives on ovarian cancer risk. *Int. J. Cancer.* 2002;102(3):262-265, [PMID: 12397647]
Exclusion code: 3

Bosse K, Rhiem K, Wappenschmidt B, et al. Screening for ovarian cancer by transvaginal ultrasound and serum CA125 measurement in women with a familial predisposition: a prospective cohort study. *Gynecol. Oncol.* 2006;103(3):1077-1082, [PMID: 16904167]
Exclusion code: 3

Boughey JC, Hartmann LC, Anderson SS, et al. Evaluation of the Tyrer-Cuzick (International Breast Cancer Intervention Study) model for breast cancer risk prediction in women with atypical hyperplasia. *J. Clin. Oncol.* 2010;28(22):3591-3596, [PMID: 20606088]
Exclusion code: 2

Bowen DJ, Bourcier E, Press N, Lewis FM, Burke W. Effects of individual and family functioning on interest in genetic testing. *Community Genet.* 2004;7(1):25-32, [PMID: 15475668]
Exclusion code: 8

Bowen DJ, Harris J, Jorgensen CM, Myers MF, Kuniyuki A. Socioeconomic influences on the effects of a genetic testing direct-to-consumer marketing campaign. *Public Health Genomics.* 2010;13(3):131-142, [PMID: 19641293]
Exclusion code: 2

Bowen DJ, Powers D. Effects of a mail and telephone intervention on breast health behaviors. *Health Educ. Behav.* 2010;37(4):479-489, [PMID: 20157016]
Exclusion code: 3

Bowen DJ, Singal R, Eng E, Crystal S, Burke W. Jewish identity and intentions to obtain breast cancer screening. *Cultur Divers Ethni Minor Psychol.* 2003;9(1):79-87, [PMID: 12647327]
Exclusion code: 4

Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. *JAMA.* 2000;283(17):2260-2265, [PMID: 10807385]
Exclusion code: 2

Boyle P, Mezzetti M, La Vecchia C, Franceschi S, Decarli A, Robertson C. Contribution of three components to individual cancer risk predicting breast cancer risk in Italy. *Eur. J. Cancer Prev.* 2004;13(3):183-191, [PMID: 15167217]
Exclusion code: 2

Bradbury AR, Ibe CN, Dignam JJ, et al. Uptake and timing of bilateral prophylactic salpingo-oophorectomy among BRCA1 and BRCA2 mutation carriers. *Genet Med.* 2008;10(3):161-166, [PMID: 18344704]
Exclusion code: 3

Bradbury AR, Olopade OI. Genetic susceptibility to breast cancer. *Rev Endocr Metab Disord.* 2007;8(3):255-267, [PMID: 17508290]
Exclusion code: 5

Bradbury AR, Patrick-Miller L, Egleston B, et al. Parent opinions regarding the genetic testing of minors for BRCA1/2. *J. Clin. Oncol.* 2010;28(21):3498-3505, [PMID: 20567018]
Exclusion code: 2

Bradbury AR, Patrick-Miller L, Pawlowski K, et al. Learning of your parent's BRCA mutation during adolescence or early adulthood: A study of offspring experiences. *Psychooncology.* 2009;18(2):200-208, [PMID: 18702049]
Exclusion code: 2

Brain A. Randomized trial of a specialist genetic assessment service for familial breast cancer. *J. Natl. Cancer Inst.* 2000;92:1345-1351, [PMID: 10944557]
Exclusion code: 8

Braithwaite D, Emery J, Walter F, Prevost AT, Sutton S. Psychological impact of genetic counseling for familial cancer: A systematic review and meta-analysis. *J. Natl. Cancer Inst.* 2004;96(2):122-133, [PMID: 14734702]
Exclusion code: 6

Braithwaite D, Emery J, Walter F, Prevost AT, Sutton S. Psychological impact of genetic counseling for familial cancer: A systematic review and meta-analysis. *Fam Cancer.* 2006;5(1):61-75, [PMID: 16528610]
Exclusion code: 2

Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *J. Gen. Intern. Med.* 2003;18(11):937-947, [PMID: 14687281]
Exclusion code: 2

Brandenburg DL, Matthews AK, Johnson TP, Hughes TL. Breast cancer risk and screening: a comparison of lesbian and heterosexual women. *Women Health.* 2007;45(4):109-130, [PMID: 18032170]
Exclusion code: 3

Brandt A, Lorenzo Bermejo J, Sundquist J, Hemminki K. Breast cancer risk in women who

fulfill high-risk criteria: at what age should surveillance start? *Breast Cancer Res. Treat.* 2010;121(1):133-141, [PMID: 19641988]
Exclusion code: 8

Brehaut JC, O'Connor AM, Wood TJ, et al. Validation of a decision regret scale. *Med. Decis. Making.* 2003;23(4):281-292, [PMID: 12926578]
Exclusion code: 2

Brekelmans CT, Seynaeve C, Bartels CC, et al. Effectiveness of breast cancer surveillance in BRCA1/2 gene mutation carries and women with high familial risk. *J. Clin. Oncol.* 2001;19(4):924-930, [PMID: 11181654]
Exclusion code: 8

Brekelmans CTM, Seynaeve C. Can bilateral prophylactic salpingo-oophorectomy reduce cancer mortality in carriers of a BRCA1 or BRCA2 mutation? *Lancet Oncol.* 2006;7(3):191-193, [PMID: 16510328]
Exclusion code: 5

Brekelmans CTM, Tilanus-Linthorst MMA, Seynaeve C, et al. Tumour characteristics, survival and prognostic factors of hereditary breast cancer from BRCA2-, BRCA1- and non-BRCA1/2 families as compared to sporadic breast cancer cases. *Eur. J. Cancer.* 2007;43(5):867-876, [PMID: 17307353]
Exclusion code: 3

Bresser PJC, Seynaeve C, Van Gool AR, et al. Satisfaction with prophylactic mastectomy and breast reconstruction in genetically predisposed women. *Plast. Reconstr. Surg.* 2006;117(6):1675-1682; discussion 1683-1674, [PMID: 16651934]
Exclusion code: 3

Bresser PJC, Seynaeve C, Van Gool AR, et al. The course of distress in women at increased risk of breast and ovarian cancer due to an (identified) genetic susceptibility who opt for prophylactic mastectomy and/or salpingo-oophorectomy. *Eur. J. Cancer.* 2007;43(1):95-103, [PMID: 17095208]
Exclusion code: 3

Bresser PJC, Van Gool AR, Seynaeve C, et al. Who is prone to high levels of distress after prophylactic mastectomy and/or salpingo-ovariectomy? *Ann. Oncol.* 2007;18(10):1641-1645, [PMID: 17660493]
Exclusion code: 3

Briasoulis E, Ziogas D, Fatouros M. Prophylactic surgery in the complex decision-making management of BRCA mutation carriers. *Ann. Surg. Oncol.* 2008;15(6):1788-1790, [PMID: 18197455]
Exclusion code: 5

Brierley KL, Campfield D, Ducaine W, et al. Errors in delivery of cancer genetics services: Implications for practice. *Conn. Med.* 2010;74(7):413-423, [PMID: 20806621]
Exclusion code: 8

Brinton JT, Barke LD, Freivogel ME, Jackson S, O'Donnell CI, Glueck DH. Breast cancer risk assessment in 64,659 women at a single high-volume mammography clinic. *Acad. Radiol.* 2012;19(1):95-99, [PMID: 22054804]
Exclusion code: 4

Brohet RM, Goldgar DE, Easton DF, et al. Oral contraceptives and breast cancer risk in the international BRCA1/2 carrier cohort study: a report from EMBRACE, GENEPSO, GEO-HEBON, and the IBCCS Collaborating Group. *J. Clin. Oncol.* 2007;25(25):3831-3836, [PMID: 17635951]
Exclusion code: 8

Brooks GA, Stopfer JE, Erlichman J, Davidson R, Nathanson KL, Domchek SM. Childhood cancer in families with and without BRCA1 or BRCA2 mutations ascertained at a high-risk breast cancer clinic. *Cancer Biol Ther.* 2006;5(9):1098-1102, [PMID: 16931905]
Exclusion code: 3

Brower V. FDA to regulate direct-to-consumer genetic tests. *J. Natl. Cancer Inst.* 2010;102(21):1610-1612, 1617, [PMID: 20966430]
Exclusion code: 5

Brower V. US court reverses gene patent ruling for BRCA1 and BRCA2. *Lancet Oncol.* 2011;12(9):835, [PMID: 21984989]
Exclusion code: 2

Brown DL, Andreotti RF, Lee SI, et al. ACR Appropriateness Criteria: ovarian cancer screening. 2009; http://www.guideline.gov. Accessed 4/4/2012
Exclusion code: 2

Brown J, Buckley D, Coulthard A, et al. Magnetic resonance imaging screening in women at genetic risk of breast cancer: Imaging and analysis protocol for the UK multicentre study. *Magn. Reson. Imaging.* 2000;18(7):765-776, [PMID: 11027869]
Exclusion code: 5

Brown J, Coulthard A, Dixon AK, et al. Protocol for a national multi-centre study of magnetic resonance imaging screening in women at genetic risk of breast cancer. *Breast.* 2000;9(2):78-82, [PMID: 14731703]
Exclusion code: 5

Brown J, Coulthard A, Dixon AK, et al. Rationale for a national multi-centre study of magnetic resonance imaging screening in women at genetic risk of breast cancer. *Breast.* 2000;9(2):72-77, [PMID: 14731702]
Exclusion code: 5

Brown KL, Moglia DM, Grumet S. Genetic counseling for breast cancer risk: general concepts, challenging themes and future directions. *Breast Dis.* 2007;27:69-96, [PMID: 17917141]
Exclusion code: 5

Brown P. Risk assessment: controversies and management of moderate- to high-risk individuals. *Breast J.* 2005;11 Suppl 1:S11-19, [PMID: 15725110]
Exclusion code: 5

Brunet J. Hereditary breast cancer and genetic counseling in young women. *Breast Cancer Res. Treat.* 2010;123 Suppl 1:7-9, [PMID: 20711663]
Exclusion code: 5

Bruno M, Digennaro M, Tommasi S, et al. Attitude towards genetic testing for breast cancer susceptibility: a comparison of affected and unaffected women. *Eur. J Cancer Care.* 2010;19(3):360-368, [PMID: 19912305]
Exclusion code: 8

Bruno M, Tommasi S, Stea B, et al. Awareness of breast cancer genetics and interest in predictive genetic testing: a survey of a southern Italian population. *Ann. Oncol.* 2004;15 Suppl 1:I48-I54, [PMID: 15280188]
Exclusion code: 8

Brunsvold AN, Wung S-F, Merkle CJ. BRCA1 genetic mutation and its link to ovarian cancer: implications for advanced practice nurses. *J. Am. Acad. Nurse Pract.* 2005;17(12):518-526, [PMID: 16293160]
Exclusion code: 5

Buckmaster AM, Gallagher P. Experiences of and perspectives on genetic testing for breast/ovarian cancer in and outside of the customary clinical setting. *Psychol Health.* 2010;25(9):1041-1059, [PMID: 20204956]
Exclusion code: 8

Budroni M, Cesaraccio R, Coviello V, et al. Role of BRCA2 mutation status on overall survival among breast cancer patients from Sardinia. *BMC Cancer.* 2009;9:62, [PMID: 19232099]
Exclusion code: 3

Bulletins ACoP. Hereditary breast and ovarian cancer syndrome. *Gynecol. Oncol.* 2009;113(1):6-11, [PMID: 19309638]
Exclusion code: 5

Burke W, Culver J, Pinsky L, et al. Genetic assessment of breast cancer risk in primary care practice. *Am J Med Genet A.* 2009;149A(3):349-356, [PMID: 19208375]
Exclusion code: 5

Burke W, Daly M, Garber JE, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2, Cancer

Genetics Study Consortium. *JAMA.* 1997;277(12):997-1003, [PMID: 9091675]
Exclusion code: 2

Burke W, Kahn MJ, Garber JE, Collins FS. "First do no harm" also applies to cancer susceptibility testing. *Cancer J. Sci. Am.* 1996;2(5):250-252, [PMID: 9166540]
Exclusion code: 5

Burki N. [Hereditary breast and ovarian cancer - indications for genetic testing, counseling and options for mutation carriers]. *Ther. Umsch.* 2010;67(7):359-366, [PMID: 20577964]
Exclusion code: 7

Burness ML, Olopade OI. Is screening with magnetic resonance imaging in BRCA mutation carriers a safe and effective alternative to prophylactic mastectomy? *J. Clin. Oncol.* 2011;29(13):1652-1654, [PMID: 21444875]
Exclusion code: 5

Burwinkel B, Wirtenberger M, Klaes R, et al. Association of NCOA3 polymorphisms with breast cancer risk. *Clin. Cancer Res.* 2005;11(6):2169-2174, [PMID: 15788663]
Exclusion code: 3

Butow P, Meiser B, Price M, et al. Psychological Morbidity in Women at Increased Risk of Developing Breast Cancer: A Controlled Study. *Psychooncology.* 2005;14(3):196-203, [PMID: 15386776]
Exclusion code: 4

Butow PN, Lobb EA. Analyzing the process and content of genetic counseling in familial breast cancer consultations. *J Genet Couns.* 2004;13(5):403-424, [PMID: 15604639]
Exclusion code: 4

Butow PN, Lobb EA, Meiser B, Barratt A, Tucker KM. Psychological outcomes and risk perception after genetic testing and counselling in breast cancer: a systematic review. *MJA.* 2003;178:77-81, [PMID: 12526728]
Exclusion code: 6

Buys SS, Partridge E, Black A, et al. Effect of screening on ovarian cancer mortality: The Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening randomized controlled trial. *JAMA.* 2011;305(22):2295-2302, [PMID: 21642681]
Exclusion code: 3

Bylund CL, Fisher CL, Brashers D, et al. Sources of uncertainty about daughters' breast cancer risk that emerge during genetic counseling consultations. *J Genet Couns.* 2012;21(2):292-304, [PMID: 21833819]
Exclusion code: 2

Byrd LM, Shenton A, Maher ER, et al. Better life expectancy in women with BRCA2 compared with BRCA1 mutations is attributable to lower frequency and later onset of ovarian cancer. *Cancer Epidemiol. Biomarkers Prev.* 2008;17(6):1535-1542, [PMID: 18559571]
Exclusion code: 3

Cabrera E, Blanco I, Yague C, Zabalegui A. The impact of genetic counseling on knowledge and emotional responses in Spanish population with family history of breast cancer. *Patient Educ. Couns.* 2010;78(3):382-388, [PMID: 19948386]
Exclusion code: 3

Calderon-Margalit R, Paltiel O. Prevention of breast cancer in women who carry BRCA1 or BRCA2 mutations: a critical review of the literature. *Int. J. Cancer.* 2004;112(3):357-364, [PMID: 15382059]
Exclusion code: 2

Calzone K, Wattendorf D, Dunn BK. The application of genetics and genomics to cancer prevention. *Semin. Oncol.* 2010;37(4):407-418, [PMID: 20816510]
Exclusion code: 5

Calzone KA, Prindiville SA, Jourkiv O, et al. Randomized comparison of group versus individual genetic education and counseling for familial breast and/or ovarian cancer. *J. Clin. Oncol.* 2005;23(15):3455-3464, [PMID: 15908654]
Exclusion code: 3

Cameron LD, Reeve J. Risk perceptions, worry, and attitudes about genetic testing for breast

cancer susceptibility. *Psychol Health.* 2006;21(2):211-230, [PMID: 21985118]
Exclusion code: 4

Campfield Bonadies D, Moyer A, Matloff ET. What I wish I'd known before surgery: BRCA carriers' perspectives after bilateral salipingo-oophorectomy. *Fam Cancer.* 2011;10(1):79-85, [PMID: 20852945]
Exclusion code: 3

Cao MZ. Analysis of BRCA1 mutations among familial and/or breast cancer praecox patients in the east of Shandong in China. *Prog Mod Biomed.* 2009;9:1028-1030.
Exclusion code: 3

Cappelli M, Verma S, Korneluk Y, et al. Psychological and genetic counseling implications for adolescent daughters of mothers with breast cancer. *Clin. Genet.* 2005;67(6):481-491, [PMID: 15857415]
Exclusion code: 3

Carcangiu ML, Peissel B, Pasini B, Spatti G, Radice P, Manoukian S. Incidental carcinomas in prophylactic specimens in BRCA1 and BRCA2 germ-line mutation carriers, with emphasis on fallopian tube lesions: report of 6 cases and review of the literature. *Am. J. Surg. Pathol.* 2006;30(10):1222-1230, [PMID: 17001151]
Exclusion code: 8

Carlson KJ. Screening for ovarian cancer 2011; http://www.uptodate.com/contents/screening-for-ovarian-cancer?source=see_link&anchor=H3#H17. Accessed 09 Nov, 2011
Exclusion code: 2

Carroll JC, Cremin C, Allanson J, et al. Hereditary breast and ovarian cancers. *Can. Fam. Physician.* 2008;54(12):1691-1692, [PMID: 19074707]
Exclusion code: 5

Carroll JC, Heisey R, Warner E. Family history and breast cancer. *CMAJ.* 2012;184(12):1391, [PMID: 22291177]
Exclusion code: 5

Carroll JC, Wilson BJ, Allanson J, et al. GenetiKit: A randomized controlled trial to enhance delivery of genetics services by family physicians. *Fam. Pract.* 2011;28(6):615-623, [PMID: 21746696]
Exclusion code: 2

Carroll PA, Nolan C, Clarke R, et al. Surgical management of an Irish cohort of BRCA-mutation carriers. *Breast.* 2011;20(5):419-423, [PMID: 21570850]
Exclusion code: 8

Carter RE. Psychological evaluation a consideration in the ethics of genetic testing for breast cancer. *Psychiatric Annals.* 2004;34(2):119-124.
Exclusion code: 5

Caruso A, Vigna C, Bigazzi V, et al. Factors associated with an individuals' decision to withdraw from genetic counseling for BRCA1 and BRCA2 genes mutations: are personality traits involved? *Fam Cancer.* 2011;10(3):581-589, [PMID: 21604195]
Exclusion code: 8

Caruso A, Vigna C, Maggi G, Sega FM, Cognetti F, Savarese A. The withdrawal from oncogenetic counselling and testing for hereditary and familial breast and ovarian cancer. A descriptive study of an Italian sample. *J. Exp. Clin. Cancer Res.* 2008;27:75, [PMID: 19025627]
Exclusion code: 3

Caruso A, Vigna C, Marozzo B, et al. Subjective versus objective risk in genetic counseling for hereditary breast and/or ovarian cancers. *J. Exp. Clin. Cancer Res.* 2009;28:157, [PMID: 20025726]
Exclusion code: 3

Carver CS, Scheier MF, Weintraub JK. Assessing Coping Strategies: A Theoretically Based Approach. *J. Pers. Soc. Psychol.* 1989;56(2):267-283, [PMID: 2926629]
Exclusion code: 2

Casey WJ, 3rd, Rebecca AM, Andres LA, et al. Safety and efficacy of perforator flap breast

reconstruction with combined intraabdominal procedures. *Ann. Plast. Surg.* 2010;64(2):144-150, [PMID: 20098096]
Exclusion code: 3

Cauley JA, Norton L, Lippman ME, et al. Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-Year results from the MORE trial. *Breast Cancer Res. Treat.* 2001;65(2):125-134, [PMID: 11261828]
Exclusion code: 5

Cavallone L, Arcand SL, Maugard CM, et al. Comprehensive BRCA1 and BRCA2 mutation analyses and review of French Canadian families with at least three cases of breast cancer. *Fam Cancer.* 2010;9(4):507-517, [PMID: 20694749]
Exclusion code: 3

Centers for Disease Control and Prevention. Genetic testing for breast and ovarian cancer susceptibility: evaluating direct-to-consumer marketing -- Atlanta, Denver, Raleigh-Durham, and Seattle, 2003. *MMWR.* 2004;53(27):603-606, [PMID: 15254451]
Exclusion code: 2

Centers for Disease Control and Prevention. Breast Cancer Statistics 2010; http://www.cdc.gov/cancer/breast/statistics/. Accessed 10 October 2012, 2012
Exclusion code: 2

Centers for Disease Control and Prevention. Breast Cancer Risk Factors. 2011; http://www.cdc.gov/cancer/breast/basic_info/risk_factors.htm. Accessed 09 Nov, 2011
Exclusion code: 2

Centers for Disease Control and Prevention. Gynecologic Cancers: Ovarian Cancer. 2011; http://www.cdc.gov/cancer/ovarian/index.htm. Accessed 09 Nov, 2011
Exclusion code: 2

What is the impact of genetic counselling in women at increased risk of developing hereditary breast cancer: a meta-analytic review (Provisional abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-10901.
Exclusion code: 5

Systematic review: using magnetic resonance imaging to screen women at high risk for breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-20229.
Exclusion code: 5

A systematic review of the effectiveness of magnetic resonance imaging (MRI) as an addition to mammography and ultrasound in screening young women at high risk of breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-19491.
Exclusion code: 5

A systematic review of perceived risks, psychological and behavioral impacts of genetic testing (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-19593.
Exclusion code: 5

Psychological outcomes and risk perception after genetic testing and counselling in breast cancer: a systematic review (Provisional abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-13579.
Exclusion code: 6

Psychological consequences of predictive genetic testing: a systematic review (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-13585.
Exclusion code: 6

Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis (Provisional abstract). 2012.

http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-26006.
Exclusion code: 2

Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers (Provisional abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-21122.
Exclusion code: 3

Magnetic resonance imaging of the breast in screening women considered to be at high genetic risk of breast cancer (Provisional abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-15169.
Exclusion code: 2

Interventions to improve risk communication in clinical genetics: systematic review (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-21188.
Exclusion code: 8

Impact of gene expression profiling tests on breast cancer outcomes (Provisional abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-15868.
Exclusion code: 5

Diagnostic accuracy of methods for the detection of BRCA1 and BRCA2 mutations: a systematic review (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-17808.
Exclusion code: 8

Cancer surveillance based on imaging techniques in carriers of BRCA1/2 gene mutations: a systematic review (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-20623.
Exclusion code: 5

Body image after bilateral prophylactic mastectomy: an integrative literature review (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-18650.
Exclusion code: 5

Comparative effectiveness of screening and prevention strategies among BRCA1/2-affected mutation carriers (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cleed&AN=NHSEED-22011000379
Exclusion code: 5

Evaluation of a surveillance programme for women with a family history of breast cancer (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cleed&AN=NHSEED-22009101998
Exclusion code: 5

Genetic nurse counsellors can be an acceptable and cost-effective alternative to clinical geneticists for breast cancer risk genetic counselling: evidence from two parallel randomised controlled equivalence trials (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cleed&AN=NHSEED-22006001753
Exclusion code: 5

Randomized trial of a specialist genetic assessment service for familial breast cancer (Structured abstract). 2012.
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cleed&AN=NHSEED-22000001369
Exclusion code: 5

Prevention with tamoxifen or other hormones versus prophylactic surgery in BRCA1/2-positive women: a decision analysis (Structured

abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cleed&AN=NHSEED-22000000354
Exclusion code: 5

Systematic review of management options for women with a hereditary predisposition to ovarian cancer (Provisional abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-12502.
Exclusion code: 5

Nonpharmacologic strategies for managing common chemotherapy adverse effects: a systematic review (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=dare&AN=00125498-100000000-21072.
Exclusion code: 3

Benefits and costs of screening Ashkenazi Jewish women for BRCA1 and BRCA2 (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=cleed&AN=NHSEED-21999000349
Exclusion code: 5

Magnetic Resonance imaging for breast cancer diagnostics. Systematic review and economic assessment (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010001463
Exclusion code: 5

Challberg J, Ashcroft L, Lalloo F, et al. Menopausal symptoms and bone health in women undertaking risk reducing bilateral salpingo-oophorectomy: significant bone health issues in those not taking HRT. *Br. J. Cancer.* 2011;105(1):22-27, [PMID: 21654687]
Exclusion code: 3

Chan K, Morris GJ. Chemoprevention of breast cancer for women at high risk. *Semin. Oncol.* 2006;33(6):642-646, [PMID: 17145342]
Exclusion code: 3

Chang J, Yang WT, Choo HF. Mammography in Asian patients with BRCA1 mutations. *Lancet.* 1999;353(9169):2070-2071, [PMID: 10376643]
Exclusion code: 2

Chan-Smutko G, Patel D, Shannon KM, Ryan PD. Professional challenges in cancer genetic testing: who is the patient? *Oncologist.* 2008;13(3):232-238, [PMID: 18378533]
Exclusion code: 5

Chapman JS, Powell CB, McLennan J, et al. Surveillance of survivors: follow-up after risk-reducing salpingo-oophorectomy in BRCA 1/2 mutation carriers. *Gynecol. Oncol.* 2011;122(2):339-343, [PMID: 21531449]
Exclusion code: 8

Chappuis PO. [Breast cancer screening different from that used for the general population: who is concerned and with which approach?]. *Rev Med Suisse.* 2006;2(66):1296-1298, 1301-1292, 1304-1295, [PMID: 16775990]
Exclusion code: 7

Charles S, Kessler L, Stopfer JE, Domchek S, Halbert CH. Satisfaction with genetic counseling for BRCA1 and BRCA2 mutations among African American women. *Patient Educ. Couns.* 2006;63(1-2):196-204, [PMID: 16533589]
Exclusion code: 8

Chart PL, Franssen E. Management of women at increased risk for breast cancer: Preliminary results from a new program. *Can. Med. Assoc. J.* 1997;157:1235-1242, [PMID: 9361645]
Exclusion code: 3

Chen J, Pee D, Ayyagari R, et al. Projecting absolute invasive breast cancer risk in white women with a model that includes mammographic density. *J. Natl. Cancer Inst.* 2006;98(17):1215-1226, [PMID: 16954474]
Exclusion code: 8

Chen L, Hsu L, Malone K. A frailty-model-based approach to estimating the age-dependent penetrance function of candidate genes using population-based case-control study designs: an application to data on the BRCA1 gene.

Biometrics. 2009;65(4):1105-1114, [PMID: 19210733]
Exclusion code: 5

Chen Z, Arendell L, Aickin M, et al. Hip bone density predicts breast cancer risk independently of Gail score: results from the Women's Health Initiative. *Cancer.* 2008;113(5):907-915, [PMID: 18666209]
Exclusion code: 5

Cherbal F, Salhi N, Bakour R, Adane S, Boualga K, Maillet P. BRCA1 and BRCA2 unclassified variants and missense polymorphisms in Algerian breast/ovarian cancer families. *Dis. Markers.* 2012;32(6):343-353, [PMID: 22684231]
Exclusion code: 4

Chereau E, Uzan C, Balleyguier C, et al. Characteristics, treatment, and outcome of breast cancers diagnosed in BRCA1 and BRCA2 gene mutation carriers in intensive screening programs including magnetic resonance imaging. *Clin Breast Cancer.* 2010;10(2):113-118, [PMID: 20299317]
Exclusion code: 8

Cheung EL, Olson AD, Yu TM, Han PZ, Beattie MS. Communication of BRCA results and family testing in 1,103 high-risk women. *Cancer Epidemiol. Biomarkers Prev.* 2010;19(9):2211-2219, [PMID: 20699375]
Exclusion code: 8

Chlebowski RT, Anderson GL, Lane DS, et al. Predicting risk of breast cancer in postmenopausal women by hormone receptor status. *J. Natl. Cancer Inst.* 2007;99(22):1695-1705, [PMID: 18000216]
Exclusion code: 2

Chun J, Schnabel F, Ogunyemi O. Assessing a Bayesian risk prediction model in a high-risk breast cancer population. *AMIA Annu Symp Proc.* 2007;Annual Symposium Proceedings/AMIA Symposium.:913, [PMID: 18694013]
Exclusion code: 8

Ciernikova S, Tomka M, Kovac M, Stevurkova V, Zajac V. Ashkenazi founder BRCA1/BRCA2 mutations in Slovak hereditary breast and/or ovarian cancer families. *Neoplasma.* 2006;53(2):97-102, [PMID: 16575464]
Exclusion code: 3

Cink TM. Breast cancer screening update and evaluation of the high-risk patient. *S. D. J. Med.* 2010;Spec No:23-30, [PMID: 20401983]
Exclusion code: 2

Cipollini G, Tommasi S, Paradiso A, et al. Genetic alterations in hereditary breast cancer. *Ann. Oncol.* 2004;15 Suppl 1:I7-I13, [PMID: 15280181]
Exclusion code: 8

Claes E, Evers-Kiebooms G, Boogaerts A, Decruyenaere M, Denayer L, Legius E. Diagnostic genetic testing for hereditary breast and ovarian cancer in cancer patients: women's looking back on the pre-test period and a psychological evaluation. *Genet Test.* 2004;8(1):13-21, [PMID: 15140370]
Exclusion code: 3

Claes E, Evers-Kiebooms G, Decruyenaere M, et al. Surveillance behavior and prophylactic surgery after predictive testing for hereditary breast/ovarian cancer. *Behav. Med.* 2005;31(3):93-105, [PMID: 16252621]
Exclusion code: 8

Claes E, Evers-Kiebooms G, Denayer L, et al. Predictive genetic testing for hereditary breast and ovarian cancer: psychological distress and illness representations 1 year following disclosure. *J Genet Couns.* 2005;14(5):349-363, [PMID: 16195942]
Exclusion code: 8

Clamp A, Danson S, Clemons M. Hormonal and genetic risk factors for breast cancer. *Surgeon.* 2003;1(1):23-31, [PMID: 15568421]
Exclusion code: 5

Clarke S, Butler K, Esplen MJ. The phases of disclosing BRCA1/2 genetic information to offspring. *Psychooncology.* 2008;17(8):797-803, [PMID: 18646247]

Exclusion code: 2

Claus EB, Risch H, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am. J. Hum. Genet.* 1991;48:232-242, [PMID: 1990835]
Exclusion code: 2

Clements A, Henderson BJ, Tyndel S, et al. Diagnosed with breast cancer while on a family history screening programme: an exploratory qualitative study. *Eur. J Cancer Care.* 2008;17(3):245-252, [PMID: 18419627]
Exclusion code: 3

Cnota W, Sodowski K, Olesiak-Andryszczak M, Pilch-Kowalczyk M. [Program for early detection of ovarian cancer for women as prophylaxis provided at a municipal hospital]. *Wiad. Lek.* 2004;57 Suppl 1:43-47, [PMID: 15884203]
Exclusion code: 7

Cohen JV, Chiel L, Boghossian L, et al. Non-cancer endpoints in BRCA1/2 carriers after risk-reducing salpingo-oophorectomy. *Fam Cancer.* 2012;11(1):69-75, [PMID: 21898151]
Exclusion code: 8

Cohen M. Breast cancer early detection, health beliefs, and cancer worries in randomly selected women with and without a family history of breast cancer. *Psychooncology.* 2006;15(10):873-883, [PMID: 16374894]
Exclusion code: 4

Col NF, Chlebowski RT. Risks and benefits of therapy with menopausal hormones versus selective estrogen-receptor modulators in peri- and postmenopausal women at increased breast cancer risk. *Menopause.* 2008;15(4 Suppl):804-809, [PMID: 18596602]
Exclusion code: 5

Colditz GA, Rosner B. Cumulative risk of breast cancer to age 70 years according to risk factor status: Data from the nurses' health study. *Am. J. Epidemiol.* 2000;152(10):950-964, [PMID: 11092437]
Exclusion code: 2

Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. *J. Natl. Cancer Inst.* 2004;96(3):218-228, [PMID: 14759989]
Exclusion code: 2

Colditz GA, Willett WC, Hunter DJ. Family history, age, and risk of breast cancer. Prospective data from the Nurses' Health Study. *JAMA.* 1993;270(3):338-343, [PMID: 8123079]
Exclusion code: 2

Colditz GA, Willett WC, Stampfer MJ. Menopause and the risk of coronary heart disease in women. *N. Engl. J. Med.* 1987;316(18):1105-1110, [PMID: 3574358]
Exclusion code: 6

Cole DEC. New genetic technologies: clinical application and ethical issues in familial ovarian cancer. *Clin Invest Med.* 2004;27(1):16-18, [PMID: 15061581]
Exclusion code: 5

Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. *Lancet.* 2001;358(9291):1389-1399, [PMID: 11705483]
Exclusion code: 6

Collier R. Young women with breast cancer genes face tough choices. *CMAJ.* 2012;184(8):E401-402, [PMID: 22508975]
Exclusion code: 5

Comen E, Balistreri L, Gonen M, et al. Discriminatory accuracy and potential clinical utility of genomic profiling for breast cancer risk in BRCA-negative women. *Breast Cancer Res. Treat.* 2011;127(2):479-487, [PMID: 20957429]
Exclusion code: 4

Condello C, Gesuita R, Pensabene M, et al. Distress and family functioning in oncogenetic counselling for hereditary and familial breast and/or ovarian cancers. *J Genet Couns.* 2007;16(5):625-634, [PMID: 17701329]

Exclusion code: 2

Contant C, van Wersch A, Menke-Pluymers M, Wai R, Eggermont A, van Geel A. Satisfaction and prosthesis related complaints in women with immediate breast reconstruction following prophylactic and oncological mastectomy. *Psychology, Health & Medicine.* 2004;9(1):71-84.
Exclusion code: 3

Contant CM, Menke-Pluijmers MB, Seynaeve C, et al. Clinical experience of prophylactic mastectomy followed by immediate breast reconstruction in women at hereditary risk of breast cancer (HB(O)C) or a proven BRCA1 and BRCA2 germ-line mutation. *Eur. J. Surg. Oncol.* 2002;28(6):627-632, [PMID: 12359199]
Exclusion code: 3

Contegiacomo A, Pensabene M, Capuano I, et al. An oncologist-based model of cancer genetic counselling for hereditary breast and ovarian cancer. *Ann. Oncol.* 2004;15(5):726-732, [PMID: 15111339]
Exclusion code: 3

Cook NR, Paynter NP. Genetics and breast cancer risk prediction--are we there yet? *J. Natl. Cancer Inst.* 2010;102(21):1605-1606, [PMID: 20956781]
Exclusion code: 5

Cook-Deegan R, DeRienzo C, Carbone J, Chandrasekharan S, Heaney C, Conover C. Impact of gene patents and licensing practices on access to genetic testing for inherited susceptibility to cancer: comparing breast and ovarian cancers with colon cancers. *Genet Med.* 2010;12(4 Suppl):S15-38, [PMID: 20393305]
Exclusion code: 2

Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. *J. Natl. Cancer Inst.* 1999;91(18):1541-1548, [PMID: 10491430]
Exclusion code: 2

Couch FJ. Genetic epidemiology of BRCA1. *Cancer Biol Ther.* 2004;3(6):509-514, [PMID: 15254414]
Exclusion code: 5

Coulson AS, Glasspool DW, Fox J, Emery J. RAGs: A novel approach to computerized genetic risk assessment and decision support from pedigrees. *Methods Inf. Med.* 2001;40(4):315-322, [PMID: 11552344]
Exclusion code: 2

Courtillot C, Touraine P. [Management of families at high risk for hereditary breast-ovarian cancers: the endocrinologist's point of view]. *Ann. Endocrinol. (Paris).* 2008;69(3):193-200, [PMID: 18294609]
Exclusion code: 7

Cox DG, Hankinson SE, Hunter DJ. The erbB2/HER2/neu receptor polymorphism Ile655Val and breast cancer risk. *Patient Educ. Couns.* 2005;15(7):447-450, [PMID: 15970791]
Exclusion code: 8

Cox SL, Zlot AI, Silvey K, et al. Patterns of cancer genetic testing: a randomized survey of Oregon clinicians. *J Cancer Epidemiol.* 2012;2012:294730, [PMID: 23150730]
Exclusion code: 2

Craft M. Hereditary breast and ovarian cancer: high-risk management. *Oncology (Williston Park).* 2007;21(11 Suppl Nurse Ed):19-21, [PMID: 18154204]
Exclusion code: 5

Crispo A, D'Aiuto G, De Marco M, et al. Gail model risk factors: impact of adding an extended family history for breast cancer. *Breast J.* 2008;14(3):221-227, [PMID: 18373641]
Exclusion code: 3

Crotser CB, Boehmke M. Survivorship considerations in adults with hereditary breast and ovarian cancer syndrome: State of the science. *J Cancer Surviv.* 2009;3(1):21-42, [PMID: 19165605]
Exclusion code: 5

Crotser CB, Dickerson SS. Women Receiving News of a Family BRCA1/2 Mutation: Messages of Fear and Empowerment. *J Nurs Scholarsh.* 2010;42(4):367-378, [PMID: 21091619]
Exclusion code: 3

Crum C, Drapkin R, Kindelberger DW. Lessons from BRCA: the tubal fimbria emerges as an origin for pelvic serous cancer. *Clin Med Res.* 2007;5(1):35-44, [PMID: 17456833]
Exclusion code: 2

Crump RJ, Fitzgerald RP, Legge M. 'Going-to-have-cancerness': a study of living with increased risk of BRCA1 and BRCA2 mutations for six South Island women. *J Prim Health Care.* 2010;2(4):311-317, [PMID: 21125072]
Exclusion code: 5

Cruzado JA, Perez-Segura P, Olivera H, et al. Necessity of psychological treatment in people with risk of hereditary cancer who initiate genetic counseling. Study of predictor variables. *Psicooncologia.* 2005;2(2-3):303-316.
Exclusion code: 8

Cruzado JA, Segura PP, Olivera H. Psychological impact and intervention needs in genetic counseling for women with hereditary breast cancer risk. *Psicooncologia.* 2007;4(2-3):465-482.
Exclusion code: 8

Cruzado JA, Segura PP, Rojo L, et al. Psychological impact of genetic testing evaluated by the multidimensional impact of cancer risk assessment (MICRA) questionnaire. A study of psychometric properties of MICRA. *Psicooncologia.* 2011;8(1):125-142.
Exclusion code: 8

Culler D, Grimes SJ, Acheson LS, Wiesner GL. Cancer genetics in primary care. *Prim. Care.* 2004;31(3):649-683, xi, [PMID: 15331253]
Exclusion code: 2

Culver J, Lowstuter K, Bowling L. Assessing breast cancer risk and BRCA1/2 carrier probability. *Breast Dis.* 2006;27:5-20, [PMID: 17917138]
Exclusion code: 5

Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. *JAMA.* 1999;281(23):2189-2197, [PMID: 10376571]
Exclusion code: 2

Cummings SR, Tice JA, Bauer S, et al. Prevention of breast cancer in postmenopausal women: Approaches to estimating and reducing risk. *J. Natl. Cancer Inst.* 2009;101(6):384-398, [PMID: 19276457]
Exclusion code: 8

Curtis MG. Comparative tolerability of first-generation selective estrogen receptor modulators in breast cancer treatment and prevention. *Drug Saf.* 2001;24(14):1039-1053, [PMID: 11735660]
Exclusion code: 2

Cusido M, Colome C, Rodriguez I, Fabregas R. [Oncologic risk counselling unit. Selection of risk groups and results]. *Med. Clin. (Barc).* 2009;132(20):779-782, [PMID: 19285318]
Exclusion code: 7

Cutuli B, Lesur A, Namer M, Kerbrat P. [Breast cancer chemoprevention. Rational, trials results and future]. *Bull. Cancer (Paris).* 2009;96(5):519-530, [PMID: 19467983]
Exclusion code: 7

Cuzick J. First results from the International Breast Cancer Intervention Study (IBIS-I): A randomised prevention trial. *Lancet.* 2002;360(9336):817-824, [PMID: 12243915]
Exclusion code: 2

Cuzick J, Powels T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. *Lancet.* 2003;361(9354):296-300, [PMID: 2559863]
Exclusion code: 2

Cypowyj C, Eisinger F, Huiart L, Sobol H, Morin M, Julian-Reynier C. Subjective interpretation of inconclusive BRCA1/2 cancer genetic test results and transmission of

information to the relatives. *Psychooncology.* 2009;18(2):209-215, [PMID: 19061202]
Exclusion code: 3

Cypowyj C, Eisinger F, Morin M, Mogoutov A, Sobol H, Julian-Reynier C. Information-seeking behaviour and psycho-social interactions during the genetic testing process. *Community Genet.* 2003;6(4):224-234, [PMID: 15331868]
Exclusion code: 8

Cyrus-David M, King J, Bevers T, Robinson E. Validity assessment of the Breast Cancer Risk Reduction Health Belief Scale. *Cancer.* 2009;115(21):4907-4916, [PMID: 19637346]
Exclusion code: 8

Cyrus-David MS. Knowledge and accuracy of perceived personal risk in underserved women who are at increased risk of breast cancer. *J. Cancer Educ.* 2010;25(4):617-623, [PMID: 20229073]
Exclusion code: 8

Dagan E, Gil S. BRCA1/2 Mutation Carriers: Psychological Distress and Ways of Coping. *J Psychosoc Oncol.* 2004;22(3):93-106.
Exclusion code: 5

d'Agincourt-Canning L. A gift or a yoke? Women's and men's responses to genetic risk information from BRCA1 and BRCA2 testing. *Clin. Genet.* 2006;70(6):462-472, [PMID: 17100990]
Exclusion code: 5

d'Agincourt-Canning L. Genetic testing for hereditary breast and ovarian cancer: Responsibility and choice. *Qual. Health Res.* 2006;16(1):97-118, [PMID: 16317179]
Exclusion code: 3

Daguet E, Malhaire C, Hardit C, et al. MR breast screening in patients with genetic mutation. *J. Radiol.* 2008;89(6):783-790, [PMID: 18641565]
Exclusion code: 7

Daly MB, Axilbund JE, Bryant E, et al. Genetic/familial high-risk assessment: breast and ovarian. *J Natl Compr Canc Netw.* 2006;4(2):156-176, [PMID: 16451772]
Exclusion code: 5

Daly MB, Axilbund JE, Buys S, et al. Genetic/familial high-risk assessment: breast and ovarian. *J Natl Compr Canc Netw.* 2010;8(5):562-594, [PMID: 20495085]
Exclusion code: 2

Daly PA. Genetic counselling in breast and colorectal cancer. *Ann. Oncol.* 2005;16 Suppl 2:ii163-169, [PMID: 15958450]
Exclusion code: 5

Dancyger C, Wiseman M, Jacobs C, Smith JA, Wallace M, Michie S. Communicating BRCA1/2 genetic test results within the family: A qualitative analysis. *Psychol Health.* 2011;26(8):1018-1035, [PMID: 21797732]
Exclusion code: 2

Danforth KN, Im TM, Whitlock EP. *Addendum to Screening for Ovarian Cancer: Evidence Update for the U.S. Preventive Services Task Force Reaffirmation Recommendation Statement.* Rockville, MD: Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services; 2012.
Exclusion code: 2

Dann RB, Kelley JL, Zorn KK. Strategies for ovarian cancer prevention. *Obstet. Gynecol. Clin. North Am.* 2007;34(4):667-686, viii, [PMID: 18061863]
Exclusion code: 5

Dapic V, Monteiro ANA. Functional implications of BRCA1 for early detection, prevention, and treatment of breast cancer. *Crit. Rev. Eukaryot. Gene Expr.* 2006;16(3):233-252, [PMID: 17073553]
Exclusion code: 5

Daum H, Sagi M, Pikarsky E, Pruss D, Hamburger T, Peretz T. [Prophylactic oophorectomy among carriers of BRCA1/2 mutations--demographic and pathologic data]. *Harefuah.* 2006;145(1):13-17, 79-80, [PMID: 16450717]
Exclusion code: 7

Surveillance of women at high risk of breast cancer: a tech brief (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32007000446
Exclusion code: 5

Davis S, Stewart S, Bloom J. Increasing the accuracy of perceived breast cancer risk: results from a randomized trial with Cancer Information Service callers. *Prev. Med.* 2004;39(1):64-73, [PMID: 15207987]
Exclusion code: 4

De Bock GH, Hesselink JW, Roorda C, et al. Model of care for women at increased risk of breast and ovarian cancer. *Maturitas.* 2012;71(1):3-5, [PMID: 22078659]
Exclusion code: 5

de Bock GH, Jacobi CE, Jonker MA, Nagelkerke NJD, van Houwelingen JC. A breast cancer prediction model. *Stat. Med.* 2005;24(10):1610-1612; author reply 1612, [PMID: 15880578]
Exclusion code: 5

de Bock GH, Vliet Vlieland TP, Hageman GC, Oosterwijk JC, Springer MP, Kievit J. The assessment of genetic risk of breast cancer: a set of GP guidelines. *Fam. Pract.* 1999;16(1):71-77, [PMID: 10321400]
Exclusion code: 2

de Hullu JA, Kets CM, Massuger LF, Ligtenberg ML, van Ham MA, Hoogerbrugge N. [Familial history of ovarian carcinoma: policy]. *Ned. Tijdschr. Geneeskd.* 2011;155:A2392, [PMID: 21262027]
Exclusion code: 7

De Leeuw JRJ, van Vliet MJ, Ausems MGEM. Predictors of choosing life-long screening or prophylactic surgery in women at high and moderate risk for breast and ovarian cancer. *Fam Cancer.* 2008;7(4):347-359, [PMID: 18338239]
Exclusion code: 8

de Silva D, Gilbert F, Needham G, Deans H, Turnpenny P, Haites N. Identification of women at high genetic risk of breast cancer through the National Health Service Breast Screening Programme (NHSBSP). *J. Med. Genet.* 1995;32(11):862-866, [PMID: 8592328]
Exclusion code: 2

Decarli A, Calza S, Masala G, Specchia C, Palli D, Gail MH. Gail model for prediction of absolute risk of invasive breast cancer: independent evaluation in the Florence-European Prospective Investigation Into Cancer and Nutrition cohort. *J. Natl. Cancer Inst.* 2006;98(23):1686-1693, [PMID: 17148770]
Exclusion code: 3

Decensi A, Maisonneuve P, Rotmensz N, et al. Effect of tamoxifen on venous thromboembolic events in a breast cancer prevention trial. *Circulation.* 2005;111(5):650-656, [PMID: 15699284]
Exclusion code: 3

Decruyenaere M, Evers-Kiebooms G, Denayer L, et al. Predictive testing for hereditary breast and ovarian cancer: A psychological framework for pre-test counselling. *Eur. J. Hum. Genet.* 2000;8(2):130-136, [PMID: 10757645]
Exclusion code: 6

Deffieux X, Touboul C, Uzan C, et al. [Chemoprevention and prophylactic surgery in ovarian carcinoma]. *J. Gynecol. Obstet. Biol. Reprod. (Paris).* 2007;36(8):756-763, [PMID: 17719183]
Exclusion code: 7

DeMarco TA, McKinnon WC. Life after BRCA1/2 testing: family communication and support issues. *Breast Dis.* 2007;27:127-136, [PMID: 17917144]
Exclusion code: 5

DeMarco TA, Peshkin BN, Mars BD, Tercyak KP. Patient satisfaction with cancer genetic counseling: a psychometric analysis of the Genetic Counseling Satisfaction Scale. *J Genet Couns.* 2004;13(4):293-304, [PMID: 19736695]
Exclusion code: 8

den Heijer M, Seynaeve C, Timman R, et al. Body image and psychological distress after

prophylactic mastectomy and breast reconstruction in genetically predisposed women: a prospective long-term follow-up study. *Eur. J. Cancer.* 2012;48(9):1263-1268, [PMID: 22105017]
Exclusion code: 3

den Heijer M, Seynaeve C, Vanheusden K, et al. Psychological distress in women at risk for hereditary breast cancer: The role of family communication and perceived social support. *Psychooncology.* 2011;20(12):1317-1323, [PMID: 20925135]
Exclusion code: 8

den Heijer M, Vos J, Seynaeve C, et al. The impact of social and personal resources on psychological distress in women at risk for hereditary breast cancer. *Psychooncology.* 2012;21(2):153-160, [PMID: 22271535]
Exclusion code: 8

Dent R, Warner E. Screening for hereditary breast cancer. *Semin. Oncol.* 2007;34(5):392-400, [PMID: 17920893]
Exclusion code: 5

Derogatis LR, Melisaratos N. The Brief Symptom Inventory: An introductory report. *Psychol. Med.* 1983;13(3):595-605, [PMID: 6622612]
Exclusion code: 2

DerSimonian R, Laird NM. Meta-analysis in clinical trials. *Control. Clin. Trials.* 1986;7:177-188, [PMID: 12111919]
Exclusion code: 2

Devilee P, Rookus MA. A tiny step closer to personalized risk prediction for breast cancer. *N. Engl. J. Med.* 2010;362(11):1043-1045, [PMID: 20237351]
Exclusion code: 5

Dhingra K. Antiestrogens--tamoxifen, SERMs and beyond. *Invest. New Drugs.* 1999;7(3):285-311, [PMID: 10665480]
Exclusion code: 2

Di Pietro ML, Giuli A, Spagnolo AG. Ethical implications of predictive DNA testing for hereditary breast cancer. *Ann. Oncol.* 2004;15 Suppl 1:I65-I70, [PMID: 15280191]
Exclusion code: 5

DiCastro M, Frydman M, Friedman I, et al. Genetic counseling in hereditary breast/ovarian cancer in Israel: Psychosocial impact and retention of genetic information. *Am. J. Med. Genet.* 2002;111(2):147-151, [PMID: 12210341]
Exclusion code: 3

Dillard AJ, Ubel PA, Smith DM, et al. The distinct role of comparative risk perceptions in a breast cancer prevention program. *Ann. Behav. Med.* 2011;42(2):262-268, [PMID: 21698518]
Exclusion code: 8

DiLorenzo TA, Schnur J, Montgomery GH, Erblich J, Winkel G, Bovbjerg DH. A model of disease-specific worry in heritable disease: the influence of family history, perceived risk and worry about other illnesses. *J. Behav. Med.* 2006;29(1):37-49, [PMID: 16470344]
Exclusion code: 5

Dite GS, Whittemore AS, Knight JA, et al. Increased cancer risks for relatives of very early-onset breast cancer cases with and without BRCA1 and BRCA2 mutations. *Br. J. Cancer.* 2010;103(7):1103-1108, [PMID: 20877337]
Exclusion code: 3

Dohany L, Gustafson S, Ducaine W, Zakalik D. Psychological Distress with Direct-to-Consumer Genetic Testing: A Case Report of an Unexpected Positive Test Result. *J Genet Couns.* 2012:1-3, [PMID: 22271377]
Exclusion code: 5

Dolbeault S, Flahault C, Stoppa-Lyonnet D, Bredart A. Communication in genetic counselling for breast/ovarian cancer. *Recent Results Cancer Res.* 2006;168:23-36, [PMID: 17073189]
Exclusion code: 5

Dombernowsky SL, Weischer M, Freiberg JJ, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Missense polymorphisms in BRCA1 and BRCA2 and risk of breast and ovarian cancer.

Cancer Epidemiol. Biomarkers Prev. 2009;18(8):2339-2342, [PMID: 19661094]
Exclusion code: 5

Domchek SM, Armstrong K, Weber BL. Clinical management of BRCA1 and BRCA2 mutation carriers. *Nat Clin Pract Oncol.* 2006;3(1):2-3, [PMID: 16407858]
Exclusion code: 5

Domchek SM, Friebel TM, Neuhausen SL, et al. Mortality after bilateral salpingo-oophorectomy in BRCA1 and BRCA2 mutation carriers: a prospective cohort study. *Lancet Oncol.* 2006;7(3):223-229, [PMID: 16510331]
Exclusion code: 2

Domchek SM, Rebbeck TR. Prophylactic oophorectomy in women at increased cancer risk. *Curr. Opin. Obstet. Gynecol.* 2007;19(1):27-30, [PMID: 17218848]
Exclusion code: 5

Domchek SM, Rebbeck TR. Preventive surgery is associated with reduced cancer risk and mortality in women with BRCA1 and BRCA2 mutations. *LDI Issue Brief.* 2010;16(2):1-4, [PMID: 21545057]
Exclusion code: 5

Domchek SM, Stopfer JE, Rebbeck TR. Bilateral risk-reducing oophorectomy in BRCA1 and BRCA2 mutation carriers. *J Natl Compr Canc Netw.* 2006;4(2):177-182, [PMID: 16451773]
Exclusion code: 5

Domchek SM, Weber BL. Clinical management of BRCA1 and BRCA2 mutation carriers. *Oncogene.* 2006;25(43):5825-5831, [PMID: 16998496]
Exclusion code: 5

Dørum A, Kristensen GB, Abeler VM, Tropé CG, Møller P. Early detection of familial ovarian cancer. *Eur. J. Cancer.* 1996;32(10):1645-1651, [PMID: 8983269]
Exclusion code: 6

Dorval M, Bouchard K, Maunsell E, et al. Health behaviors and psychological distress in women initiating BRCA1/2 genetic testing: comparison with control population. *J Genet Couns.* 2008;17(4):314-326, [PMID: 18481164]
Exclusion code: 8

Dorval M, Drolet M, LeBlanc M, Maunsell E, Dugas MJ, Simard J. Using the Impact of Event Scale to evaluate distress in the context of genetic testing for breast cancer susceptibility. *Psychol. Rep.* 2006;98(3):873-881, [PMID: 16933689]
Exclusion code: 3

Dorval M, Gauthier G, Maunsell E, et al. No evidence of false reassurance among women with an inconclusive BRCA1/2 genetic test result. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(12):2862-2867, [PMID: 16365001]
Exclusion code: 3

Dorval M, Nogués C, Berthet P, et al. Breast and ovarian cancer screening of non-carriers from BRCA1/2 mutation-positive families: 2-year follow-up of cohorts from France and Quebec. *Eur. J. Hum. Genet.* 2011;19(5):494-499, [PMID: 21248744]
Exclusion code: 8

Dougall AL, Smith AW, Somers TJ, Posluszny DM, Rubinstein WS, Baum A. Coping with genetic testing for breast cancer susceptibility. *Psychosom. Med.* 2009;71(1):98-105, [PMID: 19124622]
Exclusion code: 3

Doughty Rice C, Ruschman JG, Martin LJ, Manders JB, Miller E. Retrospective comparison of patient outcomes after in-person and telephone results disclosure counseling for BRCA1/2 genetic testing. *Fam Cancer.* 2010;9(2):203-212, [PMID: 20473602]
Exclusion code: 3

Douglas HA, Hamilton RJ, Grubs RE. The effect of BRCA gene testing on family relationships: A thematic analysis of qualitative interviews. *J Genet Couns.* 2009;18(5):418-435, [PMID: 19479365]
Exclusion code: 3

Dowdy SC, Stefanek M, Hartmann LC. Surgical risk reduction: prophylactic salpingo-oophorectomy and prophylactic mastectomy. *Am. J. Obstet. Gynecol.* 2004;191(4):1113-1123, [PMID: 15507929]
Exclusion code: 5

Drazan L, Vesely J, Hyza P, Kubek T, Foretova L, Coufal O. [Surgical prevention of breast carcinoma in patients with hereditary risk]. *Klin.* 2012;25 Suppl:S78-83, [PMID: 22920212]
Exclusion code: 7

Dudbridge F, Fletcher O, Walker K, et al. Estimating causal effects of genetic risk variants for breast cancer using marker data from bilateral and familial cases. *Cancer Epidemiol. Biomarkers Prev.* 2012;21(2):262-272, [PMID: 22028405]
Exclusion code: 8

Duffy SW, Tabar L, Olsen AH, et al. Absolute numbers of lives saved and overdiagnosis in breast cancer screening, from a randomized trial and from the Breast Screening Programme in England. *J. Med. Screen.* 2010;17(1):25-30, [PMID: 20356942]
Exclusion code: 2

Dumitrescu RG, Cotarla I. Understanding breast cancer risk -- where do we stand in 2005? *J Cell Mol Med.* 2005;9(1):208-221, [PMID: 15784178]
Exclusion code: 5

Effectiveness of magnetic resonance imaging (MRI) screening for women at high risk of breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32007000624
Exclusion code: 5

Dutil J, Colon-Colon JL, Matta JL, Sutphen R, Echenique M. Identification of the prevalent BRCA1 and BRCA2 mutations in the female population of Puerto Rico. *Cancer Genet.* 2012;205(5):242-248, [PMID: 22682623]
Exclusion code: 3

Dyer C. US judge rules that patents on breast cancer genes are unlawful. *BMJ.* 2010;340:c1870, [PMID: 20360219]
Exclusion code: 5

Dyer C. US Supreme Court is asked to rule on validity of patents on BRCA1 and BRCA2 genes. *BMJ.* 2012;345:e6624, [PMID: 23033368]
Exclusion code: 2

Easton DF, Bishop DT, Ford D, Crockford GP, The Breast Cancer Linkage Consortium. Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. *Am J Hum Genet.* 1993;52:678-701, [PMID: 8460634]
Exclusion code: 2

Easton DF, Ford D, Bishop DT, The Breast Cancer Linkage Consortium. Breast and ovarian cancer incidence in BRCA1-mutation carriers. *Am J Hum Genet.* 1995;56:265-271, [PMID: 7825587]
Exclusion code: 2

Easton DF, Hopper JL, Thomas DC, et al. Breast cancer risks for BRCA1/2 carriers. *Science.* 2004;306(5705):2187-2191; author reply 2187-2191, [PMID: 15622557]
Exclusion code: 5

Eccles D. Guidelines for a genetic risk based approach to advising women with a family history of breast cancer. *J. Med. Genet.* 2000;37:203-209, [PMID: 10699057]
Exclusion code: 2

Eccles D, Gerty S, Simmonds P, et al. Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH): study protocol. *BMC Cancer.* 2007;7:160, [PMID: 17697367]
Exclusion code: 5

Edlich RF, Cross CL, Wack CA, Chase ME, Lin KY, Long WB, 3rd. Breast cancer and ovarian cancer genetics: an update. *J. Environ. Pathol. Toxicol. Oncol.* 2008;27(4):245-256, [PMID: 19105530]
Exclusion code: 5

Edlich RF, Winters KL, Lin KY. Breast cancer and ovarian cancer genetics. *J. Long. Term Eff. Med. Implants.* 2005;15(5):533-545, [PMID: 16218901]
Exclusion code: 5

Edwards A, Gray J, Clarke A, et al. Interventions to improve risk communication in clinical genetics: systematic review. *Patient Educ. Couns.* 2008;71:4-25, [PMID: 18207694]
Exclusion code: 8

Personalised risk communication for informed decision making about taking screening tests. 2011. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=coch&AN=00075320-100000000-01239.
Exclusion code: 5

Edwards QT, Seibert D. Pre- and posttest evaluation of a breast cancer risk assessment program for nurse practitioners. *J. Am. Acad. Nurse Pract.* 2010;22(7):376-381, [PMID: 20590960]
Exclusion code: 8

Edwards QT, Seibert D, Maradiegue A, et al. Breast cancer and the family tree. An issue for all practice settings. *Adv. Nurse Pract.* 2007;15(5):34-41; quiz 41-32, [PMID: 20000211]
Exclusion code: 5

Edwards TA, Thompson HS, Kwate NOA, et al. Association between temporal orientation and attitudes about BRCA1/2 testing among women of African descent with family histories of breast cancer. *Patient Educ. Couns.* 2008;72(2):276-282, [PMID: 18479882]
Exclusion code: 3

Eerola H, Vahteristo P, Sarantaus L, et al. Survival of breast cancer patients in BRCA1, BRCA2, and non-BRCA1/2 breast cancer families: A relative survival analysis from Finland. *Int. J. Cancer.* 2001;93(3):368-372, [PMID: 11433401]
Exclusion code: 6

Einbeigi Z, Bergman A, Kindblom L-G, et al. A founder mutation of the BRCA1 gene in Western Sweden associated with a high incidence of breast and ovarian cancer. *Eur. J. Cancer.* 2001;37(15):1904-1909, [PMID: 11576847]
Exclusion code: 2

Eisen A, Lubinski J, Klijn J, et al. Breast cancer risk following bilateral oophorectomy in BRCA1 and BRCA2 mutation carriers: an international case-control study. *J. Clin. Oncol.* 2005;23(30):7491-7496, [PMID: 16234515]
Exclusion code: 3

Eisen A, Rebbeck TR, Wood WC, Weber BL. Prophylactic surgery in women with a hereditary predisposition to breast and ovarian cancer. *J. Clin. Oncol.* 2000;18(9):1980-1995, [PMID: 10784640]
Exclusion code: 5

Eisinger F. Prophylactic mastectomy: ethical issues. *Br. Med. Bull.* 2007;81-82:7-19, [PMID: 17409120]
Exclusion code: 5

Eisinger F, Horsman DE. Genetic risk assessment and BRCA mutation testing. *Ann. Intern. Med.* 2006;144(5):376; author reply 376-377, [PMID: 16520482]
Exclusion code: 5

Eisinger F, Huiart L, Sobol H. The choice of bilateral prophylactic mastectomy. *J. Clin. Oncol.* 2005;23(6):1330-1331; author reply 1331-1332, [PMID: 15718344]
Exclusion code: 5

Eisinger F, Reynier CJ, Chabal F, Luquet C, Moatti JP, Sobol H. Acceptable strategies for dealing with hereditary breast/ovarian cancer risk. *J. Natl. Cancer Inst.* 1997;89(10):731, [PMID: 9168189]
Exclusion code: 3

Ellberg C, Jonsson G, Olsson H. Can a phenotype for recessive inheritance in breast cancer be defined? *Fam Cancer.* 2010;9(4):525-530, [PMID: 20549370]
Exclusion code: 3

Ellington L, Kelly KM, Reblin M, Latimer S, Roter D. Communication in genetic counseling: cognitive and emotional processing. *Health Commun.* 2011;26(7):667-675, [PMID: 21660793]
Exclusion code: 2

Ellington L, Maxwel A, Baty BJ, Roter D, Dudley WN, Kinney AY. Genetic counseling communication with an African American BRCA1 kindred. *Soc. Sci. Med.* 2007;64(3):724-734, [PMID: 17081667]
Exclusion code: 8

Elwyn G, Iredale R, Gray J. Reactions of GPs to a triage-controlled referral system for cancer genetics. *Fam. Pract.* 2002;19(1):65-71, [PMID: 11818352]
Exclusion code: 2

Emery J. The GRAIDS Trial: the development and evaluation of computer decision support for cancer genetic risk assessment in primary care. *Ann. Hum. Biol.* 2005;32(2):218-227, [PMID: 16096220]
Exclusion code: 5

Emery J, Walton R, Coulson A, Glasspool D, Ziebland S, Fox J. Computer support for recording and interpreting family histories of breast and ovarian cancer in primary care (RAGs): qualitative evaluation with simulated patients. *BMJ.* 1999;319(7201):32-36, [PMID: 10390458]
Exclusion code: 2

Emery J, Walton R, Murphy M, et al. Computer support for interpreting family histories of breast and ovarian cancer in primary care: comparative study with simulated cases. *BMJ.* 2000;321(7252):28-32, [PMID: 10875832]
Exclusion code: 2

Eng-Wong J, Orzano-Birgani J, Chow CK, et al. Effect of raloxifene on mammographic density and breast magnetic resonance imaging in premenopausal women at increased risk for breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 2008;17(7):1696-1701, [PMID: 18583470]
Exclusion code: 8

Ensenauer RE, Michels VV, Reinke SS. Genetic testing: practical, ethical, and counseling considerations. *Mayo Clin. Proc.* 2005;80(1):63-73, [PMID: 15667031]
Exclusion code: 5

Erblich J, Brown K, Kim Y, Valdimarsdottir HB, Livingston BE, Bovbjerg DH. Development and validation of a Breast Cancer Genetic Counseling Knowledge Questionnaire. *Patient Educ. Couns.* 2005;56(2):182-191, [PMID: 15653247]
Exclusion code: 8

Escobar PF, Starks DC, Fader AN, Barber M, Rojas-Espalliat L. Single-port risk-reducing salpingo-oophorectomy with and without hysterectomy: surgical outcomes and learning curve analysis. *Gynecol. Oncol.* 2010;119(1):43-47, [PMID: 20579712]
Exclusion code: 3

Esplen MJ, Stuckless N, Hunter J, et al. The BRCA Self-Concept Scale: A new instrument to measure self-concept in BRCA 1/2 mutation carriers. *Psychooncology.* 2009;18(11):1216-1229, [PMID: 19180529]
Exclusion code: 8

Esserman L, Kaklamani V. Lessons learned from genetic testing. *JAMA.* 2010;304(9):1011-1012, [PMID: 20810382]
Exclusion code: 5

Esserman LJ, Ozanne EM, Dowsett M, Slingerland JM. Tamoxifen may prevent both ER+ and ER- breast cancers and select for ER- carcinogenesis: an alternative hypothesis. *Breast Cancer Res.* 2005;7(6):R1153-1158, [PMID: 16457695]
Exclusion code: 5

Essink-Bot ML, Rijnsburger AJ, van Dooren S, de Koning HJ, Seynaeve C. Women's acceptance of MRI in breast cancer surveillance because of a familial or genetic predisposition. *Breast.* 2006;15(5):673-676, [PMID: 16556497]
Exclusion code: 8

Esteban Cardenosa E, Bolufer Gilabert P, de Juan Jimenez I, et al. Relationship of BRCA1

and BRCA2 mutations with cancer burden in the family and tumor incidence. *Fam Cancer.* 2010;9(3):291-295, [PMID: 20711702]
Exclusion code: 8

Esteves V, Thuler L, Amendola L, et al. Prevalence of BRCA1 and BRCA2 gene mutations in families with medium and high risk of breast and ovarian cancer in Brazil. *Braz. J. Med. Biol. Res.* 2009;42(5):453-457, [PMID: 19377795]
Exclusion code: 3

Etchegary H, Miller F, deLaat S, Wilson B, Carroll J, Cappelli M. Decision-making about inherited cancer risk: Exploring dimensions of genetic responsibility. *J Genet Couns.* 2009;18(3):252-264, [PMID: 19294336]
Exclusion code: 2

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group, Berg AO, Armstrong K, et al. Recommendations from the EGAPP Working Group: Can tumor gene expression profiling improve outcomes in patients with breast cancer? *Genet Med.* 2009;11(1):66-73, [PMID: 19125125]
Exclusion code: 2

Evans DG, Gaarenstroom KN, Stirling D, et al. Screening for familial ovarian cancer: Poor survival of BRCA1/2 related cancers. *J. Med. Genet.* 2009;46(9):593-597, [PMID: 18413372]
Exclusion code: 8

Evans DG, Howell A. Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: more trouble with phenocopies. *J. Clin. Oncol.* 2012;30(10):1142-1143; author reply 1143-1144, [PMID: 22370324]
Exclusion code: 5

Evans DGR, Eccles DM, Rahman N, et al. A new scoring system for the chances of identifying a BRCA 1/2 mutation outperforms existing models including BRCAPRO. *J. Med. Genet.* 2004;41:474-480, [PMID: 15173236]
Exclusion code: 2

Evans DGR, Howell A. Breast cancer risk-assessment models. *Breast Cancer Res.* 2007;9(5):213, [PMID: 17888188]
Exclusion code: 5

Evans DGR, Young K, Bulman M, Shenton A, Wallace A, Lalloo F. Probability of BRCA1/2 mutation varies with ovarian histology: results from screening 442 ovarian cancer families. *Clin. Genet.* 2008;73(4):338-345, [PMID: 18312450]
Exclusion code: 2

Evans JP, Skrzynia C, Susswein L, Harlan M. Genetics and the young woman with breast cancer. *Breast Dis.* 2005;23:17-29, [PMID: 16823163]
Exclusion code: 3

Evans KD. Review of the US preventive services task force's statement on screening for breast cancer. *JDMS.* 2010;26(1):19-23.
Exclusion code: 5

Evans WP, Lee CH, Monsees BS, Monticciolo DL, Rebner M. U.S. Preventive Services Task Force: the unbalanced view. *Radiology.* 2010;257(1):297; author reply 297-298, [PMID: 20851949]
Exclusion code: 5

Fabian CJ, Kimler BF. Use of biomarkers for breast cancer risk assessment and prevention. *J. Steroid Biochem. Mol. Biol.* 2007;106(1-5):31-39, [PMID: 17596932]
Exclusion code: 4

Fabian CJ, Kimler BF, Mayo MS, Khan SA. Breast-tissue sampling for risk assessment and prevention. *Endocr Relat Cancer.* 2005;12(2):185-213, [PMID: 15947097]
Exclusion code: 4

Fackenthal JD, Olopade OI. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. *Nat Rev Cancer.* 2007;7(12):937-948, [PMID: 18034184]
Exclusion code: 5

Fackenthal JD, Zhang J, Zhang B, et al. High prevalence of BRCA1 and BRCA2 mutations in

unselected Nigerian breast cancer patients. *Int. J. Cancer.* 2012;131(5):1114-1123, [PMID: 22034289]
Exclusion code: 3

Fakkert IE, Jansen L, Meijer K, et al. Breast cancer screening in BRCA1 and BRCA2 mutation carriers after risk reducing salpingo-oophorectomy. *Breast Cancer Res. Treat.* 2011;129(1):157-164, [PMID: 21373873]
Exclusion code: 3

Fallowfield L, Fleissig A, Barrett J, et al. Awareness of ovarian cancer risk factors, beliefs and attitudes towards screening: baseline survey of 21,715 women participating in the UK Collaborative Trial of Ovarian Cancer Screening. *Br. J. Cancer.* 2010;103(4):454-461, [PMID: 20648018]
Exclusion code: 8

Fanale D, Amodeo V, Corsini LR, Rizzo S, Bazan V, Russo A. Breast cancer genome-wide association studies: There is strength in numbers. *Oncogene.* 2012;31(17):2121-2128, [PMID: 21996731]
Exclusion code: 2

Fang CY, Cherry C, Devarajan K, Li T, Malick J, Daly MB. A prospective study of quality of life among women undergoing risk-reducing salpingo-oophorectomy versus gynecologic screening for ovarian cancer. *Gynecol. Oncol.* 2009;112(3):594-600, [PMID: 19141360]
Exclusion code: 3

Farooq A, Naveed AK, Azeem Z, Ahmad T. Breast and ovarian cancer risk due to prevalence of BRCA1 and BRCA2 variants in pakistani population: A Pakistani database report. *J Oncol.* 2011, [PMID: 21559243]
Exclusion code: 3

Fasching PA, Bani MR, Nestle-Kramling C, et al. Evaluation of mathematical models for breast cancer risk assessment in routine clinical use. *Eur. J. Cancer Prev.* 2007;16(3):216-224, [PMID: 17415092]
Exclusion code: 8

Federico M, Maiorana A, Mangone L, et al. Identification of families with hereditary breast and ovarian cancer for clinical and mammographic surveillance: The Modena Study Group proposal. *Breast Cancer Res. Treat.* 1999;55(3):213-221, [PMID: 10517166]
Exclusion code: 6

Fialho AM, Chakrabarty AM. Law-medicine interfacing: patenting of human genes and mutations. *Recent Pat DNA Gene Seq.* 2011;5(2):81-85, [PMID: 21574951]
Exclusion code: 5

Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 Mutation. *JAMA.* 2006;296(2):185-192, [PMID: 16835424]
Exclusion code: 3

Finch A, Metcalfe K, Lui J, et al. Breast and ovarian cancer risk perception after prophylactic salpingo-oophorectomy due to an inherited mutation in the BRCA1 or BRCA2 gene. *Clin. Genet.* 2009;75(3):220-224, [PMID: 19263514]
Exclusion code: 3

Finlay E, Stopfer JE, Burlingame E, et al. Factors determining dissemination of results and uptake of genetic testing in families with known BRCA1/2 mutations. *Genet Test.* 2008;12(1):81-91, [PMID: 18373407]
Exclusion code: 2

Firth C, Jacobs C, Evison M, Pichert G, Izatt L, Hunter MS. Novel one-stop multidisciplinary follow-up clinic for BRCA1/2 carriers: patient satisfaction and decision making. *Psychooncology.* 2011;20(12):1301-1308, [PMID: 22114045]
Exclusion code: 8

Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J. Natl. Cancer Inst.* 1998;90(18):1371-1388, [PMID: 9747868]
Exclusion code: 2

Fishman DA, Cohen L, Blank SV, et al. The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. *Am. J. Obstet. Gynecol.* 2005;192(4):1214-1222, [PMID: 15846205]
Exclusion code: 3

The clinical effectiveness and cost-effectiveness of genotyping for CYP2D6 for the management of women with breast cancer treated with tamoxifen: a systematic review (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000281
Exclusion code: 3

Fletcher KE, Clemow L, Peterson BA, Lemon SC, Estabrook B, Zapka JG. A path analysis of factors associated with distress among first-degree female relatives of women with breast cancer diagnosis. *Health Psychol.* 2006;25(3):413-424, [PMID: 16719614]
Exclusion code: 4

Fletcher SW. Screening for breast cancer. 2011; http://www.uptodate.com/contents/screening-for-breast-cancer?source=search_result&search=breast+cancer+screening&selectedTitle=1%7E34#H22. Accessed 09 Nov, 2011
Exclusion code: 2

Force USPST. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: recommendation statement. *Ann. Intern. Med.* 2005;143(5):355-361, [PMID: 16144894]
Exclusion code: 2

Ford D, Easton DF. The genetics of breast and ovarian cancer. *Br. J. Cancer.* 1995;72(4):805-812, [PMID: 7547224]
Exclusion code: 2

Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. *Lancet.* 1994;343(8899):692-695, [PMID: 7907678]
Exclusion code: 2

Ford ME, Alford SH, Britton D, McClary B, Gordon HS. Factors influencing perceptions of breast cancer genetic counseling among women in an urban health care system. *J Genet Couns.* 2007;16(6):735-753, [PMID: 17701328]
Exclusion code: 8

Forman AD, Hall MJ. Influence of race/ethnicity on genetic counseling and testing for hereditary breast and ovarian cancer. *Breast J.* 2009;15 Suppl 1:S56-62, [PMID: 19775331]
Exclusion code: 8

Foster C, Evans DGR, Eeles R, et al. Non-uptake of predictive genetic testing for BRCA1/2 among relatives of known carriers: attributes, cancer worry, and barriers to testing in a multicenter clinical cohort. *Genet Test.* 2004;8(1):23-29, [PMID: 15140371]
Exclusion code: 8

Foulkes WD, Chappuis PO, Wong N, et al. Primary node negative breast cancer in BRCA1 mutation carriers has a poor outcome. *Ann. Oncol.* 2000;11(3):307-313, [PMID: 10811497]
Exclusion code: 6

Foulkes WD, Wong N, Brunet JS, et al. Germline BRCA1 mutation is an adverse prognostic factor in Ashkenazi Jewish women with breast cancer. *Clin. Cancer Res.* 1997;3(12 I):2465-2469, [PMID: 9815648]
Exclusion code: 6

Freedman AN, Seminara D, Gail MH, et al. Cancer risk prediction models: a workshop on development, evaluation, and application. *J. Natl. Cancer Inst.* 2005;97(10):715-723, [PMID: 15900041]
Exclusion code: 2

Freedman AN, Wideroff L, Olson L, et al. US physicians' attitudes toward genetic testing for cancer susceptibility. *Am. J. Med. Genet.* 2003;120 A(1):63-71, [PMID: 12794694]
Exclusion code: 3

Friebel TM, Domchek SM, Neuhausen SL, et al. Bilateral prophylactic oophorectomy and bilateral prophylactic mastectomy in a prospective cohort of unaffected BRCA1 and

BRCA2 mutation carriers. *Clin Breast Cancer.* 2007;7(11):875-882, [PMID: 18269778]
Exclusion code: 8

Friedenson B. Assessing and managing breast cancer risk: clinical tools for advising patients. *MedGenMed.* 2004;6(1):8, [PMID: 15208521]
Exclusion code: 5

Friedman LC. Psychological impact of receiving negative BRCA1 mutation test results in Ashkenazim. *Genet Med.* 1999;1(3):74-79, [PMID: 11336456]
Exclusion code: 3

Friedman LC, Kramer RM. Reproductive issues for women with BRCA mutations. *J. Natl. Cancer Inst.* 2005;Monographs.(34):83-86, [PMID: 15784831]
Exclusion code: 5

Fries MH, Holt C, Carpenter I, et al. Guidelines for evaluation of patients at risk for inherited breast and ovarian cancer: recommendations of the Department of Defense Familial Breast/Ovarian Cancer Research Project. *Mil. Med.* 2002;167(2):93-98, [PMID: 11873549]
Exclusion code: 2

Frost CJ, Venne V, Cunningham D, Gerritsen-McKane R. Decision making with uncertain information: learning from women in a high risk breast cancer clinic. *J Genet Couns.* 2004;13(3):221-236, [PMID: 15604633]
Exclusion code: 5

Frost MH, Schaid DJ, Sellers TA, et al. Long-term satisfaction and psychological and social function following bilateral prophylactic mastectomy. *JAMA.* 2000;284(3):319-324, [PMID: 10891963]
Exclusion code: 5

Fry A, Busby-Earle C, Rush R, Cull A. Prophylactic oophorectomy versus screening: psychosocial outcomes in women at increased risk of ovarian cancer. *Psychooncology.* 2001;10(3):231-241, [PMID: 11351375]
Exclusion code: 5

Fuller S, Anderson RC. Adjustment issues related to bilateral prophylactic mastectomy in women at elevated risk of developing breast cancer. *Plast. Surg. Nurs.* 2006;26(2):60-65; quiz 66-67, [PMID: 16783176]
Exclusion code: 5

Gaarenstroom KN, Van Der Hiel B, Tollenaar RAEM, et al. Efficacy of screening women at high risk of hereditary ovarian cancer: Results of an 11-year cohort study. *Int J Gynecol Cancer.* 2006;16(SUPPL. 1):54-59, [PMID: 16515568]
Exclusion code: 8

Gabrovska PN, Smith RA, O'Leary G, Haupt LM, Griffiths LR. Investigation of the 1758G>C and 2880A>G variants within the NCOA3 gene in a breast cancer affected Australian population. *Gene.* 2011;482(1-2):68-72, [PMID: 21601620]
Exclusion code: 3

Gadzicki D, Wingen LU, Teige B, et al. Communicating BRCA1 and BRCA2 genetic test results *J. Clin. Oncol.* 2006;24(18):2969-2970, [PMID: 16782939]
Exclusion code: 5

Gail M, Brinton L, Byar D, Corle D, Green S, Schairer C. Projecting individualized probablities of developing breast cancer for white females who are being examined annually. *J. Natl. Cancer Inst.* 1989;81(24):1879-1886, [PMID: 2593165]
Exclusion code: 2

Gail MH. Discriminatory accuracy from single-nucleotide polymorphisms in models to predict breast cancer risk. *J. Natl. Cancer Inst.* 2008;100(14):1037-1041, [PMID: 18612136]
Exclusion code: 8

Gail MH, Anderson WF, Garcia-Closas M, Sherman ME. Absolute risk models for subtypes of breast cancer. *J. Natl. Cancer Inst.* 2007;99(22):1657-1659, [PMID: 18000214]
Exclusion code: 2

Gail MH, Costantino JP, Pee D, et al. Projecting individualized absolute invasive breast cancer risk in African American women. *J. Natl.*

Cancer Inst. 2007;99(23):1782-1792, [PMID: 18042936]
Exclusion code: 3

Gail MH, Mai PL. Comparing breast cancer risk assessment models. *J. Natl. Cancer Inst.* 2010;102(10):665-668, [PMID: 20427429]
Exclusion code: 2

Gallagher KM, Updegraff JA, Rothman AJ, Sims L. Perceived susceptibility to breast cancer moderates the effect of gain- and loss-framed messages on use of screening mammography. *Health Psychol.* 2011;30(2):145-152, [PMID: 21401248]
Exclusion code: 8

Gammon AD, Rothwell E, Simmons R, et al. Awareness and preferences regarding BRCA1/2 genetic counseling and testing among Latinas and non-Latina White women at increased risk for hereditary breast and ovarian cancer. *J Genet Couns.* 2011;20(6):625-638, [PMID: 21691939]
Exclusion code: 3

Ganz PA. Breast cancer, menopause, and long-term survivorship: critical issues for the 21st century. *Am. J. Med.* 2005;118 Suppl 12B:136-141, [PMID: 16414339]
Exclusion code: 3

Garber JE, Hartman AR. Prophylactic oophorectomy and hormone replacement therapy: Protection at what price? *J. Clin. Oncol.* 2004;22(6):978-980, [PMID: 14981100]
Exclusion code: 5

Geiger AM, Yu O, Herrinton LJ, et al. A population-based study of bilateral prophylactic mastectomy efficacy in women at elevated risk for breast cancer in community practices. *Arch. Intern. Med.* 2005;165(5):516-520, [PMID: 15767526]
Exclusion code: 3

Geirdal AO, Dahl AA. The relationship between psychological distress and personality in women from families with familial breast/ovarian or hereditary non-polyposis colorectal cancer in the absence of demonstrated mutations. *J Genet Couns.* 2008;17(4):384-393, [PMID: 18607701]
Exclusion code: 8

Gerdes A-MA, Ejlertsen B. [Inherited breast and ovarian cancers]. *Ugeskr. Laeger.* 2006;168(24):2367-2369, [PMID: 16822422]
Exclusion code: 7

Gershoni-Baruch R, Dagan E, Fried G, et al. Significantly lower rates of BRCA1/BRCA2 founder mutations in Ashkenazi women with sporadic compared with familial early onset breast cancer. *Eur. J. Cancer.* 2000;36(8):983-986, [PMID: 10885601]
Exclusion code: 2

Gevensleben H, Serce N, Buttner R. [Hereditary breast and ovarian cancers]. *Pathologe.* 2010;31(6):438-444, [PMID: 20848107]
Exclusion code: 7

Gibbon S. Re-examining geneticization: family trees in breast cancer genetics. *Sci Cult.* 2002;11(4):429-457, [PMID: 15971366]
Exclusion code: 5

Gil F, Méndez I, Sirgo A, Llort G, Blanco I, Cortés-Funes H. Perception of breast cancer risk and surveillance behaviours of women with family history of breast cancer: A brief report on a Spanish cohort. *Psychooncology.* 2003;12(8):821-827, [PMID: 14681955]
Exclusion code: 8

Gil FL, Costa G. Psychological aspects of risk reduction surgery (mastectomy and ooforectomy). *Psicooncologia.* 2005;2(2-3):317-328.
Exclusion code: 5

Gilpin CA, Carson N, Hunter AG. A preliminary validation of a family history assessment form to select women at risk for breast or ovarian cancer for referral to a genetics center. *Clin. Genet.* 2000;58(4):299-308, [PMID: 11076055]
Exclusion code: 2

Giuliano AE, Boolbol S, Degnim A, Kuerer H, Leitch AM, Morrow M. Society of Surgical Oncology: position statement on prophylactic mastectomy. Approved by the Society of Surgical Oncology Executive Council, March

2007. *Ann. Surg. Oncol.* 2007;14(9):2425-2427, [PMID: 17597344]
Exclusion code: 5

Gjorgov NA. Breast cancer risk assessments to barrier contraception exposure. A new approach. *Makedonska Akademija na Naukite i Umetnostite Oddelenie Za Bioloshki i Meditsinski Nauki Prilozi.* 2009;30(1):217-232, [PMID: 19736543]
Exclusion code: 4

Glenn BA, Chawla N, Bastani R. Barriers to genetic testing for breast cancer risk among ethnic minority women: an exploratory study. *Ethn. Dis.* 2012;22(3):267-273, [PMID: 22870568]
Exclusion code: 8

Goffin JR, Chappuis PO, Bégin LR, et al. Impact of germline BRCA1 mutations and overexpression of p53 on prognosis and response to treatment following breast carcinoma: 10-Year follow-up data. *Cancer.* 2003;97(3):527-536, [PMID: 12548593]
Exclusion code: 3

Gold ER, Carbone J. Myriad Genetics: In the eye of the policy storm. *Genet Med.* 2010;12(4 Suppl):S39-70, [PMID: 20393310]
Exclusion code: 2

Goldberg JI, Borgen PI. Breast cancer susceptibility testing: past, present and future. *Expert Rev Anticancer Ther.* 2006;6(8):1205-1214, [PMID: 16925486]
Exclusion code: 5

Goldfrank D, Chuai S, Bernstein JL, et al. Effect of mammography on breast cancer risk in women with mutations in BRCA1 or BRCA2. *Cancer Epidemiol. Biomarkers Prev.* 2006;15(11):2311-2313, [PMID: 17119064]
Exclusion code: 4

Golshan M, Miron A, Nixon AJ, et al. The prevalence of germline BRCA1 and BRCA2 mutations in young women with breast cancer undergoing breast-conservation therapy. *Am. J. Surg.* 2006;192(1):58-62, [PMID: 16769276]
Exclusion code: 3

Gomez-Garcia EB, Ambergen T, Blok MJ, van den Wijngaard A. Patients with an unclassified genetic variant in the BRCA1 or BRCA2 genes show different clinical features from those with a mutation. *J. Clin. Oncol.* 2005;23(10):2185-2190, [PMID: 15800311]
Exclusion code: 8

Gonzalez-Angulo AM, Timms KM, Liu S, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. *Clin. Cancer Res.* 2011;17(5):1082-1089, [PMID: 21233401]
Exclusion code: 3

Goodnight JE, Quagliana JM, Morton DL. Failure of subcutaneous mastectomy to prevent the development of breast cancer. *J. Surg. Oncol.* 1984;26:198-201, [PMID: 6330460]
Exclusion code: 2

Goodwin PJ, Phillips K-A, West DW, et al. Breast cancer prognosis in BRCA1 and BRCA2 mutation carriers: an International Prospective Breast Cancer Family Registry population-based cohort study. *J. Clin. Oncol.* 2012;30(1):19-26, [PMID: 22147742]
Exclusion code: 8

Goozner M. Ruling on BRCA gene patents could have limited impact. *J. Natl. Cancer Inst.* 2010;102(11):754-757, [PMID: 20498427]
Exclusion code: 5

Górski B, Jakubowska A, Huzarski T, et al. A high proportion of founder BRCA1 mutations in Polish breast cancer families. *Int. J. Cancer.* 2004;110(5):683-686, [PMID: 15146557]
Exclusion code: 8

Goss PE, Ingle JN, Ales-Martinez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N. Engl. J. Med.* 2011;364(25):2381-2391, [PMID: 21639806]
Exclusion code: 2

Gotlieb WH, Barchana M, Ben-Baruch G, Friedman E. Malignancies following bilateral salpingo-oophorectomy (BSO). *Eur. J. Surg. Oncol.* 2006;32(10):1231-1234, [PMID: 16626923]

Exclusion code: 3

Graeser MK, Engel C, Rhiem K, et al. Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. *J. Clin. Oncol.* 2009;27(35):5887-5892, [PMID: 19858402]
Exclusion code: 2

Gramling R, Anthony D, Lowery J, et al. Association between screening family medical history in general medical care and lower burden of cancer worry among women with a close family history of breast cancer. *Genet Med.* 2005;7(9):640-645, [PMID: 16301866]
Exclusion code: 4

Grana G, Daly M, Sands C. The role of prophylactic mastectomy in managing genetic risk. *Breast Cancer Res. Treat.* 1994;32(Suppl):72.
Exclusion code: 5

Granader EJ, Dwamena B, Carlos RC. MRI and mammography surveillance of women at increased risk for breast cancer: recommendations using an evidence-based approach. *Acad. Radiol.* 2008;15(12):1590-1595, [PMID: 19000876]
Exclusion code: 5

Grandner MA, Kripke DF, Yoon IY, Youngstedt SD. Criterion validity of the Pittsburgh Sleep Quality Index: Investigation in a non-clinical sample. *Sleep Biol Rhythms.* 2006;4(2):129-136, [PMID: 22822303]
Exclusion code: 2

Grann VR, Patel P, Bharthuar A, et al. Breast cancer-related preferences among women with and without BRCA mutations. *Breast Cancer Res. Treat.* 2010;119(1):177-184, [PMID: 19322653]
Exclusion code: 8

Grant AM, Van Teijlingen ER, Forrest-Keenan K, Torrance N, Wilson BJ, Haites NE. Does breast cancer genetic counselling meet women's expectations? A qualitative study. *Critical Public Health.* 2006;16(4):281-293.
Exclusion code: 8

Graubard BI, Freedman AN, Gail MH. Five-year and lifetime risk of breast cancer among U.S. subpopulations: implications for magnetic resonance imaging screening. *Cancer Epidemiol. Biomarkers Prev.* 2010;19(10):2430-2436, [PMID: 20841391]
Exclusion code: 2

Gray SW. Direct-to-consumer genetic testing and its potential impact on patient care: What oncologists need to know. *Community Oncology.* 2011;8(9):49-50.
Exclusion code: 5

Gray SW, Hornik RC, Schwartz JS, Armstrong K. The impact of risk information exposure on women's beliefs about direct-to-consumer genetic testing for BRCA mutations. *Clin. Genet.* 2012;81(1):29-37, [PMID: 21992449]
Exclusion code: 2

Gray SW, O'Grady C, Karp L, et al. Risk information exposure and direct-to-consumer genetic testing for BRCA mutations among women with a personal or family history of breast or ovarian cancer. *Cancer Epidemiol. Biomarkers Prev.* 2009;18(4):1303-1311, [PMID: 19318436]
Exclusion code: 2

Green MJ, Peterson SK, Baker MW, et al. Use of an educational computer program before genetic counseling for breast cancer susceptibility: effects on duration and content of counseling sessions. *Genet Med.* 2005;7(4):221-229, [PMID: 15834239]
Exclusion code: 3

Green MJ, Peterson SK, Baker MW, et al. Effect of a computer-based decision aid on knowledge, perceptions, and intentions about genetic testing for breast cancer susceptibility: a randomized controlled trial. *JAMA.* 2004;292(4):442-452, [PMID: 15280342]
Exclusion code: 3

Greene MH, Mai PL. What have we learned from risk-reducing salpingo-oophorectomy? *J. Natl. Cancer Inst.* 2009;101(2):70-71, [PMID: 19141782]
Exclusion code: 2

Greene MH, Piedmonte M, Alberts D, et al. A prospective study of risk-reducing salpingo-oophorectomy and longitudinal CA-125 screening among women at increased genetic risk of ovarian cancer: design and baseline characteristics: a Gynecologic Oncology Group study. *Cancer Epidemiol. Biomarkers Prev.* 2008;17(3):594-604, [PMID: 18349277]
Exclusion code: 5

Gregory H, Wordsworth S, Gibbons B, Wilson B, Haites N. Risk estimation for familial breast cancer: improving the system of counselling. *Eur. J. Hum. Genet.* 2007;15(11):1139-1144, [PMID: 17637807]
Exclusion code: 8

Griebsch I, Brown J, Boggis C, et al. Cost-effectiveness of screening with contrast enhanced magnetic resonance imaging vs X-ray mammography of women at a high familial risk of breast cancer. *Br. J. Cancer.* 2006;95(7):801-810, [PMID: 17016484]
Exclusion code: 8

Grindedal EM, Renkonen-Sinisalo L, Vasen H, et al. Survival in women with MMR mutations and ovarian cancer: a multicentre study in Lynch syndrome kindreds. *J. Med. Genet.* 2010;47(2):99-102, [PMID: 19635727]
Exclusion code: 3

Gronwald J, Byrski T, Huzarski T, et al. A survey of preventive measures among BRCA1 mutation carriers from Poland. *Clin. Genet.* 2007;71(2):153-157, [PMID: 17250664]
Exclusion code: 8

Gronwald J, Byrski T, Huzarski T, et al. Influence of selected lifestyle factors on breast and ovarian cancer risk in BRCA1 mutation carriers from Poland. *Breast Cancer Res. Treat.* 2006;95(2):105-109, [PMID: 16261399]
Exclusion code: 8

Gronwald J, Elsakov P, Gorski B, Lubinski J. High incidence of 4153delA BRCA1 gene mutations in Lithuanian breast- and breast-ovarian cancer families. *Breast Cancer Res. Treat.* 2005;94(2):111-113, [PMID: 16261409]
Exclusion code: 3

Gronwald J, Huzarski T, Byrski B, et al. Cancer risks in first degree relatives of BRCA1 mutation carriers: effects of mutation and proband disease status. *J. Med. Genet.* 2006;43(5):424-428, [PMID: 16227521]
Exclusion code: 3

Gronwald J, Huzarski T, Byrski T, et al. Direct-to-patient BRCA1 testing: the Twoj Styl experience. *Breast Cancer Res. Treat.* 2006;100(3):239-245, [PMID: 16807675]
Exclusion code: 5

Gronwald J, Tung N, Foulkes WD, et al. Tamoxifen and contralateral breast cancer in BRCA1 and BRCA2 carriers: an update. *Int. J. Cancer.* 2006;118(9):2281-2284, [PMID: 16331614]
Exclusion code: 3

Grzybowska E, Zientek H, Jasinska A, et al. High frequency of recurrent mutations in BRCA1 and BRCA2 genes in polish families with breast and ovarian cancer. *Hum. Mutat.* 2000;16(6):482-490, [PMID: 11102977]
Exclusion code: 8

Gu D, Zhuang L, Huang H, et al. TGFB1 T29C polymorphism and breast cancer risk: a meta-analysis based on 10,417 cases and 11,455 controls. *Breast Cancer Res. Treat.* 2010;123(3):857-861, [PMID: 20157775]
Exclusion code: 8

Guenard F, Labrie Y, Ouellette G, et al. Germline mutations in the breast cancer susceptibility gene PTEN are rare in high-risk non-BRCA1/2 French Canadian breast cancer families. *Fam Cancer.* 2007;6(4):483-490, [PMID: 17636424]
Exclusion code: 8

Gui GPH, Hogben RKF, Walsh G, A'Hern R, Eeles R. The incidence of breast cancer from screening women according to predicted family history risk: Does annual clinical examination add to mammography? *Eur. J. Cancer.* 2001;37(13):1668-1673, [PMID: 11527694]
Exclusion code: 8

Gui GPH, Kadayaprath G, Darhouse N, et al. Clinical outcome and service implications of screening women at increased breast cancer risk from a family history. *Eur. J. Surg. Oncol.* 2006;32(7):719-724, [PMID: 16784834]
Exclusion code: 4

Guirguis-Blake J. Cancer genetic risk assessment for individuals at risk of familial breast cancer. *Am. Fam. Physician.* 2008;77(4):449-450, [PMID: 18326163]
Exclusion code: 5

Gulden C, Olopade OI. Risk assessment and genetic testing for ovarian cancer. *Am. J. Roentgenol.* 2010;194(2):309-310, [PMID: 20093589]
Exclusion code: 5

Guo S, Yang S, Taylor C, Sonenshein GE. Green tea polyphenol epigallocatechin-3 gallate (EGCG) affects gene expression of breast cancer cells transformed by the carcinogen 7,12-dimethylbenz[a]anthracene. *J. Nutr.* 2005;135(12 Suppl):2978S-2986S, [PMID: 16317158]
Exclusion code: 4

Gurmankin Levy A, Micco E, Putt M, Armstrong K. Value for the future and breast cancer-preventive health behavior. *Cancer Epidemiol. Biomarkers Prev.* 2006;15(5):955-960, [PMID: 16702376]
Exclusion code: 8

Gutierrez-Enriquez S, de la Hoya M, Martinez-Bouzas C, et al. Screening for large rearrangements of the BRCA2 gene in Spanish families with breast/ovarian cancer. *Breast Cancer Res. Treat.* 2007;103(1):103-107, [PMID: 17063271]
Exclusion code: 3

Haakensen VD, Biong M, Lingjaerde OC, et al. Expression levels of uridine 5'-diphospho-glucuronosyltransferase genes in breast tissue from healthy women are associated with mammographic density. *Breast Cancer Res.* 2010;12(4):R65, [PMID: 20799965]
Exclusion code: 8

Haas JS, Kaplan CP, Des Jarlais G, Gildengoin V, Perez-Stable EJ, Kerlikowske K. Perceived risk of breast cancer among women at average and increased risk. *J Womens Health.* 2005;14(9):845-851, [PMID: 16313212]
Exclusion code: 8

Hadley DW, Ashida S, Jenkins JF, et al. Generation after generation: exploring the psychological impact of providing genetic services through a cascading approach. *Genet Med.* 2010;12(12):808-815, [PMID: 20921894]
Exclusion code: 3

Haffty BG, Choi DH, Goyal S, et al. Breast cancer in young women (YBC): prevalence of BRCA1/2 mutations and risk of secondary malignancies across diverse racial groups. *Ann. Oncol.* 2009;20(10):1653-1659, [PMID: 19491284]
Exclusion code: 3

Haffty BG, Silber A, Matloff E, Chung J, Lannin D. Racial differences in the incidence of BRCA1 and BRCA2 mutations in a cohort of early onset breast cancer patients: African American compared to white women. *J. Med. Genet.* 2006;43(2):133-137, [PMID: 15983021]
Exclusion code: 3

Hagoel L, Neter E, Dishon S, Barnett O, Rennert G. BRCA1/2 mutation carriers: living with susceptibility. *Community Genet.* 2003;6(4):242-248, [PMID: 15331870]
Exclusion code: 3

Haile RW, Thomas DC, McGuire V, et al. BRCA1 and BRCA2 mutation carriers, oral contraceptive use, and breast cancer before age 50. *Cancer Epidemiol. Biomarkers Prev.* 2006;15(10):1863-1870, [PMID: 17021353]
Exclusion code: 3

Haiman CA, Hankinson SE, Spiegelman D, et al. Polymorphic repeat in AIB1 does not alter breast cancer risk. *Breast Cancer Res.* 2000;2(5):378-385, [PMID: 11056690]
Exclusion code: 8

Halapy E, Chiarelli AM, Klar N, Knight JA. Accuracy of breast screening among women

with and without a family history of breast and/or ovarian cancer. *Breast Cancer Res. Treat.* 2005;90(3):299-305, [PMID: 15830144]
Exclusion code: 3

Halbert C, Kessler L, Collier A, Paul Wileyto E, Brewster K, Weathers B. Psychological functioning in African American women at an increased risk of hereditary breast and ovarian cancer. *Clin. Genet.* 2005;68(3):222-227, [PMID: 16098010]
Exclusion code: 3

Halbert CH. Decisions and outcomes of genetic testing for inherited breast cancer risk. *Ann. Oncol.* 2004;15 (Suppl 1):I35-I39, [PMID: 15280185]
Exclusion code: 8

Halbert CH. Genetic counseling and testing for breast cancer risk in African Americans. *LDI Issue Brief.* 2006;12(1):1-4, [PMID: 17302016]
Exclusion code: 5

Halbert CH, Kessler L, Stopfer JE, Domchek S, Wileyto EP. Low rates of acceptance of BRCA1 and BRCA2 test results among African American women at increased risk for hereditary breast-ovarian cancer. *Genet Med.* 2006;8(9):576-582, [PMID: 16980814]
Exclusion code: 8

Halbert CH, Kessler L, Troxel AB, Stopfer JE, Domchek S. Effect of genetic counseling and testing for BRCA1 and BRCA2 mutations in African American women: a randomized trial. *Public Health Genomics.* 2010;13(7-8):440-448, [PMID: 20234119]
Exclusion code: 3

Halbert CH, Kessler L, Wileyto EP, et al. Breast cancer screening behaviors among African American women with a strong family history of breast cancer. *Prev. Med.* 2006;43(5):385-388, [PMID: 16860380]
Exclusion code: 8

Halbert CH, Kessler LJ, Mitchell E. Genetic testing for inherited breast cancer risk in African Americans. *Cancer Invest.* 2005;23(4):285-295, [PMID: 16100940]
Exclusion code: 6

Halbert CH, Schwartz MD, Wenzel L, et al. Predictors of cognitive appraisals following genetic testing for BRCA1 and BRCA2 mutations. *J. Behav. Med.* 2004;27(4):373-392, [PMID: 15559734]
Exclusion code: 3

Halbert CH, Stopfer JE, McDonald J, et al. Long-Term Reactions to Genetic Testing for BRCA1 and BRCA2 Mutations: Does Time Heal Women's Concerns? *J. Clin. Oncol.* 2011;29(32):4302-4306, [PMID: 21990416]
Exclusion code: 3

Halbert CH, Wenzel L, Lerman C, et al. Predictors of participation in psychosocial telephone counseling following genetic testing for BRCA1 and BRCA2 mutations. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(5):875-881, [PMID: 15159322]
Exclusion code: 8

Haldar K, Crawford R. Risk-reducing salpingo-oophorectomy for BRCA mutation carriers. *Maturitas.* 2010;67(3):290, [PMID: 20869823]
Exclusion code: 5

Hall J, Gray S, A'Hern R, et al. Genetic testing for BRCA1: effects of a randomised study of knowledge provision on interest in testing and long term test uptake; implications for the NICE guidelines. *Fam Cancer.* 2009;8(1):5-13, [PMID: 18679829]
Exclusion code: 8

Hall MJ, Reid JE, Burbidge LA, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. *Cancer.* 2009;115(10):2222-2233, [PMID: 19241424]
Exclusion code: 2

Hallowell N, Baylock B, Heiniger L, et al. Looking different, feeling different: women's reactions to risk-reducing breast and ovarian surgery. *Fam Cancer.* 2012;11(2):215-224, [PMID: 22198037]
Exclusion code: 8

Hamann HA, Somers TJ, Smith AW, Inslicht SS, Baum A. Posttraumatic stress associated with cancer history and BRCA1/2 genetic testing. *Psychosom. Med.* 2005;67(5):766-772, [PMID: 16204436]
Exclusion code: 3

Hamann U, Sinn HP. Survival and tumor characteristics of German hereditary breast cancer patients. *Breast Cancer Res. Treat.* 2000;59(2):185-192, [PMID: 10817354]
Exclusion code: 6

Hamilton JG, Lobel M, Moyer A. Emotional distress following genetic testing for hereditary breast and ovarian cancer: a meta-analytic review. *Health Psychol.* 2009;28(4):510-518, [PMID: 19594276]
Exclusion code: 3

Hamilton R, William JK, Bowers BJ, Calzone K. Life trajectories, genetic testing, and risk reduction decisions in 18-39 year old women at risk for hereditary breast and ovarian cancer. *J Genet Couns.* 2009;18(2):147-159, [PMID: 18979190]
Exclusion code: 3

Hamilton R, Williams JK, Skirton H, Bowers BJ. Living with genetic test results for hereditary breast and ovarian cancer. *J Nurs Scholarsh.* 2009;41(3):276-283, [PMID: 19723276]
Exclusion code: 2

Hamilton RJ, Bowers BJ, Williams JK. Disclosing genetic test results to family members. *J Nurs Scholarsh.* 2005;37(1):18-24, [PMID: 15813582]
Exclusion code: 8

Hampel H, Sweet K, Westman JA, Offit K, Eng C. Referral for cancer genetics consultation: a review and compilation of risk assessment criteria. *J. Med. Genet.* 2004;41:81-91, [PMID: 14757853]
Exclusion code: 2

Hankinson SE, Colditz GA, Hunter DJ, Spencer TL, Rosner B, Stampfer MJ. A quantitative assessment of oral contraceptive use and risk of ovarian cancer. *Obstet. Gynecol.* 1992;80(4):708-714, [PMID: 1407899]
Exclusion code: 6

Hanoch Y, Miron-Shatz T, Himmelstein M. Genetic testing and risk interpretation: How do women understand lifetime risk results? *Judgment and Decision Making.* 2010;5(2):116-123.
Exclusion code: 8

Harlid S, Ivarsson MIL, Butt S, et al. Combined effect of low-penetrant SNPs on breast cancer risk. *Br. J. Cancer.* 2012;106(2):389-396, [PMID: 22045194]
Exclusion code: 4

Harris H, Nippert I, Julian-Reynier C, et al. Familial breast cancer: is it time to move from a reactive to a proactive role? *Fam Cancer.* 2011;10(3):501-503, [PMID: 21400222]
Exclusion code: 5

Harris RP, Helfand M, Woolf SH, et al. Current methods of the U.S. Preventive Services Task Force: A review of the process. *Am. J. Prev. Med.* 2001;20(3, Supplement 1):21-35, [PMID: 11306229]
Exclusion code: 2

Hartmaier RJ, Tchatchou S, Richter AS, et al. Nuclear receptor coregulator SNP discovery and impact on breast cancer risk. *BMC Cancer.* 2009;9, [PMID: 20003447]
Exclusion code: 4

Hartman AR, Daniel BL, Kurian AW, et al. Breast Magnetic Resonance Image Screening and Ductal Lavage in Women at High Genetic Risk for Breast Carcinoma. *Cancer.* 2004;100(3):479-489, [PMID: 14745863]
Exclusion code: 3

Hartmann LC, Degnim A, Schaid DJ. Prophylactic mastectomy for BRCA1/2 carriers: Progress and more questions. *J. Clin. Oncol.* 2004;22(6):981-983, [PMID: 14981099]
Exclusion code: 5

Hashemian AH, Hajizadeh E, Kazemnejad A, Atri M, Mehdipour P. Penetrance of

BRCA1/BRCA2 specific gene mutations in Iranian women with breast cancer. *Saudi Med J.* 2009;30(1):41-44, [PMID: 19139771]
Exclusion code: 3

Hatcher MB, Fallowfield L, A'Hern R. The psychosocial impact of bilateral prophylactic mastectomy: prospective study using questionnaires and semistructured interviews. *BMJ.* 2001;322(7278):76, [PMID: 11154619]
Exclusion code: 5

Hay JL, McCaul KD, Magnan RE. Does worry about breast cancer predict screening behaviors? A meta-analysis of the prospective evidence. *Prev. Med.* 2006;42(6):401-408, [PMID: 16626796]
Exclusion code: 8

Genetic testing for susceptibility to ovarian cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006000101
Exclusion code: 5

Breast cancer susceptibility 1 and 2 (BRCA1/2) sequence variant testing for susceptibility to hereditary breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000063
Exclusion code: 5

Comprehensive screening for large rearrangements in BRCA1/2 for assessment of breast cancer risk (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000070
Exclusion code: 5

Gene expression profiling for prognosis of recurrence of primary breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000100
Exclusion code: 5

BRCA1 and BRCA2 sequence variant analysis for susceptibility to hereditary ovarian cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000959
Exclusion code: 5

MammaPrint for prognosis of breast cancer recurrence (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32011001341
Exclusion code: 5

Impact of intensive screening in women with an increased risk of breast cancer because of a familiar predisposition - primary research (Brief record). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32005000528
Exclusion code: 5

Mammography in women under 50 by GP in families with hereditary breast cancer - primary research (Brief record). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32005000531
Exclusion code: 5

Evaluation of mammographic surveillance services in women under 50 with a family history of breast cancer (Project record). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32003000271
Exclusion code: 5

Heemskerk-Gerritsen BAM, Brekelmans CTM, Menke-Pluymers MBE, et al. Prophylactic mastectomy in BRCA1/2 mutation carriers and women at risk of hereditary breast cancer: long-term experiences at the Rotterdam Family Cancer Clinic. *Ann. Surg. Oncol.* 2007;14(12):3335-3344, [PMID: 17541692]
Exclusion code: 3

Heijnsdijk EAM, Warner E, Gilbert FJ, et al. Differences in natural history between breast

cancers in BRCA1 and BRCA2 mutation carriers and effects of MRI screening-MRISC, MARIBS, and Canadian studies combined. *Cancer Epidemiol. Biomarkers Prev.* 2012;21(9):1458-1468, [PMID: 22744338]
Exclusion code: 2

Helmes AW, Bowen DJ, Bowden R, Bengel J. Predictors of participation in genetic research in a primary care physician network. *Cancer Epidemiol. Biomarkers Prev.* 2000;9(12):1377-1379, [PMID: 11142425]
Exclusion code: 8

Herman J. Evaluating women with ovarian cancer for BRCA1 and BRCA2 mutations: missed opportunities. *Obstet. Gynecol.* 2010;116(2 Pt 1):440-441; author reply 441, [PMID: 20664408]
Exclusion code: 5

Herman JD, Appelbaum H. Hereditary breast and ovarian cancer syndrome and issues in pediatric and adolescent practice. *J. Pediatr. Adolesc. Gynecol.* 2010;23(4):253-258, [PMID: 20632459]
Exclusion code: 3

Herrinton LJ, Barlow WE, Yu O, et al. Efficacy of prophylactic mastectomy in women with unilateral breast cancer: A cancer research network project. *J. Clin. Oncol.* 2005;23(19):4275-4286, [PMID: 15795415]
Exclusion code: 3

Heshka JT, Palleschi C, Howley H, Wilson B, Wells PS. A systematic review of perceived risks, psychological and behavioral impacts of genetic testing. *Genet Med.* 2008;10(1):19-32, [PMID: 18197053]
Exclusion code: 2

Higgins J, Thompson S. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 2002;21:1539-1558, [PMID: 12111919]
Exclusion code: 2

Hilgart JS, Coles B, Iredale R. Cancer genetic risk assessment for individuals at risk of familial breast cancer. *Cochrane Database Syst Rev.* 2012;2:CD003721, [PMID: 22336791]
Exclusion code: 5

Ho L, Fivecoat H, Wang J, Pasinetti GM. Alzheimer's disease biomarker discovery in symptomatic and asymptomatic patients: Experimental approaches and future clinical applications. *Exp. Gerontol.* 2010;45(1):15-22, [PMID: 19796674]
Exclusion code: 3

Hofman KJ, Tambor ES, Chase GA, Geller G, Faden RR, Holtzman NA. Physicians' knowledge of genetics and genetic tests. *Acad. Med.* 1993;68(8):625-632, [PMID: 835287]
Exclusion code: 6

Holloway S, Porteous M, Cetnarskyj R, et al. Patient satisfaction with two different models of cancer genetic services in south-east Scotland. *Br. J. Cancer.* 2004;90(3):582-589, [PMID: 14760368]
Exclusion code: 6

Holloway SM, Bernhard B, Campbell H, Lam WWK. Uptake of testing for BRCA1/2 mutations in South East Scotland. *Eur. J. Hum. Genet.* 2008;16(8):906-912, [PMID: 18285832]
Exclusion code: 8

Hooker GW, Leventhal K-G, DeMarco T, et al. Longitudinal changes in patient distress following interactive decision aid use among BRCA1/2 carriers: a randomized trial. *Med. Decis. Making.* 2011;31(3):412-421, [PMID: 20876346]
Exclusion code: 3

Hopwood P. Psychosocial aspects of risk communication and mutation testing in familial breast-ovarian cancer. *Curr. Opin. Oncol.* 2005;17(4):340-344, [PMID: 15933464]
Exclusion code: 8

Hopwood P, Fletcher I, Lee A, Al Ghazal S. A body image scale for use with cancer patients. *Eur. J. Cancer.* 2001;37(2):189-197, [PMID: 11166145]
Exclusion code: 2

Hopwood P, Howell A, Lalloo F, Evans G. Do women understand the odds? Risk perceptions

and recall of risk information in women with a family history of breast cancer. *Community Genet.* 2003;6(4):214-223, [PMID: 15331867]
Exclusion code: 6

Hopwood P, Shenton A, Lalloo F, Evans DGR, Howell A. Risk perception and cancer worry: An exploratory study of the impact of genetic risk counselling in women with a family history of breast cancer [12]. *J. Med. Genet.* 2001;38(2):139-142, [PMID: 11288719]
Exclusion code: 6

Hopwood P, van Asperen CJ, Borreani G, et al. Cancer genetics service provision: a comparison of seven European centres. *Community Genet.* 2003;6(4):192-205, [PMID: 15331865]
Exclusion code: 8

Hopwood P, Watson M. Genetic susceptibility to breast/ovarian cancer. *Psycho-Oncology*. 2nd ed. New York, NY: Oxford University Press; US; 2010:105-109.
Exclusion code: 5

Horowitz M, Wilner N, Alvarez W. Impact of event scale: A measure of subjective stress. *Psychosom. Med.* 1979;41(3):209-218, [PMID: 472086]
Exclusion code: 6

Horsman D, Wilson BJ, Avard D, et al. Clinical management recommendations for surveillance and risk-reduction strategies for hereditary breast and ovarian cancer among individuals carrying a deleterious BRCA1 or BRCA2 mutation. *J Obstet Gynaecol Can.* 2007;29(1):45-60, [PMID: 17346477]
Exclusion code: 5

Hoskins LM. *Negotiation of health risks and risk management for young adult brca1/2-positive women: Implications for partnering and family formation*, Hoskins, Lindsey M : U Maryland, Coll Park, US; 2011
Exclusion code: 5

Hoskins LM, Roy K, Peters JA, Loud JT, Greene MH. Disclosure of positive BRCA1/2-mutation status in young couples: The journey from uncertainty to bonding through partner support. *Fam Syst Health.* 2008;26(3):296-316.
Exclusion code: 8

Hoskins LM, Roy KM, Greene MH. Toward a new understanding of risk perception among young female BRCA1/2 "previvors". *Fam Syst Health.* 2012;30(1):32-46, [PMID: 22429077]
Exclusion code: 5

Howard A, Balneaves LG, Bottorff JL. Women's decision making about risk-reducing strategies in the context of hereditary breast and ovarian cancer: A systematic review. *J Genet Couns.* 2009;18(6):578-597, [PMID: 19802692]
Exclusion code: 8

Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2008. 2011; http://seer.cancer.gov/csr/1975_2008/. Accessed 16 Oct, 2012
Exclusion code: 2

Hu Z, Song C-G, Lu J-S, et al. A multigenic study on breast cancer risk associated with genetic polymorphisms of ER Alpha, COMT and CYP19 gene in BRCA1/BRCA2 negative Shanghai women with early onset breast cancer or affected relatives. *J Cancer Res Clin Oncol.* 2007;133(12):969-978, [PMID: 17562079]
Exclusion code: 8

Hu Z, Wu J, Lu JS, et al. Analysis of BRCA1 mutations in Chinese patients with early onset breast cancer and affected relatives. *Zhonghua Zhong Liu Za Zhi.* 2004;26(11):657-659, [PMID: 15777502]
Exclusion code: 7

Hughes DJ. Use of association studies to define genetic modifiers of breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Fam Cancer.* 2008;7(3):233-244, [PMID: 18283561]
Exclusion code: 8

Hughes DJ, Ginolhac SM, Coupier I, et al. Breast cancer risk in BRCA1 and BRCA2 mutation carriers and polyglutamine repeat length in the AIB1 gene. *Int. J. Cancer.* 2005;117(2):230-233, [PMID: 15900600]
Exclusion code: 8

Hulbert-Williams N, Neal R, Morrison V, Hood K, Wilkinson C. Anxiety, depression and quality of life after cancer diagnosis: What psychosocial variables best predict how patients adjust? *Psychooncology.* 2012;21(8):857-867, [PMID: 21695744]
Exclusion code: 3

Huo D, Senie RT, Daly M, et al. Prediction of BRCA Mutations Using the BRCAPRO Model in Clinic-Based African American, Hispanic, and Other Minority Families in the United States. *J. Clin. Oncol.* 2009;27(8):1184-1190, [PMID: 19188678]
Exclusion code: 3

Huo X, Hu Z, Zhai X, et al. Common non-synonymous polymorphisms in the BRCA1 Associated RING Domain (BARD1) gene are associated with breast cancer susceptibility: a case-control analysis. *Breast Cancer Res. Treat.* 2007;102(3):329-337, [PMID: 17028982]
Exclusion code: 8

Huo X, Lu C, Huang X, et al. Polymorphisms in BRCA1, BRCA1-interacting genes and susceptibility of breast cancer in Chinese women. *J. Cancer Res. Clin. Oncol.* 2009;135(11):1569-1575, [PMID: 19484476]
Exclusion code: 8

Huys I, Van Overwalle G, Matthijs G. Gene and genetic diagnostic method patent claims: a comparison under current European and US patent law. *Eur. J. Hum. Genet.* 2011;19(10):1104-1107, [PMID: 21654725]
Exclusion code: 5

Hwang ES, McLennan JL, Moore DH, Crawford BB, Esserman LJ, Ziegler JL. Ductal carcinoma in situ in BRCA mutation carriers. *J. Clin. Oncol.* 2007;25(6):642-647, [PMID: 17210933]
Exclusion code: 3

IBIS Investigators. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. *Lancet.* 2002;360(9336):817-824, [PMID: 12243915]
Exclusion code: 2

Ibrahim SS, Hafez EE, Hashishe MM. Presymptomatic breast cancer in Egypt: role of BRCA1 and BRCA2 tumor suppressor genes mutations detection. *J. Exp. Clin. Cancer Res.* 2010;29:82, [PMID: 20579331]
Exclusion code: 3

Ikeda N, Miyoshi Y, Yoneda K, et al. Frequency of BRCA1 and BRCA2 germline mutations in Japanese breast cancer families. *Int. J. Cancer.* 2001;91(1):83-88, [PMID: 11149425]
Exclusion code: 8

Infante M, Durán M, Acedo A, et al. BRCA1 5272-1G>A and BRCA2 5374delTATG are founder mutations of high relevance for genetic counselling in breast/ovarian cancer families of Spanish origin. *Clin. Genet.* 2010;77(1):60-69, [PMID: 19912264]
Exclusion code: 4

Inumaru LE, Silveira EA, Naves MM. Risk and protective factors for breast cancer: a systematic review. *Cad. Saude Publica.* 2011;27(7):1259-1270, [PMID: 21808811]
Exclusion code: 7

Iredale R, Rapport F, Sivell S, et al. Exploring the requirements for a decision aid on familial breast cancer in the UK context: a qualitative study with patients referred to a cancer genetics service. *J. Eval. Clin. Pract.* 2008;14(1):110-115, [PMID: 18211652]
Exclusion code: 8

Jackson C. The General Health Questionnaire. *Occup. Med.* 2007;57(1):79.
Exclusion code: 2

Jacobi CE, de Bock GH, Siegerink B, van Asperen CJ. Differences and similarities in breast cancer risk assessment models in clinical practice: which model to choose? *Breast Cancer Res. Treat.* 2009;115(2):381-390, [PMID: 18516672]
Exclusion code: 6

Jacobs I. Screening for familial ovarian cancer: The need for well-designed prospective studies. *J. Clin. Oncol.* 2005;23(24):5443-5445, [PMID: 16110006]

Exclusion code: 5

Jager M, Oosterwijk J, Middel B, Hoekstra-Weebers J. Motivation to participate in genetic counseling and cancer worry of women with a high family risk for hereditary breast/ovarian cancer: Differences between women who choose for DNA research and those who decline. *Gedrag & Gezondheid: Tijdschrift voor Psychologie en Gezondheid.* 2004;32(1):18-27.
Exclusion code: 7

Jakubowska A, Gronwald J, Menkiszak J, et al. BRCA1-associated breast and ovarian cancer risks in Poland: no association with commonly studied polymorphisms. *Breast Cancer Res. Treat.* 2010;119(1):201-211, [PMID: 19360465]
Exclusion code: 8

Jakubowska A, Jaworska K, Cybulski C, et al. Do BRCA1 modifiers also affect the risk of breast cancer in non-carriers? *Eur. J. Cancer.* 2009;45(5):837-842, [PMID: 19071013]
Exclusion code: 3

Janiszewska H, Haus O, Lauda-Świeciak A, et al. Frequency of three BRCA1 gene founder mutations in breast/ovarian cancer families from the Pomerania-Kujawy region of Poland. *Clin. Genet.* 2003;64(6):502-508, [PMID: 14986830]
Exclusion code: 4

Jara L, Ampuero S, Santibanez E, et al. BRCA1 and BRCA2 mutations in a South American population. *Cancer Genet. Cytogenet.* 2006;166(1):36-45, [PMID: 16616110]
Exclusion code: 3

Jhanwar SM. *Influence of body image on intentions for prophylactic mastectomy*, Jhanwar, Sabrina M : Yeshiva U , US; 2012
Exclusion code: 8

John E, Miron A, Gong G, et al. Prevalence of pathogenic BRCA1 mutation carriers in 5 U.S. racial/ethnic groups. *JAMA.* 2007;298(24):2869-2876, [PMID: 18159056]
Exclusion code: 2

Johnson N, Lancaster T, Fuller A. The prevalence of a family history of cancer in general practice. *Fam. Pract.* 1995;12(3):287-289, [PMID: 8536831]
Exclusion code: 2

Jones AL. Chemoprevention of breast cancer (The British tamoxifen trials). *Canadian Journal of Infectious Diseases.* 1995;6(Suppl C):193C.
Exclusion code: 5

Jones JL, Hughes KS, Kopans DB, et al. Evaluation of hereditary risk in a mammography population. *Clin Breast Cancer.* 2005;6(1):38-44, [PMID: 15899071]
Exclusion code: 8

Joo JG, Csanad M, Toth K, Mate S, Nagy BZ. Risk assessment in familial breast cancer. *Orv. Hetil.* 2011;152(19):758-762, [PMID: 21498166]
Exclusion code: 7

Joo JG, Ladi S, Nagy BZ, Langmar Z. [Management of hereditary ovarian cancer]. *Orv. Hetil.* 2011;152(40):1596-1608, [PMID: 21945869]
Exclusion code: 7

Joo JG, Ladi S, Nagy BZ, Langmar Z. Managment of herediatry ovarian cancer. *Orv. Hetil.* 2011;152(40):1596-1608, [PMID: 21945869]
Exclusion code: 7

Joosse SA, van Beers EH, Tielen IHG, et al. Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH. *Breast Cancer Res. Treat.* 2009;116(3):479-489, [PMID: 18704682]
Exclusion code: 3

Jorgensen TJ, Helzlsouer KJ, Clipp SC, Bolton JH, Crum RM, Visvanathan K. DNA repair gene variants associated with benign breast disease in high cancer risk women. *Cancer Epidemiol. Biomarkers Prev.* 2009;18(1):346-350, [PMID: 19124519]
Exclusion code: 8

Joseph G, Beattie MS, Lee R, et al. Pre-counseling education for low literacy women at risk of Hereditary Breast and Ovarian Cancer

(HBOC): patient experiences using the Cancer Risk Education Intervention Tool (CREdIT). *J Genet Couns.* 2010;19(5):447-462, [PMID: 20490636]
Exclusion code: 8

Julian-Reynier C. [Genetic predisposition to breast and ovarian cancer: importance of test results]. *Med Sci (Paris).* 2011;27(6-7):657-661, [PMID: 21718651]
Exclusion code: 7

Julian-Reynier C, Bouhnik AD, Mouret-Fourme E, et al. Time to prophylactic surgery in BRCA1/2 carriers depends on psychological and other characteristics. *Genet Med.* 2010;12(12):801-807, [PMID: 20921896]
Exclusion code: 8

Kadouri L, Hubert A, Rotenberg Y, et al. Cancer risks in carriers of the BRCA1/2 Ashkenazi founder mutations. *J. Med. Genet.* 2007;44(7):467-471, [PMID: 17307836]
Exclusion code: 3

Kadouri L, Kote-Jarai Z, Easton DF, et al. Polyglutamine repeat length in the AIB1 gene modifies breast cancer susceptibility in BRCA1 carriers. *Int. J. Cancer.* 2004;108(3):399-403, [PMID: 14648706]
Exclusion code: 8

Kalfoglou EA, Faikoglu R, Ozcan S, Petridis G, Yukseloglu H, Atasoy S. DNA analysis as a tool for breast cancer malpractice determination: an interdisciplinary approach. *Oncol. Rep.* 2006;16(1):203-206, [PMID: 16786147]
Exclusion code: 5

Kandel MJ, Stadler Z, Masciari S, et al. Prevalence of BRCA1 mutations in triple negative breast cancer (BC). *Journal of Clinical Oncology: ASCO annual meeting proceedings.* Vol 242006:508
Exclusion code: 3

Kang HH, Williams R, Leary J, et al. Evaluation of models to predict BRCA germline mutations. *Br. J. Cancer.* 2006;95(7):914-920, [PMID: 17016486]
Exclusion code: 3

Kang P, Mariapun S, Phuah SY, et al. Large BRCA1 and BRCA2 genomic rearrangements in Malaysian high risk breast-ovarian cancer families. *Breast Cancer Res. Treat.* 2010;124(2):579-584, [PMID: 20617377]
Exclusion code: 3

Kaphingst KA, McBride CM. Patient responses to genetic information: Studies of patients with hereditary cancer syndromes identify issues for use of genetic testing in nephrology practice. *Semin. Nephrol.* 2010;30(2):203-214, [PMID: 20347649]
Exclusion code: 8

Karlan BY, Baldwin RL, Lopez-Luevanos E, et al. Peritoneal serous papillary carcinoma, a phenotypic variant of familial ovarian cancer: Implications for ovarian cancer screening. *Am. J. Obstet. Gynecol.* 1999;180(4):917-928, [PMID: 10203660]
Exclusion code: 3

Karlan BY, Berchuck A, Mutch D. The role of genetic testing for cancer susceptibility in gynecologic practice. *Obstet. Gynecol.* 2007;110(1):155-167, [PMID: 17601911]
Exclusion code: 5

Karliner LS, Napoles-Springer A, Kerlikowske K, Haas JS, Gregorich SE, Kaplan CP. Missed opportunities: family history and behavioral risk factors in breast cancer risk assessment among a multiethnic group of women. *J. Gen. Intern. Med.* 2007;22(3):308-314, [PMID: 17356960]
Exclusion code: 8

Kasparian NA, Wakefield CE, Meiser B. Assessment of psychosocial outcomes in genetic counseling research: an overview of available measurement scales. *J Genet Couns.* 2007;16(6):693-712, [PMID: 17694397]
Exclusion code: 5

Katapodi MC, Aouizerat BE. Do women in the community recognize hereditary and sporadic breast cancer risk factors? *Oncol. Nurs. Forum.* 2005;32(3):617-623, [PMID: 15897936]
Exclusion code: 8

Katapodi MC, Dodd MJ, Lee KA, Facione NC. Underestimation of breast cancer risk: influence on screening behavior. *Oncol. Nurs. Forum.* 2009;36(3):306-314, [PMID: 19403452]
Exclusion code: 4

Katapodi MC, Northouse L, Pierce P, Milliron KJ, Liu C, Merajver SD. Differences between women who pursued genetic testing for hereditary breast and ovarian cancer and their at-risk relatives who did not. *Oncol. Nurs. Forum.* 2011;38(5):572-581, [PMID: 21875844]
Exclusion code: 8

Kauff ND, Barakat RR. Risk-reducing salpingo-oophorectomy in patients with germline mutations in BRCA1 or BRCA2. *J. Clin. Oncol.* 2007;25(20):2921-2927, [PMID: 17617523]
Exclusion code: 5

Kauff ND, Domcheck SM, Friebel TM, et al. Multi-center prospective analysis of risk-reducing salpingo-oophorectomy to prevent BRCA-associated breast and ovarian cancer. *Journal of Clinical Oncology : ASCO annual meeting proceedings.* Vol 242006:1003
Exclusion code: 5

Kauff ND, Domchek SM, Friebel TM, et al. Risk-reducing salpingo-oophorectomy for the prevention of BRCA1- and BRCA2-associated breast and gynecologic cancer: a multicenter, prospective study. *J. Clin. Oncol.* 2008;26(8):1331-1337, [PMID: 18268356]
Exclusion code: 3

Kauff ND, Offit K. Modeling genetic risk of breast cancer. *JAMA.* 2007;297(23):2637-2639, [PMID: 17579233]
Exclusion code: 5

Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N. Engl. J. Med.* 2002;346(21):1609-1615, [PMID: 12023992]
Exclusion code: 3

Keating NL, Stoeckert KA, Regan MM, DiGianni L, Garber JE. Physicians' experiences with BRCA1/2 testing in community settings. *J. Clin. Oncol.* 2008;26(35):5789-5796, [PMID: 19001322]
Exclusion code: 3

Kelly K, Leventhal H, Andrykowski M, et al. The decision to test in women receiving genetic counseling for BRCA1 and BRCA2 mutations. *J Genet Couns.* 2004;13(3):237-257, [PMID: 15604634]
Exclusion code: 3

Kelly K, Leventhal H, Andrykowski M, et al. Using the common sense model to understand perceived cancer risk in individuals testing for BRCA1/2 mutations. *Psychooncology.* 2005;14(1):34-48, [PMID: 15386791]
Exclusion code: 3

Kelly K, Leventhal H, Marvin M, Toppmeyer D, Baran J, Schwalb M. Cancer genetics knowledge and beliefs and receipt of results in Ashkenazi Jewish individuals receiving counseling for BRCA1/2 mutations. *Cancer Control.* 2004;11(4):236-244, [PMID: 15284715]
Exclusion code: 3

Kelly K, Leventhal H, Marvin M, et al. Subjective and objective risk of breast cancer in Ashkenazi Jewish individuals at risk for BRCA1/2 mutations. *Genet Test.* 2004;8(2):139-147, [PMID: 15345111]
Exclusion code: 8

Kenen R, Ardern-Jones A, Eeles R. Family stories and the use of heuristics: women from suspected hereditary breast and ovarian cancer (HBOC) families. *Sociol. Health Illn.* 2003;25(7):838-865, [PMID: 19774749]
Exclusion code: 8

Kenen R, Ardern-Jones A, Lynch E, Eeles R. Ownership of uncertainty: healthcare professionals counseling and treating women from hereditary breast and ovarian cancer families who receive an inconclusive BRCA1/2 genetic test result. *Genet Test Mol Biomarkers.* 2011;15(4):243-250, [PMID: 21254913]
Exclusion code: 3

Keogh LA, Hopper JL, Rosenthal D, Phillips K. Australian clinicians and chemoprevention for

women at high familial risk for breast cancer. *Hered Cancer Clin Pract.* 2009;7(1), [PMID: 19409108]
Exclusion code: 3

Keogh LA, Southey MC, Maskiell J, et al. Uptake of offer to receive genetic information about BRCA1 and BRCA2 mutations in an Australian population-based study. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(12):2258-2263, [PMID: 15598789]
Exclusion code: 8

Kerber RA, Slattery ML. Comparison of self-reported and database-linked family history of cancer data in a case-control study. *Am. J. Epidemiol.* 1997;146(3):244-248, [PMID: 9247008]
Exclusion code: 2

Kerlikowske K, Carney PA, Geller B, et al. Performance of screening mammography among women with and without a first-degree relative with breast cancer. *Ann. Intern. Med.* 2000;133(11):855-863, [PMID: 11103055]
Exclusion code: 2

Kerlikowske K, Grady D, Barclay J, Sickles EA, Ernster V. Effect of age, breast density, and family history on the sensitivity of first screening mammography. *JAMA.* 1996;276(1):33-38, [PMID: 8667536]
Exclusion code: 2

Keshavarzi F, Javadi GR, Zeinali S. BRCA1 and BRCA2 germline mutations in 85 Iranian breast cancer patients. *Fam Cancer.* 2012;11(1):57-67, [PMID: 21918854]
Exclusion code: 3

Kessler L, Collier A, Brewster K, et al. Attitudes about genetic testing and genetic testing intentions in African American women at increased risk for hereditary breast cancer. *Genet Med.* 2005;7(4):230-238, [PMID: 15834240]
Exclusion code: 3

Kessler L, Domchek S, Stopfer J, Halbert CH. BRCA1 and BRCA2 risk perceptions among African American women at increased risk for hereditary breast-ovarian cancer. *Community Genet.* 2008;11(4):193-200, [PMID: 18417966]
Exclusion code: 3

Kets CM, Niermeijer MF, Massuger LFAG, Hoogerbrugge N. [In cases of familial ovarian cancer, always consider the risk of breast cancer]. *Ned. Tijdschr. Geneeskd.* 2004;148(35):1709-1711, [PMID: 15468897]
Exclusion code: 7

Khoury MJ, Feero WG, Reyes M, et al. The genomic applications in practice and prevention network. *Genet Med.* 2009;11(7):488-494, [PMID: 19471162]
Exclusion code: 5

Kiechle M. [Genetic testing in patients at risk for carcinoma of the breast]. *Dtsch. Med. Wochenschr.* 2006;131(43):2420-2422, [PMID: 17054060]
Exclusion code: 7

Kiely BE, Jenkins MA, McKinley JM, et al. Contralateral risk-reducing mastectomy in BRCA1 and BRCA2 mutation carriers and other high-risk women in the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab). *Breast Cancer Res. Treat.* 2010;120(3):715-723, [PMID: 19669874]
Exclusion code: 3

King HM. *Risk reduction decision making in women with BRCA1/2 gene mutations*, King, Heidi M : U South Florida, US; 2009
Exclusion code: 4

King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science.* 2003;302(5645):643-646, [PMID: 14576434]
Exclusion code: 2

King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in brca1 and brca2 national surgical adjuvant breast and bowel project (nsabp-p1) breast cancer prevention trial. *JAMA.* 2001;286(18):2251-2256, [PMID: 11710890]
Exclusion code: 5

Kinney AY, Gammon A, Coxworth J, Simonsen SE, Arce-Laretta M. Exploring attitudes, beliefs, and communication preferences of Latino community members regarding BRCA1/2 mutation testing and preventive strategies. *Genet Med.* 2010;12(2):105-115, [PMID: 20061960]
Exclusion code: 3

Kinney AY, Simonsen SE, Baty BJ, et al. Risk reduction behaviors and provider communication following genetic counseling and BRCA1 mutation testing in an African American kindred. *J Genet Couns.* 2006;15(4):293-305, [PMID: 16865561]
Exclusion code: 8

Kinney AY, Simonsen SE, Baty BJ, et al. Acceptance of genetic testing for hereditary breast ovarian cancer among study enrollees from an African American kindred. *Am J Med Genet A.* 2006;140(8):813-826, [PMID: 16523520]
Exclusion code: 8

Kirova YM, Stoppa-Lyonnet D, Savignoni A, Sigal-Zafrani B, Fabre N, Fourquet A. Risk of breast cancer recurrence and contralateral breast cancer in relation to BRCA1 and BRCA2 mutation status following breast-conserving surgery and radiotherapy. *Eur. J. Cancer.* 2005;41(15):2304-2311, [PMID: 16140006]
Exclusion code: 2

Kleibl Z, Havranek O, Kormunda S, et al. The AIB1 gene polyglutamine repeat length polymorphism and the risk of breast cancer development. *J. Cancer Res. Clin. Oncol.* 2011;137(2):331-338, [PMID: 20422428]
Exclusion code: 8

Klitzman R. Genetic testing creates new versions of ancient dilemmas. *NY Times.* 2006:F5, [PMID: 16429628]
Exclusion code: 5

Kodl MM, Lee JW, Matthews AK, Cummings SA, Olopade OI. Correlates of depressive symptoms among women seeking cancer genetic counseling and risk assessment at a high-risk cancer clinic. *J Genet Couns.* 2006;15(4):267-276, [PMID: 16897360]
Exclusion code: 4

Kollias J, Sibbering DM, Blarney RW, et al. Screening women aged less than 50 years with a family history of breast cancer. *Eur. J. Cancer.* 1998;34(6):878-883, [PMID: 9797701]
Exclusion code: 8

Kolor K, Duquette D, Zlot A, et al. Public awareness and use of direct-to-consumer personal genomic tests from four state population-based surveys, and implications for clinical and public health practice. *Genet Med.* 2012;14(10):860-867, [PMID: 22814860]
Exclusion code: 2

Komenaka IK, Ditkoff BA, Joseph KA, et al. The Development of Interval Breast Malignancies in Patients with BRCA Mutations. *Cancer.* 2004;100(10):2079-2083, [PMID: 15139048]
Exclusion code: 8

Kong CY, Lee JM, McMahon PM, et al. Using radiation risk models in cancer screening simulations: important assumptions and effects on outcome projections. *Radiology.* 2012;262(3):977-984, [PMID: 22357897]
Exclusion code: 5

Konstantopoulou I, Rampias T, Ladopoulou A, et al. Greek BRCA1 and BRCA2 mutation spectrum: Two BRCA1 mutations account for half the carriers found among high-risk breast/ovarian cancer patients. *Breast Cancer Res. Treat.* 2008;107(3):431-441, [PMID: 17453335]
Exclusion code: 3

Kopans DB. The U.S. Preventive Services Task Force guidelines are not supported by the scientific evidence. *Radiology.* 2010;257(1):294-295; author reply 295, [PMID: 20851947]
Exclusion code: 5

Kotalik J, Silverman M. Genetic medicine: ethical and practical issues of application of the new technology. *Clin Invest Med.* 2004;27(1):14-15, [PMID: 15061580]
Exclusion code: 5

Kote-Jarai Z, Powles TJ, Mitchell G, et al. BRCA1/BRCA2 mutation status and analysis of cancer family history in participants of the Royal Marsden Hospital tamoxifen chemoprevention trial. *Cancer Lett.* 2007;247(2):259-265, [PMID: 16777318]
Exclusion code: 8

Kotsopoulos J, Lubinski J, Lynch HT, et al. Oophorectomy after menopause and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Cancer Epidemiol. Biomarkers Prev.* 2012;21(7):1089-1096, [PMID: 22564871]
Exclusion code: 2

Kotsopoulos J, Lubinski J, Lynch HT, et al. Age at first birth and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Breast Cancer Res. Treat.* 2007;105(2):221-228, [PMID: 17245541]
Exclusion code: 8

Kotsopoulos J, Lubinski J, Lynch HT, et al. Age at menarche and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Cancer Causes Control.* 2005;16(6):667-674, [PMID: 16049805]
Exclusion code: 8

Kowalska E, Narod SA, Huzarski T, et al. Increased rates of chromosome breakage in BRCA1 carriers are normalized by oral selenium supplementation. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(5):1302-1306, [PMID: 15894690]
Exclusion code: 8

Kram V, Peretz T, Sagi M. Acceptance of preventive surgeries by Israeli women who had undergone BRCA testing. *Fam Cancer.* 2006;5(4):327-335, [PMID: 16724248]
Exclusion code: 8

Kramer R, Brown P. Should tamoxifen be used in breast cancer prevention? *Drug Saf.* 2004;27(13):979-989, [PMID: 15471505]
Exclusion code: 5

Kriege M, Brekelmans CTM, Boetes C, et al. MRI screening for breast cancer in women with familial or genetic predisposition: Design of the Dutch National Study (MRISC). *Fam Cancer.* 2001;1(3-4):163-168, [PMID: 14574173]
Exclusion code: 2

Kriege M, Brekelmans CTM, Peterse H, et al. Tumor characteristics and detection method in the MRISC screening program for the early detection of hereditary breast cancer. *Breast Cancer Res. Treat.* 2007;102(3):357-363, [PMID: 17051427]
Exclusion code: 5

Kriege M, Jager A, Hooning MJ, et al. The efficacy of taxane chemotherapy for metastatic breast cancer in BRCA1 and BRCA2 mutation carriers. *Cancer.* 2012;118(4):899-907, [PMID: 21761396]
Exclusion code: 3

Kroiss R, Winkler V, Bikas D, et al. Younger birth cohort correlates with higher breast and ovarian cancer risk in European BRCA1 mutation carriers. *Hum. Mutat.* 2005;26(6):583-589, [PMID: 16287141]
Exclusion code: 8

Kroiss R, Winkler V, Kalteis K, et al. Prevalence of pre-malignant and malignant lesions in prophylactic mastectomy specimens of BRCA1 mutation carriers: comparison with a control group. *J. Cancer Res. Clin. Oncol.* 2008;134(10):1113-1121, [PMID: 18392852]
Exclusion code: 4

Kuhl CK. High-risk screening: Multi-modality surveillance of women at high risk for breast cancer (proven or suspected carriers of a breast cancer susceptibility gene). *J. Exp. Clin. Cancer Res.* 2002;21(3 SUPPL.):103-106, [PMID: 12585663]
Exclusion code: 6

Kuhl CK. [Familial breast cancer: what the radiologist needs to know]. *Rofo.* 2006;178(7):680-687, [PMID: 16817122]
Exclusion code: 7

Kuhl CK, Kuhn W, Schild H. Management of women at high risk for breast cancer: New imaging beyond mammography. *Breast.* 2005;14(6):480-486, [PMID: 16185869]

Exclusion code: 5

Kuhl CK, Schmutzler RK, Leutner CS, et al. Breast MR imaging screening in 192 women proved or suspected to be carriers of a breast cancer susceptibility gene: preliminary results. *Radiology.* 2000;215(1):267-279, [PMID: 10751498]
Exclusion code: 2

Kuhl CK, Schrading S, Leutner CC, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. *J. Clin. Oncol.* 2005;23(33):8469-8476, [PMID: 16293877]
Exclusion code: 3

Kuijper TM, Ruigrok-Ritstier K, Verhoef-Post M, et al. LH receptor gene expression is essentially absent in breast tumor tissue: Implications for treatment. *Mol. Cell. Endocrinol.* 2009;302(1):58-64, [PMID: 19356624]
Exclusion code: 3

Kurian AW, Gong GD, John EM, et al. Performance of prediction models for BRCA mutation carriage in three racial/ethnic groups: findings from the Northern California Breast Cancer Family Registry. *Cancer Epidemiol. Biomarkers Prev.* 2009;18(4):1084-1091, [PMID: 19336551]
Exclusion code: 3

Kurian AW, Hartman A-R, Mills MA, Ford JM, Daniel BL, Plevritis SK. Opinions of women with high inherited breast cancer risk about prophylactic mastectomy: an initial evaluation from a screening trial including magnetic resonance imaging and ductal lavage. *Health Expect.* 2005;8(3):221-233, [PMID: 16098152]
Exclusion code: 3

Kurian AW, Mills MA, Jaffee M, et al. Ductal lavage of fluid-yielding and non-fluid-yielding ducts in BRCA1 and BRCA2 mutation carriers and other women at high inherited breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(5):1082-1089, [PMID: 15894656]
Exclusion code: 8

Kurian AW, Munoz DF, Rust P, et al. Online tool to guide decisions for BRCA1/2 mutation carriers. *J. Clin. Oncol.* 2012;30(5):497-506, [PMID: 22231042]
Exclusion code: 5

Kurian AW, Sigal BM, Plevritis SK. Survival analysis of cancer risk reduction strategies for BRCA1/2 mutation carriers. *J. Clin. Oncol.* 2010;28(2):222-231, [PMID: 19996031]
Exclusion code: 5

Kuusisto KM, Bebel A, Vihinen M, Schleutker J, Sallinen S. Screening for BRCA1, BRCA2, CHEK2, PALB2, BRIP1, RAD50, and CDH1 mutations in high-risk Finnish BRCA1/2-founder mutation-negative breast and/or ovarian cancer individuals. *Breast Cancer Res.* 2011;13(1), [PMID: 21356067]
Exclusion code: 3

Kwiatkowski F, Dessenne P, Laquet C, Petit M-F, Bignon Y-J. Permanence of the information given during oncogenetic counseling to persons at familial risk of breast/ovarian and/or colon cancer. *Eur. J. Hum. Genet.* 2012;20(2):141-147, [PMID: 21934710]
Exclusion code: 3

Kwon JS, Daniels MS, Sun CC, Lu KH. Preventing future cancers by testing women with ovarian cancer for BRCA mutations. *J. Clin. Oncol.* 2010;28(4):675-682, [PMID: 19841329]
Exclusion code: 3

Kwon JS, Gutierrez-Barrera AM, Young D, et al. Expanding the criteria for BRCA mutation testing in breast cancer survivors. *J. Clin. Oncol.* 2010;28(27):4214-4220, [PMID: 20733129]
Exclusion code: 3

Kwon JS, Lenehan J, Carey M, Ainsworth P. Prolonged survival among women with BRCA germline mutations and advanced endometrial cancer: a case series. *Int J Gynecol Cancer.* 2008;18(3):546-549, [PMID: 17645508]
Exclusion code: 3

Kwong A, Ng EKO, Law FBF, et al. High-resolution melting analysis for rapid screening of BRCA2 founder mutations in Southern

Chinese breast cancer patients. *Breast Cancer Res. Treat.* 2010;122(2):605-607, [PMID: 20396944]
Exclusion code: 5

Kwong A, Wong CHN, Shea C, Suen DTK, Choi CLY. Choice of management of southern Chinese BRCA mutation carriers. *World J. Surg.* 2010;34(7):1416-1426, [PMID: 20182723]
Exclusion code: 8

Kwong A, Wong CHN, Suen DTK, et al. Accuracy of BRCA1/2 mutation prediction models for different ethnicities and genders: experience in a southern Chinese cohort. *World J. Surg.* 2012;36(4):702-713, [PMID: 22290208]
Exclusion code: 8

Lacey JV, Greene MH, Buys SS, et al. Ovarian cancer screening in women with a family history of breast or ovarian cancer. *Obstet. Gynecol.* 2006;108(5):1176-1184, [PMID: 17077240]
Exclusion code: 8

Lacour RA, Westin SN, Meyer LA, et al. Improved survival in non-Ashkenazi Jewish ovarian cancer patients with BRCA1 and BRCA2 gene mutations. *Gynecol. Oncol.* 2011;121(2):358-363, [PMID: 21276604]
Exclusion code: 3

Lacroix M, Nycum G, Godard B, Knoppers BM. Should physicians warn patients' relatives of genetic risks? *CMAJ.* 2008;178(5):593-595, [PMID: 18299550]
Exclusion code: 5

Lagos VI, Perez MA, Ricker CN, et al. Social-cognitive aspects of underserved Latinas preparing to undergo genetic cancer risk assessment for hereditary breast and ovarian cancer. *Psychooncology.* 2008;17(8):774-782, [PMID: 18646245]
Exclusion code: 3

Lai M, Yen M, Kuo H, al. e. Efficacy of breast-cancer screening for female relatives of breast-cancer-index cases: Taiwan multicentre cancer screening (TAMCAS). *Int. J. Cancer.* 1998;78:21-26, [PMID: 9724089]
Exclusion code: 8

Laitman Y, Kaufman B, Lahad EL, Papa MZ, Friedman E. Germline CHEK2 mutations in Jewish Ashkenazi women at high risk for breast cancer. *Isr Med Assoc J.* 2007;9(11):791-796, [PMID: 18085035]
Exclusion code: 3

Lakhani S, Manek S, Penault-Llorca F. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. *Clin. Cancer Res.* 2004;10(7):2473-2481, [PMID: 15073127]
Exclusion code: 2

Laki F, Kirova YM, This P, et al. Prophylactic salpingo-oophorectomy in a series of 89 women carrying a BRCA1 or a BRCA2 mutation. *Cancer.* 2007;109(9):1784-1790, [PMID: 17351952]
Exclusion code: 3

Lalloo F, Boggis CR, Evans DG, Shenton A, Threlfall AG, Howell A. Screening by mammography, women with a family history of breast cancer. *Eur. J. Cancer.* 1998;34(6):937-940, [PMID: 9797712]
Exclusion code: 8

Lancaster DR. Coping with appraised breast cancer risk among women with family histories of breast cancer. *Res. Nurs. Health.* 2005;28(2):144-158, [PMID: 15779052]
Exclusion code: 8

Lancaster JM, Powell CB, Kauff ND, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol. Oncol.* 2007;107(2):159-162, [PMID: 17950381]
Exclusion code: 2

Lancastle D, Brain K, Phelps C. Illness representations and distress in women undergoing screening for familial ovarian cancer. *Psychol Health.* 2011;26(12):1659-1677, [PMID: 21736431]
Exclusion code: 8

Lanceley A, Eagle Z, Ogden G, et al. Family history and women with ovarian cancer: is it asked and does it matter?: An observational

study. *Int J Gynecol Cancer.* 2012;22(2):254-259, [PMID: 22274317]
Exclusion code: 4

Land SR, Wickerham D, Costantino JP, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer Prevention: The NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. *JAMA.* 2006;295(23):2742-2751, [PMID: 16754728]
Exclusion code: 3

Landon G, Stewart J, Deavers M, Lu K, Sneige N. Peritoneal washing cytology in patients with BRCA1 or BRCA2 mutations undergoing risk-reducing salpingo-oophorectomies: a 10-year experience and reappraisal of its clinical utility. *Gynecol. Oncol.* 2012;125(3):683-686, [PMID: 22425664]
Exclusion code: 8

Landsbergen K, Verhaak C, Kraaimaat F, Hoogerbrugge N. Genetic uptake in BRCA-mutation families is related to emotional and behavioral communication characteristics of index patients. *Fam Cancer.* 2005;4(2):115-119, [PMID: 15951961]
Exclusion code: 8

Landsbergen KM, Brunner HG, Manders P, Hoogerbrugge N, Prins JB. Educational-support groups for BRCA mutation carriers satisfy need for information but do not affect emotional distress. *Genet. Couns.* 2010;21(4):423-437, [PMID: 21290972]
Exclusion code: 4

Landsbergen KM, Prins JB, Kamm YJL, Brunner HG, Hoogerbrugge N. Female BRCA mutation carriers with a preference for prophylactic mastectomy are more likely to participate an educational-support group and to proceed with the preferred intervention within 2 years. *Fam Cancer.* 2010;9(2):213-220, [PMID: 19967456]
Exclusion code: 8

Lapointe J, Abdous B, Camden S, et al. Influence of the family cluster effect on psychosocial variables in families undergoing BRCA1/2 genetic testing for cancer susceptibility. *Psychooncology.* 2012;21(5):515-523, [PMID: 21370312]
Exclusion code: 3

Lapointe J, Bouchard K, Patenaude AF, Maunsell E, Simard J, Dorval M. Incidence and predictors of positive and negative effects of BRCA1/2 genetic testing on familial relationships: A 3-year follow-up study. *Genet Med.* 2012;14(1):60-68, [PMID: 22237432]
Exclusion code: 3

Larouche G, Bouchard K, Chiquette J, Desbiens C, Simard J, Dorval M. Self-reported mammography use following BRCA1/2 genetic testing may be overestimated. *Fam Cancer.* 2012;11(1):27-32, [PMID: 22080962]
Exclusion code: 8

Leach MO, Eeles RA, Turnbull LW, et al. The UK national study of magnetic resonance imaging as a method of screening for breast cancer (MARIBS). *J. Exp. Clin. Cancer Res.* 2002;21(3 SUPPL.):107-114, [PMID: 12585664]
Exclusion code: 6

Leblond D, Bredart A, Dolbeault S, et al. [Cognitive, emotional and behavioral impact of an uncertain outcome after study of BRCA1/2: review of the literature]. *Bull. Cancer (Paris).* 2011;98(2):184-198, [PMID: 21382771]
Exclusion code: 7

Lech R, Przemyslaw O. Epidemiological models for breast cancer risk estimation. *Ginekol. Pol.* 2011;82(6):451-454, [PMID: 21853936]
Exclusion code: 5

Lee E, Ma H, McKean-Cowdin R, et al. Effect of reproductive factors and oral contraceptives on breast cancer risk in BRCA1/2 mutation carriers and noncarriers: results from a population-based study. *Cancer Epidemiol. Biomarkers Prev.* 2008;17(11):3170-3178, [PMID: 18990759]
Exclusion code: 3

Lee E-H, Park SK, Park B, et al. Effect of BRCA1/2 mutation on short-term and long-term

breast cancer survival: a systematic review and meta-analysis. *Breast Cancer Res. Treat.* 2010;122(1):11-25, [PMID: 20376556]
Exclusion code: 3

Lee E-O, Ahn S-H, You C, et al. Determining the main risk factors and high-risk groups of breast cancer using a predictive model for breast cancer risk assessment in South Korea. *Cancer Nurs.* 2004;27(5):400-406, [PMID: 15525868]
Exclusion code: 2

Lee JS, John EM, McGuire V, et al. Breast and ovarian cancer in relatives of cancer patients, with and without BRCA mutations. *Cancer Epidemiol. Biomarkers Prev.* 2006;15(2):359-363, [PMID: 16492929]
Exclusion code: 3

Lee R, Beattie M, Crawford B, et al. Recruitment, genetic counseling, and BRCA testing for underserved women at a public hospital. *Genet Test.* 2005;9(4):306-312, [PMID: 16379544]
Exclusion code: 4

Leeflang MMG, Deeks JJ, Gatsonis C, Bossuyt PMM, Cochrane Diagnostic Test Accuracy Working G. Systematic reviews of diagnostic test accuracy. *Ann. Intern. Med.* 2008;149(12):889-897, [PMID: 19075208]
Exclusion code: 2

Lehman CD, Blume JD, Weatherall P, et al. Screening women at high risk for breast cancer with mammography and magnetic resonance imaging. *Cancer.* 2005;103(9):1898-1905, [PMID: 15800894]
Exclusion code: 8

Lehman CD, Isaacs C, Schnall MD, et al. Cancer yield of mammography, MR, and US in high-risk women: prospective multi-institution breast cancer screening study. *Radiology.* 2007;244(2):381-388, [PMID: 17641362]
Exclusion code: 3

Lerman C, Hughes C, Lemon SJ, et al. What you don't know can hurt you: Adverse psychologic effects in members of BRCA1-linked and BRCA2-linked families who decline genetic testing. *J. Clin. Oncol.* 1998;16(5):1650-1654, [PMID: 9586874]
Exclusion code: 3

Lerman C, Narod S, Schulman K, et al. BRCA1 testing in families with hereditary breast-ovarian cancer. A prospective study of patient decision making and outcomes. *JAMA.* 1996;275(24):1885-1892, [PMID: 8648868]
Exclusion code: 8

Lessick M. Genetic testing for breast and ovarian cancer: ethical, legal, and psychosocial considerations. *Nurs Womens Health.* 2007;11(4):390-399; quiz 400-391, [PMID: 17883756]
Exclusion code: 5

Leunen K, Drijkoningen M, Neven P, et al. Prophylactic mastectomy in familial breast carcinoma. What do the pathologic findings learn us? *Breast Cancer Res. Treat.* 2008;107(1):79-86, [PMID: 17431765]
Exclusion code: 8

Levine D, Argenta P, Yee C. Fallopian tube and primary perirtoneal carcinomas associated with BRCA mutations. *J. Clin. Oncol.*;21(22):4222-4227, [PMID: 14615451]
Exclusion code: 2

Levy D, Garber J, Shields A. Guidelines for Genetic Risk Assessment of Hereditary Breast and Ovarian Cancer: Early Disagreements and Low Utilization. *J. Gen. Intern. Med.* 2009;24(7):822-828, [PMID: 19455369]
Exclusion code: 3

Levy DE, Byfield SD, Comstock CB, et al. Underutilization of BRCA1/2 testing to guide breast cancer treatment: black and Hispanic women particularly at risk. *Genet Med.* 2011;13(4):349-355, [PMID: 21358336]
Exclusion code: 3

Lewis MJ, Peterson SK. Perceptions of genetic testing for cancer predisposition among Ashkenazi Jewish women. *Community Genet.* 2007;10(2):72-81, [PMID: 17380056]
Exclusion code: 4

Li W-F, Hu Z, Rao N-Y, et al. The prevalence of BRCA1 and BRCA2 germline mutations in high-risk breast cancer patients of Chinese Han nationality: two recurrent mutations were identified. *Breast Cancer Res Treat.* 2008;110(1):99-109, [PMID: 17851763]
Exclusion code: 3

Lidén A, Berglund G, Hansson MG, Rosenquist R, Sjödén PO, Nordin K. Genetic Counselling for Cancer and Risk Perception. *Acta Oncol.* 2003;42(7):726-734, [PMID: 14690158]
Exclusion code: 6

Liede A, Karlan BY, Narod SA. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. *J. Clin. Oncol.* 2004;22(4):735-742, [PMID: 14966099]
Exclusion code: 2

Lifford KJ, Fraser L, Rosenthal AN, et al. Withdrawal from familial ovarian cancer screening for surgery: findings from a psychological evaluation study (PsyFOCS). *Gynecol. Oncol.* 2012;124(1):158-163, [PMID: 21999920]
Exclusion code: 8

Lim J, Macluran M, Price M, Bennett B, Butow P, kConFab Psychosocial G. Short- and long-term impact of receiving genetic mutation results in women at increased risk for hereditary breast cancer. *J Genet Couns.* 2004;13(2):115-133, [PMID: 15612172]
Exclusion code: 6

Lindberg NM, Wellisch DK. Identification of Traumatic Stress Reactions in Women at Increased Risk for Breast Cancer. *Psychosomatics.* 2004;45(1):7-16, [PMID: 14709756]
Exclusion code: 4

Lindor NM, Greene MH, Mayo Familial Cancer Program. The concise handbook of family cancer syndromes. *J. Natl. Cancer Inst.* 1998;90(14):1039-1071, [PMID: 9672254]
Exclusion code: 2

Lindor NM, Guidugli L, Wang X, et al. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). *Hum. Mutat.* 2012;33(1):8-21, [PMID: 21990134]
Exclusion code: 5

Lindor NM, Johnson KJ, Harvey H, et al. Predicting BRCA1 and BRCA2 gene mutation carriers: comparison of PENN II model to previous study. *Fam Cancer.* 2010;9(4):495-502, [PMID: 20512419]
Exclusion code: 3

Litton JK, Westin SN, Ready K, et al. Perception of screening and risk reduction surgeries in patients tested for a BRCA deleterious mutation. *Cancer.* 2009;115(8):1598-1604, [PMID: 19280625]
Exclusion code: 3

Lo S-H, Chernoff H, Cong L, Ding Y, Zheng T. Discovering interactions among BRCA1 and other candidate genes associated with sporadic breast cancer. *Proc. Natl. Acad. Sci. U. S. A.* 2008;105(34):12387-12392, [PMID: 18711133]
Exclusion code: 5

Loader S, Shields CG, Rowley PT. Impact of genetic testing for breast-ovarian cancer susceptibility. *Genet Test.* 2004;8(1):1-12, [PMID: 15140369]
Exclusion code: 6

Lobb E, Butow P, Meiser B, et al. The use of audiotapes in consultations with women from high risk breast cancer families: A randomised trial. *J. Med. Genet.* 2002;39(9):697-703, [PMID: 12205117]
Exclusion code: 3

Lobb E, Meiser B. Genetic counselling and prophylactic surgery in women from families with hereditary breast or ovarian cancer. *Lancet.* 2004;363(9424):1-4, [PMID: 15183619]
Exclusion code: 5

Lobb EA, Barlow-Stewart K, Suthers G, Hallowell N. Treatment-focused DNA testing for newly diagnosed breast cancer patients: some implications for clinical practice. *Clin. Genet.* 2010;77(4):350-354, [PMID: 19930416]
Exclusion code: 5

Lobb EA, Butow P, Barratt A, Meiser B, Tucker K. Differences in individual approaches: communication in the familial breast cancer consultation and the effect on patient outcomes. *J Genet Couns.* 2005;14(1):43-53, [PMID: 15789155]
Exclusion code: 8

Locke I, Kote-Jarai Z, Bancroft E, et al. Loss of heterozygosity at the BRCA1 and BRCA2 loci detected in ductal lavage fluid from BRCA gene mutation carriers and controls. *Cancer Epidemiol. Biomarkers Prev.* 2006;15(7):1399-1402, [PMID: 16835343]
Exclusion code: 5

Loescher LJ, Lim KH, Leitner O, Ray J, D'Souza J, Armstrong CM. Cancer surveillance behaviors in women presenting for clinical BRCA genetic susceptibility testing. *Oncol. Nurs. Forum.* 2009;36(2):E57-67, [PMID: 19273395]
Exclusion code: 8

Loizidou M, Marcou Y, Anastasiadou V, Newbold R, Hadjisavvas A, Kyriacou K. Contribution of BRCA1 and BRCA2 germline mutations to the incidence of early-onset breast cancer in Cyprus. *Clin. Genet.* 2007;71(2):165-170, [PMID: 17250666]
Exclusion code: 3

Loizidou MA, Cariolou MA, Neuhausen SL, et al. Genetic variation in genes interacting with BRCA1/2 and risk of breast cancer in the Cypriot population. *Breast Cancer Res. Treat.* 2010;121(1):147-156, [PMID: 19714462]
Exclusion code: 4

Loman N, Borg A. Improving surveillance and quality of life of BRCA mutation carriers. *J. Clin. Oncol.* 2010;28(22):e376-377, [PMID: 20458044]
Exclusion code: 5

Lorenzo Bermejo J, Hemminki K. A population-based assessment of the clustering of breast cancer in families eligible for testing of BRCA1 and BRCA2 mutations. *Ann. Oncol.* 2005;16(2):322-329, [PMID: 15668291]
Exclusion code: 3

Lostumbo L, Carbine NE, Wallace J. Prophylactic mastectomy for the prevention of breast cancer. *Cochrane Database Syst Rev.* 2010;11:CD002748, [PMID: 21069671]
Exclusion code: 3

Love RR. Population health, global bioethics and breast cancer treatment. *Oncology (Williston Park).* 2006;20(7):675, [PMID: 16841793]
Exclusion code: 5

Lowenfels AB, Maisonneuve P, Whitcomb DC. Risk factors for cancer in hereditary pancreatitis. *Med. Clin. North Am.* 2000;84(3):565-573, [PMID: 10872414]
Exclusion code: 5

Lowery JT, Byers T, Axell L, Ku L, Jacobellis J. The impact of direct-to-consumer marketing of cancer genetic testing on women according to their genetic risk. *Genet Med.* 2008;10(12):888-894, [PMID: 19092441]
Exclusion code: 2

Lubinski J, Korzen M, Gorski B, et al. Breast cancer susceptibility genes. *Journal of B.U.ON.* 2007;12 Suppl 1:S23-29, [PMID: 17935274]
Exclusion code: 5

Lubitz RJ, Komaromy M, Crawford B, et al. Development and pilot evaluation of novel genetic educational materials designed for an underserved patient population. *Genet Test.* 2007;11(3):276-290, [PMID: 17949289]
Exclusion code: 5

Lucassen A, Watson E, Harcourt J, Rose P, O'Grady J. Guidelines for referral to a regional genetics service: GPs respond by referring more appropriate cases. *Fam. Pract.* 2001;18(2):135-140, [PMID: 11264262]
Exclusion code: 2

Lux MP, Ackermann S, Bani MR, et al. Age of uptake of early cancer detection facilities by low-risk and high-risk patients with familial breast and ovarian cancer. *Eur. J. Cancer Prev.* 2005;14(6):503-511, [PMID: 16284494]
Exclusion code: 8

Lux MP, Ackermann S, Nestle-Kramling C, et al. Use of intensified early cancer detection in high-risk patients with familial breast and ovarian cancer. *Eur. J. Cancer Prev.* 2005;14(4):399-411, [PMID: 16030432]
Exclusion code: 3

Lux MP, Bani MR, Fasching PA, Beckmann MW. [Prophylactic surgery of mammary and ovarian carcinoma]. *Chirurg.* 2005;76(12):1145-1154, [PMID: 16237564]
Exclusion code: 7

Lux MP, Fasching PA, Beckmann MW. Hereditary breast and ovarian cancer: review and future perspectives. *J. Mol. Med.* 2006;84(1):16-28, [PMID: 16283147]
Exclusion code: 5

Lynch HT, Silva E, Snyder C, Lynch JF. Hereditary breast cancer: part I. Diagnosing hereditary breast cancer syndromes. *Breast J.* 2008;14(1):3-13, [PMID: 18086272]
Exclusion code: 5

Lynch HT, Snyder C, Lynch J. Hereditary breast cancer: practical pursuit for clinical translation. *Ann. Surg. Oncol.* 2012;19(6):1723-1731, [PMID: 22434244]
Exclusion code: 5

Lynch HT, Snyder C, Lynch JF, et al. Patient responses to the disclosure of BRCA mutation tests in hereditary breast-ovarian cancer families. *Cancer Genet. Cytogenet.* 2006;165(2):91-97, [PMID: 16527602]
Exclusion code: 3

Lynch HT, Snyder CL, Lynch JF, Ghate S, Narod SA, Gong G. Family information service participation increases the rates of mutation testing among members of families with BRCA1/2 mutations. *Breast J.* 2009;15 Suppl 1:S20-24, [PMID: 19775326]
Exclusion code: 8

MacDonald DJ, Sarna L, Weitzel JN, Ferrell B. Women's perceptions of the personal and family impact of genetic cancer risk assessment: focus group findings. *J Genet Couns.* 2010;19(2):148-160, [PMID: 19902342]
Exclusion code: 8

Machackova E, Foretova L, Lukesova M, et al. Spectrum and characterisation of BRCA1 and BRCA2 deleterious mutations in high-risk Czech patients with breast and/or ovarian cancer. *BMC Cancer.* 2008;8:140, [PMID: 18489799]
Exclusion code: 3

Mackay J, Schulz P, Rubinelli S, Pithers A. Online patient education and risk assessment: project OPERA from Cancerbackup. Putting inherited breast cancer risk information into context using argumentation theory. *Patient Educ. Couns.* 2007;67(3):261-266, [PMID: 17590305]
Exclusion code: 5

Mackenzie A, Patrick-Miller L, Bradbury AR. Controversies in communication of genetic risk for hereditary breast cancer. *Breast J.* 2009;15 Suppl 1:S25-32, [PMID: 19775327]
Exclusion code: 5

MacNew HG, Rudolph R, Brower ST, Beck AN, Meister EA. Assessing the knowledge and attitudes regarding genetic testing for breast cancer risk in our region of southeastern Georgia. *Breast J.* 2010;16(2):189-192, [PMID: 20030654]
Exclusion code: 3

Madalinska JB, Hollenstein J, Bleiker E, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. *J. Clin. Oncol.* 2005;23(28):6890-6898, [PMID: 16129845]
Exclusion code: 3

Madalinska JB, van Beurden M, Bleiker EMA, et al. The impact of hormone replacement therapy on menopausal symptoms in younger high-risk women after prophylactic salpingo-oophorectomy. *J. Clin. Oncol.* 2006;24(22):3576-3582, [PMID: 16877724]
Exclusion code: 3

Maheu C. Implications of living with a strong family history of breast cancer. *Can. J. Nurs. Res.* 2009;41(2):100-112, [PMID: 19650516]

Exclusion code: 8

Maheu C, Thorne S. Receiving inconclusive genetic test results: an interpretive description of the BRCA1/2 experience. *Res. Nurs. Health.* 2008;31(6):553-562, [PMID: 18449940]
Exclusion code: 3

Mahfoudh W, Bouaouina N, Ahmed SB, et al. Hereditary breast cancer in Middle Eastern and North African (MENA) populations: identification of novel, recurrent and founder BRCA1 mutations in the Tunisian population. *Mol. Biol. Rep.* 2012;39(2):1037-1046, [PMID: 21603858]
Exclusion code: 8

Mahon SM. Managing families with a hereditary cancer syndrome. *Oncol. Nurs. Forum.* 2011;38(6):641-644, [PMID: 22037327]
Exclusion code: 5

Mahoney MC. Breast cancer risk reduction and counseling: lifestyle, chemoprevention, and surgery. *J Natl Compr Canc Netw.* 2007;5(8):702-710, [PMID: 17927927]
Exclusion code: 5

Mai PL, Garceau AO, Graubard BI, et al. Confirmation of family cancer history reported in a population-based survey. *J. Natl. Cancer Inst.* 2011;103(10):788-797, [PMID: 21562245]
Exclusion code: 2

Mai PL, Lagos VI, Palomares MR, Weitzel JN. Contralateral risk-reducing mastectomy in young breast cancer patients with and without genetic cancer risk assessment. *Ann. Surg. Oncol.* 2008;15(12):3415-3421, [PMID: 18836779]
Exclusion code: 3

Mai PL, Wideroff L, Greene MH, Graubard BI. Prevalence of family history of breast, colorectal, prostate, and lung cancer in a population-based study. *Public Health Genomics.* 2010;13(7-8):495-503, [PMID: 20389042]
Exclusion code: 8

Majdak EJ, Debniak J, Milczek T, et al. Prognostic impact of BRCA1 pathogenic and BRCA1/BRCA2 unclassified variant mutations in patients with ovarian carcinoma. *Cancer.* 2005;104(5):1004-1012, [PMID: 16047333]
Exclusion code: 3

Majdak-Paredes EJ, Fatah F. Hereditary breast cancer syndromes and clinical implications. *J Plast Reconstr Aesthet Surg.* 2009;62(2):181-189, [PMID: 19027378]
Exclusion code: 5

Gene expression profiling tests for breast cancer (Project record). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010001443
Exclusion code: 5

Malacrida S, Agata S, Callegaro M, et al. BRCA1 p.Val1688del is a deleterious mutation that recurs in breast and ovarian cancer families from Northeast Italy. *J. Clin. Oncol.* 2008;26(1):26-31, [PMID: 18165637]
Exclusion code: 3

Malone KE, Begg CB, Haile RW, et al. Population-based study of the risk of second primary contralateral breast cancer associated with carrying a mutation in BRCA1 or BRCA2. *J. Clin. Oncol.* 2010;28(14):2404-2410, [PMID: 20368571]
Exclusion code: 3

Malone KE, Daling JR, Doody DR, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. *Cancer Res.* 2006;66(16):8297-8308, [PMID: 16912212]
Exclusion code: 2

Maloney E, Edgerson S, Robson M, et al. What women with breast cancer discuss with clinicians about risk for their adolescent daughters. *J Psychosoc Oncol.* 2012;30(4):484-502, [PMID: 22747109]
Exclusion code: 3

Malpas P. The right to remain in ignorance about genetic information--can such a right be

defended in the name of autonomy? *N. Z. Med. J.* 2005;118(1220):U1611, [PMID: 16132072]
Exclusion code: 5

Manchanda R, Burnell M, Abdelraheim A, et al. Factors influencing uptake and timing of risk reducing salpingo-oophorectomy in women at risk of familial ovarian cancer: a competing risk time to event analysis. *Bjog.* 2012;119(5):527-536, [PMID: 22260402]
Exclusion code: 8

Mancini J, Nogues C, Adenis C, et al. Impact of an information booklet on satisfaction and decision-making about BRCA genetic testing. *Eur. J. Cancer.* 2006;42(7):871-881, [PMID: 16563745]
Exclusion code: 8

Manders P, Pijpe A, Hooning MJ, et al. Body weight and risk of breast cancer in BRCA1/2 mutation carriers. *Breast Cancer Res. Treat.* 2011;126(1):193-202, [PMID: 20730487]
Exclusion code: 8

Mangerich B, Stichler JF. Breast and ovarian cancer: a new model for educating women. *Nurs Womens Health.* 2008;12(6):490-499, [PMID: 19121054]
Exclusion code: 5

Manguoglu E, Guran S, Yamac D, et al. Germline mutations of BRCA1 and BRCA2 genes in Turkish breast, ovarian, and prostate cancer patients. *Cancer Genet. Cytogenet.* 2010;203(2):230-237, [PMID: 21156238]
Exclusion code: 3

Manguoglu E, Guran S, Yamac D, et al. Genomic large rearrangement screening of BRCA1 and BRCA2 genes in high-risk Turkish breast/ovarian cancer patients by using multiplex ligation-dependent probe amplification assay. *Cancer Invest.* 2011;29(1):73-77, [PMID: 20919953]
Exclusion code: 3

Manne S, Audrain J, Schwartz M, Main D, Finch C, Lerman C. Associations between relationship support and psychological reactions of participants and partners to BRCA1 and BRCA2 testing in a clinic-based sample. *Ann. Behav. Med.* 2004;28(3):211-225, [PMID: 15576260]
Exclusion code: 8

Mannis G, Fehniger J, Creasman J, Jacoby VL, Beattie MS. Risk-Reducing Salpingo-oophorectomy and Ovarian Cancer Screening in 1077 Women After BRCA Testing. *JAMA.* 2013;173(2):96-103, [PMID: 23247828]
Exclusion code: 2

Marchbanks PA, McDonald JA, Wilson HG, et al. The NICHD Women's Contraceptive and Reproductive Experiences Study: Methods and operational results. *Ann. Epidemiol.* 2002;12(4):213-221, [PMID: 11988408]
Exclusion code: 3

Marchetti P, Di Rocco CZ, Ricevuto E, et al. Reducing breast cancer incidence in familial breast cancer: overlooking the present panorama. *Ann. Oncol.* 2004;15 (Suppl 1):I27-I34, [PMID: 15280184]
Exclusion code: 5

Marchina E, Fontana MG, Speziani M, et al. BRCA1 and BRCA2 genetic test in high risk patients and families: counselling and management. *Oncol. Rep.* 2010;24(6):1661-1667, [PMID: 21042765]
Exclusion code: 3

Impact of gene expression profiling tests on breast cancer outcomes (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32008000072
Exclusion code: 5

Margolin S, Lindblom A. Familial breast cancer, underlying genes, and clinical implications: a review. *Crit. Rev. Oncog.* 2006;12(1-2):75-113, [PMID: 17078207]
Exclusion code: 5

Margolin S, Werelius B, Fornander T, Lindblom A. BRCA1 mutations in a population-based study of breast cancer in Stockholm County.

Genet Test. 2004;8(2):127-132, [PMID: 15345109]
Exclusion code: 3

Markopoulos C, Tsaroucha AK, Kouskos E, Mantas D, Antonopoulou Z, Karvelis S. Impact of breast cancer surgery on the self-esteem and sexual life of female patients. *J. Int. Med. Res.* 2009;37(1):182-188, [PMID: 19215689]
Exclusion code: 3

Marpeau O, Ancel PY, Antoine M, Uzan S, Barranger E. [Synchronous bilateral breast cancer: risk factors, diagnosis, histology and treatment]. *Gynecol Obstet Fertil.* 2008;36(1):35-44, [PMID: 18178120]
Exclusion code: 7

Diagnostic performance of digital mammography in breast cancer screening (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000799.
Exclusion code: 5

Marshall E. European patents. BRCA2 claim faces new challenge. *Science.* 2005;308(5730):1851, [PMID: 15976277]
Exclusion code: 5

Marshall E. Patents. U.S. appeals court hears gene patent arguments. *Science.* 2012;337(6092):277-278, [PMID: 22822118]
Exclusion code: 2

Marshall M, Solomon S. Hereditary breast-ovarian cancer: clinical findings and medical management. *Plast. Surg. Nurs.* 2007;27(3):124-127, [PMID: 17901820]
Exclusion code: 5

Effects of communicating DNA-based disease risk estimates on risk-reducing behaviours. 2011. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=coch&AN=00075320-100000000-05884.
Exclusion code: 5

Martin W, Degner L. Perception of risk and surveillance practices of women with a family history of breast cancer. *Cancer Nurs.* 2006;29(3):227-235, [PMID: 16783123]
Exclusion code: 8

Marzo Castillejo M. [Prevention of breast cancer 2007. New evidence?]. *Aten. Primaria.* 2007;39(3):115-117, [PMID: 17386201]
Exclusion code: 7

Masood S. The knowledge about risk for breast cancer: the patients' right to know. *Breast J.* 2008;14(3):219-220, [PMID: 18476881]
Exclusion code: 5

Matalqah LM, Radaideh KM, Yusoff ZM, Awaisu A. Health-related quality of life using EQ-5D among breast cancer survivors in comparison with age-matched peers from the general population in the state of Penang, Malaysia. *Journal of Public Health.* 2011;19(5):475-480.
Exclusion code: 2

Matloff E, Caplan A. Direct to confusion: lessons learned from marketing BRCA testing. *Am J Bioeth.* 2008;8(6):5-8, [PMID: 18726769]
Exclusion code: 5

Matloff ET, Brierley KL. The double-helix derailed: the story of the BRCA patent. *Lancet.* 2010;376(9738):314-315, [PMID: 20674708]
Exclusion code: 5

Matthijs G. The European opposition against the BRCA gene patents. *Fam Cancer.* 2006;5(1):95-102, [PMID: 16528613]
Exclusion code: 5

Maurice A, Evans DGR, Shenton A, et al. Screening younger women with a family history of breast cancer--does early detection improve outcome? *Eur. J. Cancer.* 2006;42(10):1385-1390, [PMID: 16750910]
Exclusion code: 3

Mavaddat N, Barrowdale D, Andrulis I. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *Cancer*

Epidemiol. Biomarkers Prev. 2012;21(1):134-147, [PMID: 22144499]
Exclusion code: 2

McClain MR, Nathanson KL, Palomaki GE, Haddow JE. An evaluation of BRCA1 and BRCA2 founder mutations penetrance estimates for breast cancer among Ashkenazi Jewish women. *Genet Med.* 2005;7(1):34-39, [PMID: 15654226]
Exclusion code: 5

McClain MR, Palomaki GE, Nathanson KL, Haddow JE. Adjusting the estimated proportion of breast cancer cases associated with BRCA1 and BRCA2 mutations: public health implications. *Genet Med.* 2005;7(1):28-33, [PMID: 15654225]
Exclusion code: 8

McDaniel SH. The psychotherapy of genetics. *Fam. Process.* 2005;44(1):25-44, [PMID: 15807076]
Exclusion code: 5

BRCA1 and BRCA2 predictive genetic testing for breast and ovarian cancers: a systematic review of clinical evidence (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006000281
Exclusion code: 5

A clinical systematic review of BRCA1 and BRCA2 genetic testing for breast and ovarian cancers (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006000282
Exclusion code: 5

McGaughey A. Body image after bilateral prophylactic mastectomy: An integrative literature review. *J Midwifery Womens Health.* 2006;51(6):e45-e49, [PMID: 17081926]
Exclusion code: 5

McGuire V, Felberg A, Mills M, et al. Relation of contraceptive and reproductive history to ovarian cancer risk in carriers and noncarriers of BRCA1 gene mutations. *Am. J. Epidemiol.* 2004;160(7):613-618, [PMID: 15383404]
Exclusion code: 6

McHorney CA, Ware Jr JE, Lu JF, Sherbourne CD. The MOS 36-item Short-Form Health Survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patient groups. *Med. Care.* 1994;32(1):40-66, [PMID: 8277801]
Exclusion code: 2

McInerney-Leo A, Biesecker BB, Hadley DW, et al. BRCA1/2 testing in hereditary breast and ovarian cancer families: effectiveness of problem-solving training as a counseling intervention. *Am J Med Genet A.* 2004;130A(3):221-227, [PMID: 15378542]
Exclusion code: 3

McInerney-Leo A, Biesecker BB, Hadley DW, et al. BRCA1/2 testing in hereditary breast and ovarian cancer families II: impact on relationships. *Am J Med Genet A.* 2005;133A(2):165-169, [PMID: 15633195]
Exclusion code: 8

McInerney-Leo A, Hadley D, Kase RG, Giambarresi TR, Struewing JP, Biesecker BB. BRCA1/2 testing in hereditary breast and ovarian cancer families III: risk perception and screening. *Am J Med Genet A.* 2006;140(20):2198-2206, [PMID: 16969872]
Exclusion code: 8

McKinnon W, Naud S, Ashikaga T, Colletti R, Wood M. Results of an intervention for individuals and families with BRCA mutations: a model for providing medical updates and psychosocial support following genetic testing. *J Genet Couns.* 2007;16(4):433-456, [PMID: 17594133]
Exclusion code: 8

McLaughlin JR, Risch HA, Lubinski J, et al. Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study. *Lancet Oncol.* 2007;8(1):26-34, [PMID: 17196508]
Exclusion code: 8

McPherson E. Genetic diagnosis and testing in clinical practice. *Clin Med Res.* 2006;4(2):123-129, [PMID: 16809405]
Exclusion code: 5

Mealiffe ME, Stokowski RP, Rhees BK, Prentice RL, Pettinger M, Hinds DA. Assessment of clinical validity of a breast cancer risk model combining genetic and clinical information. *J. Natl. Cancer Inst.* 2010;102(21):1618-1627, [PMID: 20956782]
Exclusion code: 3

Medeiros F, Muto M, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am. J. Surg. Pathol.* 2006;30(2):230-236, [PMID: 16434898]
Exclusion code: 2

Meeuwissen PAM, Seynaeve C, Brekelmans CTM, Meijers-Heijboer HJ, Klijn JGM, Burger CW. Outcome of surveillance and prophylactic salpingo-oophorectomy in asymptomatic women at high risk for ovarian cancer. *Gynecol. Oncol.* 2005;97(2):476-482, [PMID: 15863147]
Exclusion code: 3

Mefford H, Baumbach L, Panguluri R. Evidence for BRCA1 founder mutation in families of West African ancestry. *Am J Hum Genet.* 1999;65(2):575-578, [PMID: 10417303]
Exclusion code: 2

Meijers-Heijboer H, van Geel B, van Putten WLJ, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. *N. Engl. J. Med.* 2001;345(3):159-164, [PMID: 1463009]
Exclusion code: 3

Meilleur KG, Littleton-Kearney MT. Interventions to improve patient education regarding multifactorial genetic conditions: a systematic review. *Am J Med Genet A.* 2009;149A(4):819-830, [PMID: 19291763]
Exclusion code: 8

Meindl A. Comprehensive analysis of 989 patients with breast or ovarian cancer provides BRCA1 and BRCA2 mutation profiles and frequencies for the German population. *Int. J. Cancer.* 2002;97(4):472-480, [PMID: 11802209]
Exclusion code: 8

Meiser B. Psychological impact of genetic testing for cancer susceptibility: an update of the literature. *Psychooncology.* 2005;14(12):1060-1074, [PMID: 15937976]
Exclusion code: 6

Meiser B, Gaff C, Julian-Reynier C, et al. International perspectives on genetic counseling and testing for breast cancer risk. *Breast Dis.* 2006;27:109-125, [PMID: 17917143]
Exclusion code: 5

Mellon S, Gold R, Janisse J, et al. Risk perception and cancer worries in families at increased risk of familial breast/ovarian cancer. *Psychooncology.* 2008;17(8):756-766, [PMID: 18613300]
Exclusion code: 3

Menon U, Harper J, Sharma A, et al. Views of BRCA gene mutation carriers on preimplantation genetic diagnosis as a reproductive option for hereditary breast and ovarian cancer. *Hum. Reprod.* 2007;22(6):1573-1577, [PMID: 17428877]
Exclusion code: 8

Menon U, Skates SJ, Lewis S, et al. Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer. *J. Clin. Oncol.* 2005;23(31):7919-7926, [PMID: 16258091]
Exclusion code: 3

Metcalfe A, Werrett J, Burgess L, Clifford C. Psychosocial impact of the lack of information given at referral about familial risk for cancer. *Psychooncology.* 2007;16(5):458-465, [PMID: 16933207]
Exclusion code: 4

Metcalfe K, Gershman S, Lynch HT, et al. Predictors of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. *Br. J. Cancer.* 2011;104(9):1384-1392, [PMID: 21487411]
Exclusion code: 3

Metcalfe K, Lynch HT, Ghadirian P, et al. Contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. *J. Clin. Oncol.* 2004;22(12):2328-2335, [PMID: 15197194]
Exclusion code: 3

Metcalfe KA, Birenbaum-Carmeli D, Lubinski J, et al. International variation in rates of uptake of preventive options in BRCA1 and BRCA2 mutation carriers. *Int. J. Cancer.* 2008;122(9):2017-2022, [PMID: 18196574]
Exclusion code: 8

Metcalfe KA, Foulkes WD, Kim-Sing C, et al. Family history as a predictor of uptake of cancer preventive procedures by women with a BRCA1 or BRCA2 mutation. *Clin. Genet.* 2008;73(5):474-479, [PMID: 18341607]
Exclusion code: 8

Metcalfe KA, Poll A, Llacuachaqui M, et al. Patient satisfaction and cancer-related distress among unselected Jewish women undergoing genetic testing for BRCA1 and BRCA2. *Clin. Genet.* 2010;78(5):411-417, [PMID: 20653694]
Exclusion code: 3

Metcalfe KA, Poll A, O'Connor A, et al. Development and testing of a decision aid for breast cancer prevention for women with a BRCA1 or BRCA2 mutation. *Clin. Genet.* 2007;72(3):208-217, [PMID: 17718858]
Exclusion code: 8

Metcalfe KA, Semple JL, Narod SA. Satisfaction with breast reconstruction in women with bilateral prophylactic mastectomy: A descriptive study. *Plast. Reconstr. Surg.* 2004;114(2):360-366, [PMID: 15277800]
Exclusion code: 8

Michigan Department of Community Health (MDCH). *BRCA 1/2 Surveillance in Michigan, 2008-2012*. Lansing, MI; 2012.
Exclusion code: 2

Miki Y, Swensen J, Shattuck-Eidens D, et al. A Strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science.* 1994;266(5182):66-71, [PMID: 7545954]
Exclusion code: 2

Mikkelsen EM, Sunde L, Johansen C, Johnsen SP. Psychosocial conditions of women awaiting genetic counseling: a population-based study. *J Genet Couns.* 2008;17(3):242-251, [PMID: 18256912]
Exclusion code: 3

Miller SM, Fleisher L, Roussi P, et al. Facilitating informed decision making about breast cancer risk and genetic counseling among women calling the NCI's Cancer Information Service. *J Health Commun.* 2005;10 Suppl 1:119-136, [PMID: 16377604]
Exclusion code: 3

Miller SM, Roussi P, Daly MB, et al. Enhanced counseling for women undergoing BRCA1/2 testing: impact on subsequent decision making about risk reduction behaviors. *Health Educ. Behav.* 2005;32(5):654-667, [PMID: 16148211]
Exclusion code: 3

Milne RL, Antoniou AC. Genetic modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. *Ann. Oncol.* 2011;22(SUPPL. 1):i11-i17, [PMID: 21285145]
Exclusion code: 2

Milne RL, Knight JA, John EM, et al. Oral contraceptive use and risk of early-onset breast cancer in carriers and noncarriers of BRCA1 and BRCA2 mutations. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(2):350-356, [PMID: 15734957]
Exclusion code: 3

Minckwitz GV, Prieshof B, Jackisch C, et al. First experience with gosereline and ibandronate as medical prevention in premenopausal patients with increased familiary breast cancer risk: The GISS study [abstract]. *Third International Conference and Ninth Annual Meeting of the International Society of Cancer Chemoprevention.* Vol 2. 2004
Exclusion code: 5

Miramar MD, Calvo MT, Rodriguez A, et al. Genetic analysis of BRCA1 and BRCA2 in breast/ovarian cancer families from Aragon (Spain): two novel truncating mutations and a large genomic deletion in BRCA1. *Breast*

Cancer Res. Treat. 2008;112(2):353-358, [PMID: 18176857]
Exclusion code: 4

Miser WF. Cancer screening in the primary care setting: the role of the primary care physician in screening for breast, cervical, colorectal, lung, ovarian, and prostate cancers. *Prim. Care.* 2007;34(1):137-167, [PMID: 17481991]
Exclusion code: 5

Mislowsky A, Domchek S, Stroede C, et al. Breast cancer surgery trend changes since the introduction of BRCA1/2 mutation screening: a retrospective cohort analysis of 158 mutation carriers treated at a single institution. *Ann. Surg. Oncol.* 2011;18(3):745-751, [PMID: 20972632]
Exclusion code: 8

Mitchell RJ, Farrington SM, Dunlop MG, Campbell H. Mismatch repair genes hMLH1 and hMSH2 and colorectal cancer: A HuGE review. *Am. J. Epidemiol.* 2002;156(10):885-902, [PMID: 12419761]
Exclusion code: 6

Moatter T, Aban M, Khan S, Azam I, Pervez S. BRCA1 status in Pakistani breast cancer patients with moderate family history. *J Coll Physicians Surg Pak.* 2011;21(11):680-684, [PMID: 22078348]
Exclusion code: 3

Modan B, Hartge P, Hirsh-Yechezkel G, et al. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a BRCA1 or BRCA2 mutation. *N. Engl. J. Med.* 2001;345(4):235-240, [PMID: 11474660]
Exclusion code: 3

Møller P, Borg A, Gareth Evans D, et al. Survival in prospectively ascertained familial breast cancer: Analysis of a series stratified by tumour characteristics, BRCA mutations and oophorectomy. *Int. J. Cancer.* 2002;101(6):555-559, [PMID: 12237897]
Exclusion code: 6

Moller P, Evans DG, Reis MM, et al. Surveillance for familial breast cancer: Differences in outcome according to BRCA mutation status. *Int. J. Cancer.* 2007;121(5):1017-1020, [PMID: 17471561]
Exclusion code: 3

Moller P, Evans G, Haites N, et al. Guidelines for follow-up of women at high risk for inherited breast cancer: consensus statement from the Biomed 2 Demonstration Programme on Inherited Breast Cancer. *Dis. Markers.* 1999;15(1-3):207-211, [PMID: 10595280]
Exclusion code: 2

Møller P, Hagen AI, Apold J, et al. Genetic epidemiology of BRCA mutations - family history detects less than 50% of the mutation carriers. *Eur. J. Cancer.* 2007;43(11):1713-1717, [PMID: 17574839]
Exclusion code: 3

Moller P, Maehle L, Heimdal K, et al. Inherited breast carcinoma--prospective findings in 1,194 women at risk. *Acta Oncol.* 1996;35 Suppl 8:7-11, [PMID: 9073043]
Exclusion code: 8

Moller P, Reis MM, Evans G, et al. Efficacy of early diagnosis and treatment in women with a family history of breast cancer. *Dis. Markers.* 1999;15(1-3):179-186, [PMID: 10595275]
Exclusion code: 6

Montgomery KG, Chang J-H, Gertig DM, et al. The AIB1 glutamine repeat polymorphism is not associated with risk of breast cancer before age 40 years in Australian women. *Breast Cancer Res.* 2005;7(3):R353-356, [PMID: 15987430]
Exclusion code: 3

Moorman PG, Iversen ES, Marcom PK, et al. Evaluation of established breast cancer risk factors as modifiers of BRCA1 or BRCA2: a multi-center case-only analysis. *Breast Cancer Res. Treat.* 2010;124(2):441-451, [PMID: 20309627]
Exclusion code: 5

Moorman PG, Skinner CS, Evans JP, et al. Racial differences in enrolment in a cancer genetics registry. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(8):1349-1354, [PMID: 15298957]

Exclusion code: 5

Mor P, Oberle K. Ethical issues related to BRCA gene testing in orthodox Jewish women. *Nurs. Ethics.* 2008;15(4):512-522, [PMID: 18515440]
Exclusion code: 5

Mora S, Robertson C, Guerrieri GA, et al. A randomized 2 x 2 biomarker trial of low-dose tamoxifen and fenretinide in premenopausal women at high risk for breast cancer [abstract P41]. *Third International Conference and Ninth Annual Meeting of the International Society of Cancer Chemoprevention.* Vol 2. 2004.
Exclusion code: 5

Morgan D, Sylvester H, Lucas FL, Miesfeldt S. Cancer prevention and screening practices among women at risk for hereditary breast and ovarian cancer after genetic counseling in the community setting. *Fam Cancer.* 2009;8(4):277-287, [PMID: 19347608]
Exclusion code: 8

Morgan D, Sylvester H, Lucas FL, Miesfeldt S. Hereditary breast and ovarian cancer: referral source for genetic assessment and communication regarding assessment with nongenetic clinicians in the community setting. *Genet Med.* 2010;12(1):25-31, [PMID: 20027114]
Exclusion code: 3

Morgan D, Sylvester H, Lucas FL, Miesfeldt S. Perceptions of high-risk care and barriers to care among women at risk for hereditary breast and ovarian cancer following genetic counseling in the community setting. *J Genet Couns.* 2010;19(1):44-54, [PMID: 19809867]
Exclusion code: 8

Morris EA, Liberman L, Ballon DJ, et al. MRI of occult breast carcinoma in a high-risk population. *Am. J. Roentgenol.* 2003;181(3):619-626, [PMID: 12933450]
Exclusion code: 2

Morrison V, Henderson BJ, Taylor C, A'Ch Dafydd N, Unwin A. The impact of information order on intentions to undergo predictive genetic testing: an experimental study. *J Health Psychol.* 2010;15(7):1082-1092, [PMID: 20472604]
Exclusion code: 8

Mosca L, Barrett-Connor E, Wenger NK, et al. Design and methods of the Raloxifene Use for The Heart (RUTH) study. *Am. J. Cardiol.* 2001;88(4):392-395, [PMID: 11545760]
Exclusion code: 2

Moslehi R, Chu W, Karlan B, et al. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. *Am. J. Hum. Genet.* 2000;66(4):1259-1272, [PMID: 10739756]
Exclusion code: 2

Mouchawar J, Hensley-Alford S, Laurion S, et al. Impact of direct-to-consumer advertising for hereditary breast cancer testing on genetic services at a managed care organization: a naturally-occurring experiment. *Genet Med.* 2005;7(3):191-197, [PMID: 15775755]
Exclusion code: 2

Mouchawar J, Laurion S, Ritzwoller DP, Ellis J, Kulchak-Rahm A, Hensley-Alford S. Assessing controversial direct-to-consumer advertising for hereditary breast cancer testing: reactions from women and their physicians in a managed care organization. *Am. J. Manag. Care.* 2005;11(10):601-608, [PMID: 16232001]
Exclusion code: 2

Mouchawar J, Valentine Goins K, Somkin C, et al. Guidelines for breast and ovarian cancer genetic counseling referral: Adoption and implementation in HMOs. *Genet Med.* 2003;5(6):444-450, [PMID: 14614396]
Exclusion code: 2

MRI screening for breast cancer in genetically high-risk women (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006000584
Exclusion code: 5

MRI screening for breast cancer: screening for breast cancer with MRI in genetically high-risk women. Horizon Scanning Prioritising Summary

- Volume 3 (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006000592
Exclusion code: 5

Murday V, Pears R, Ball J, Eeles R, Hodgson S. An audit of screening for familial breast cancer before 50 years in the South Thames Region - Have we got it right? *Fam Cancer.* 2004;3(1):29-34, [PMID: 15131403]
Exclusion code: 8

Murff HJ, Spigel DR, Syngal S. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. *JAMA.* 2004;292(12):1480-1489, [PMID: 15383520]
Exclusion code: 2

Murray ML, Cerrato F, Bennett RL, Jarvik GP. Follow-up of carriers of BRCA1 and BRCA2 variants of unknown significance: variant reclassification and surgical decisions. *Genet Med.* 2011;13(12):998-1005, [PMID: 21811163]
Exclusion code: 3

Genomic tests for ovarian cancer detection and management (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006001022
Exclusion code: 5

Myers MF, Chang M-H, Jorgensen C, et al. Genetic testing for susceptibility to breast and ovarian cancer: evaluating the impact of a direct-to-consumer marketing campaign on physicians' knowledge and practices. *Genet Med.* 2006;8(6):361-370, [PMID: 16778598]
Exclusion code: 2

Myriad. BRAC Analysis. 2010; www.myriad.com/products/brcanalysis.php. Accessed 24 Oct, 2012
Exclusion code: 2

Myriad. Myriad PRO BRCA1 and BRCA2 Prevalence Tables. 2012; https://www.myriadpro.com/bracanalysis-prevalence-tables. Accessed 26 Feb 2013Exclusion code: 2

Myriad Genetic Laboratories. *BRACAnalysis Technical Specifications.* 2011.
Exclusion code: 2

Nagy Z, Csanad M, Toth K, Mate S, Joo JG. [Clinical-genetic care of BRCA-mutation carrier women: prevention, diagnosis and therapy]. *Orv. Hetil.* 2011;152(23):913-918, [PMID: 21592951]
Exclusion code: 7

Narod SA. Modifiers of risk of hereditary breast cancer. *Oncogene.* 2006;25(43):5832-5836, [PMID: 16998497]
Exclusion code: 5

Narod SA. Testing for CHEK2 in the cancer genetics clinic: ready for prime time? *Clin. Genet.* 2010;78(1):1-7, [PMID: 20597917]
Exclusion code: 3

Narod SA. BRCA mutations in the management of breast cancer: the state of the art. *Nat Rev Clin Oncol.* 2010;7(12):702-707, [PMID: 20956982]
Exclusion code: 5

Narod SA. The impact of contralateral mastectomy on mortality in BRCA1 and BRCA2 mutation carriers with breast cancer. *Breast Cancer Res. Treat.* 2011;128(2):581-583, [PMID: 21455666]
Exclusion code: 3

Narod SA. Genetic variants associated with breast-cancer risk. *Lancet Oncol.* 2011;12(5):415-416, [PMID: 21514220]
Exclusion code: 5

Narod SA. Screening of women at high risk for breast cancer. *Prev. Med.* 2011;53(3):127-130, [PMID: 21745498]
Exclusion code: 5

Narod SA, Brunet JS, Ghadirian P, et al. Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: A case-control study. *Lancet.* 2000;356(9245):1876-1881, [PMID: 11130383]

Exclusion code: 3

Narod SA, Dubé MP, Klijn J, et al. Oral contraceptives and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *J. Natl. Cancer Inst.* 2002;94(23):1773-1779, [PMID: 12464649]
Exclusion code: 8

Narod SA, Lubinski J, Ghadirian P, et al. Screening mammography and risk of breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. *Lancet Oncol.* 2006;7(5):402-406, [PMID: 16648044]
Exclusion code: 4

Narod SA, Offit K. Prevention and management of hereditary breast cancer. *J. Clin. Oncol.* 2005;23(8):1656-1663, [PMID: 15755973]
Exclusion code: 5

Narod SA, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. *N. Engl. J. Med.* 1998;339(7):424-428, [PMID: 9700175]
Exclusion code: 6

Narod SA, Sun P, Ghadirian P, et al. Tubal ligation and risk of ovarian cancer in carriers of BRCA1 or BRCA2 mutations: A case-control study. *Lancet.* 2001;357(9267):1467-1470, [PMID: 11377596]
Exclusion code: 6

National Breast Cancer Center. *Advice about familial aspects of breast cancer and ovarian cancer - A guide for health professionals.* Sydney: National Breast Cancer Center; 2000.
Exclusion code: 2

National Cancer Institute. Cancer Statistics Review, 1975–2005. http://seer.cancer.gov/csr/1975_2005/index.html. Accessed 10 October 2012.
Exclusion code: 2

National Cancer Institute. PDQ® Cancer Information Summary. 2009; http://www.cancer.gov/cancertopics/pdq/genetics/breast-and-ovarian/healthprofessional. Accessed 10 October 2012.
Exclusion code: 2

National Cancer Institute. Breast Cancer. 2012; http://www.cancer.gov/cancertopics/types/breast. Accessed 10 October 2012.
Exclusion code: 2

National Cancer Institute. Ovarian Cancer. 2012; http://www.cancer.gov/cancertopics/types/ovarian. Accessed 10 October 2012. Exclusion code: 2

National Cancer Institute. (PDQ®) Breast Cancer Screening. 2013; http://www.cancer.gov/cancertopics/pdq/screening/breast/healthprofessional/page1/AllPages. Accessed 20 Feb, 2013
Exclusion code: 2

National Cancer Institute. Breast Cancer. 2013; http://www.cancer.gov/cancertopics/types/breast. Accessed 4 March 2013
Exclusion code: 2

National Cancer Institute. Ovarian Cancer. 2013; http://www.cancer.gov/cancertopics/types/ovarian. Accessed 4 March 2013
Exclusion code: 2

National Cancer Institute. PDQ® Breast Cancer Treatment. 2013; http://www.cancer.gov/cancertopics/pdq/treatment/breast/healthprofessional. Accessed 6 Mar, 2013
Exclusion code: 2

National Cancer Institute. Ovarian Epithelial Cancer Treatment (PDQ®). 2013; http://cancer.gov/cancertopics/pdq/treatment/ovarianepithelial/HealthProfessional. Accessed 06 Mar, 2013
Exclusion code: 2

NCCN Clinical Practice Guidelines in Oncology: Breast Cancer Screening and Diagnosis. 2011. http://www.nccn.org/professionals/physician_gls/pdf/breast-screening.pdf. Accessed 16 Oct 2012
Exclusion code: 5

National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Breast

and Ovarian. 2012; http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf. Accessed 27 Feb, 2013
Exclusion code: 2

MammaPrint (gene test) for breast cancer - prognostic test: horizon scanning technology briefing (Project record). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32008100372
Exclusion code: 5

Oncotype DX prognostic and predictive test for early breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010000622
Exclusion code: 5

Familial breast cancer: the classification and care of women at risk of familial breast cancer in primary, secondary and tertiary care (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32004000675
Exclusion code: 5

Negura L, Uhrhammer N, Negura A, Artenie V, Carasevici E, Bignon Y-J. Complete BRCA mutation screening in breast and ovarian cancer predisposition families from a North-Eastern Romanian population. *Fam Cancer.* 2010;9(4):519-523, [PMID: 20567915]
Exclusion code: 3

Nelson H, Huffman L, Fu R, Harris E, Walker M, Bougatsos C. *Genetic Risk Assessment and BRCA Mutation Testing for Breast and Ovarian Cancer Susceptibility: Evidence Synthesis.* Rockville, MD: Agency for Healthcare Research and Quality; 2005
Exclusion code: 2

Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: evidence synthesis (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32005001123
Exclusion code: 5

Nelson H, Smith M, Griffin J, Fu R. Use of Medications to Reduce Risk for Primary Breast Cancer: Systematic Review for the 1 U.S. Preventive Services Task Force [in press]. *Ann. Intern. Med.* 2013.
Exclusion code: 2

Nelson H, Smith M, Griffin J, Fu R. Use of Medications to Reduce Risk for Primary Breast Cancer: Systematic Review for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 2012 (In press), [PMID: 20722176]
Exclusion code: 2

Screening for breast cancer: systematic evidence review update for the U.S. Preventive Services Task Force (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32011000569
Exclusion code: 5

Nelson HD, Fu R, Griffin JC, Nygren P, Smith MEB, Humphrey L. Systematic review: comparative effectiveness of medications to reduce risk for primary breast cancer. *Ann. Intern. Med.* 2009;151(10):703-715, W-226-735, [PMID: 19920271]
Exclusion code: 2

Nelson HD, Fu R, Humphrey L, Smith M, Griffin JC, Nygren P. *Comparative Effectiveness of Medications to Reduce Risk of Primary Breast Cancer in Women*: Agency for Healthcare Research and Quality; 2009
Exclusion code: 2

Nelson HD, Zakher B, Cantor A, et al. Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. *Ann. Intern. Med.* 2012;156(9):635-648, [PMID: 22547473]
Exclusion code: 2

Nelson NJ. Breast cancer prevention in high-risk women: searching for new options. *J. Natl.*

Cancer Inst. 2011;103(9):710-711, [PMID: 21515834]
Exclusion code: 5

Neuhausen S, Gilewski T, Norton L, et al. Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nat. Genet.* 1996;13(1):126-128, [PMID: 8673092]
Exclusion code: 2

Neuman B. *The Neuman Systems Model*. 3 ed. Norwalk, Conn: Appleton & Lange; 1995.
Exclusion code: 5

The early detection and diagnosis of breast cancer: a literature review - an update (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-31998009908
Exclusion code: 5

Newman LA. Applicability of the Gail model for breast cancer risk assessment in Turkish female population and evaluation of breastfeeding as a risk factor. *Breast Cancer Res. Treat.* 2010;120(2):425-426, [PMID: 19967560]
Exclusion code: 5

NIH Consensus Development Panel. NIH Consensus conference. Ovarian cancer: Screening, treatment, and follow-up *JAMA.* 1995;273(6):491-497, [PMID: 7837369]
Exclusion code: 2

Nisker JA. The need for public education: "Surveillance and risk reduction strategies" for women at risk for carrying BRCA gene mutations. *J Obstet Gynaecol Can.* 2007;29(6):510-511, [PMID: 17568484]
Exclusion code: 5

Predictive genetic testing for breast and prostate cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-31999008425
Exclusion code: 5

Nordin K, Roshanai A, Bjorvatn C, et al. Is genetic counseling a stressful event? *Acta Oncol.* 2011;50(7):1089-1097, [PMID: 21864049]
Exclusion code: 3

Notaridou M, Quaye L, Dafou D, et al. Common alleles in candidate susceptibility genes associated with risk and development of epithelial ovarian cancer. *Int. J. Cancer.* 2011;128(9):2063-2074, [PMID: 20635389]
Exclusion code: 4

Nusbaum R, Isaacs C. Management updates for women with a BRCA1 or BRCA2 mutation. *Mol Diagn Ther.* 2007;11(3):133-144, [PMID: 17570734]
Exclusion code: 5

Obdeijn I-MA, Loo CE, Rijnsburger AJ, et al. Assessment of false-negative cases of breast MR imaging in women with a familial or genetic predisposition. *Breast Cancer Res. Treat.* 2010;119(2):399-407, [PMID: 19876732]
Exclusion code: 5

Ockhuysen-Vermey CF, Henneman L, van Asperen CJ, Oosterwijk JC, Menko FH, Timmermans DRM. Design of the BRISC study: a multicentre controlled clinical trial to optimize the communication of breast cancer risks in genetic counselling. *BMC Cancer.* 2008;8:283, [PMID: 18834503]
Exclusion code: 5

O'Connell G, Arnold A. Tamoxifen and cancer of the endometrium. . *Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne.* 1993;148:2113-2114, [PMID: 8324682]
Exclusion code: 5

O'Doherty K. Agency and choice in genetic counseling: Acknowledging patients' concerns. *J Genet Couns.* 2009;18(5):464-474, [PMID: 19714455]
Exclusion code: 8

O'Doherty K, Suthers GK. Risky communication: pitfalls in counseling about risk, and how to avoid them. *J Genet Couns.* 2007;16(4):409-417, [PMID: 17473963]

Exclusion code: 5

Oei AL, Massuger LF, Bulten J, Ligtenberg MJ, Hoogerbrugge N, De Hullu JA. Surveillance of women at high risk for hereditary ovarian cancer is inefficient. *Br. J. Cancer.* 2006;94(6):814-819, [PMID: 16495917]
Exclusion code: 3

Oestreicher N, Ramsey SD, Linden HM, et al. Gene expression profiling and breast cancer care: what are the potential benefits and policy implications? *Genet Med.* 2005;7(6):380-389, [PMID: 16024969]
Exclusion code: 5

Offit K. BRCA Mutation Frequency and Penetrance: New Data, Old Debate. *J. Natl. Cancer Inst.* 2006;98(23):1675-1677, [PMID: 17148764]
Exclusion code: 5

Offit K, Groeger E, Turner S, Wadsworth EA, Weiser MA. The "duty to warn" a patient's family members about hereditary disease risks. *JAMA.* 2004;292(12):1469-1473, [PMID: 15383518]
Exclusion code: 2

Olaya W, Esquivel P, Wong JH, et al. Disparities in BRCA testing: when insurance coverage is not a barrier. *Am. J. Surg.* 2009;198(4):562-565, [PMID: 19800469]
Exclusion code: 8

Olivier RI, Lubsen-Brandsma MAC, Verhoef S, Van Beurden M. CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer. *Gynecol. Oncol.* 2006;100(1):20-26, [PMID: 16188302]
Exclusion code: 8

Olivier RI, Van Beurden M, Lubsen MAC, et al. Clinical outcome of prophylactic oophorectomy in BRCA1/BRCA2 mutation carriers and events during follow-up. *Br. J. Cancer.* 2004;90(8):1492-1497, [PMID: 15083174]
Exclusion code: 3

Olopade OI. Using genetic analysis to individualize preventive measures for breast and ovarian cancers. *Nat Clin Pract Oncol.* 2006;3(4):182-183, [PMID: 16596140]
Exclusion code: 5

Olopade OI, Artioli G. Efficacy of Risk-Reducing Salpingo-Oophorectomy in Women with BRCA-1 and BRCA-2 Mutations. *Breast J.* 2004;10(1 SUPPL.):S5-S9, [PMID: 14984481]
Exclusion code: 5

Onay VU, Briollais L, Knight JA, et al. SNP-SNP interactions in breast cancer susceptibility. *BMC Cancer.* 2006;6:114, [PMID: 16672066]
Exclusion code: 3

O'Neill SC, DeMarco T, Peshkin BN, et al. Tolerance for uncertainty and perceived risk among women receiving uninformative BRCA1/2 test results. *Am J Med Genet C Semin Med Genet.* 2006;142(4):251-259, [PMID: 17024668]
Exclusion code: 3

O'Neill SC, Rini C, Goldsmith RE, Valdimarsdottir H, Cohen LH, Schwartz MD. Distress among women receiving uninformative BRCA1/2 results: 12-month outcomes. *Psychooncology.* 2009;18(10):1088-1096, [PMID: 19214961]
Exclusion code: 3

O'Neill SC, Valdimarsdottir HB, Demarco TA, et al. BRCA1/2 test results impact risk management attitudes, intentions, and uptake. *Breast Cancer Res. Treat.* 2010;124(3):755-764, [PMID: 20383578]
Exclusion code: 8

O'Neill SM, Peters JA, Vogel VG, Feingold E, Rubinstein WS. Referral to cancer genetic counseling: are there stages of readiness? *Am J Med Genet C Semin Med Genet.* 2006;142C(4):221-231, [PMID: 17068804]
Exclusion code: 8

O'Quinn C, Steele P, Ludman MD, Kieser K. Hereditary breast ovarian cancer syndromes in the Maritimes. *J Obstet Gynaecol Can.* 2010;32(2):155-159, [PMID: 20181317]

Exclusion code: 5

Ormondroyd E, Moynihan C, Ardern-Jones A, et al. Communicating genetics research results to families: problems arising when the patient participant is deceased. *Psychooncology.* 2008;17(8):804-811, [PMID: 18688787]
Exclusion code: 8

Ormondroyd E, Moynihan C, Watson M, et al. Disclosure of genetics research results after the death of the patient participant: a qualitative study of the impact on relatives. *J Genet Couns.* 2007;16(4):527-538, [PMID: 17492498]
Exclusion code: 5

Hysterectomy versus hysterectomy plus oophorectomy for premenopausal women. 2010. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=coch&AN=00075320-100000000-04582.
Exclusion code: 3

Osorio A, Milne RL, Alonso R, et al. Evaluation of the XRCC1 gene as a phenotypic modifier in BRCA1/2 mutation carriers. Results from the consortium of investigators of modifiers of BRCA1/BRCA2. *Br. J. Cancer.* 2011;104(8):1356-1361, [PMID: 21427728]
Exclusion code: 8

Ozakinci G. *Psychological and behavioral outcomes of genetic testing for BRCA1/2 mutations among Ashkenazi Jewish women.* New Brunswick, NJ, Rutgers, The State University of New Jersey; 2004
Exclusion code: 2

Ozakinci G, Humphris G, Steel M. Provision of breast cancer risk information to women at the lower end of the familial risk spectrum. *Community Genet.* 2007;10(1):41-44, [PMID: 17167250]
Exclusion code: 8

Ozanne EM, Wittenberg E, Garber JE, Weeks JC. Breast cancer prevention: patient decision making and risk communication in the high risk setting. *Breast J.* 2010;16(1):38-47, [PMID: 19889168]
Exclusion code: 3

Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer.* 2005;104(12):2807-2816, [PMID: 16284991]
Exclusion code: 3

Pal T, Permuth-Wey J, Holtje T, Sutphen R. BRCA1 and BRCA2 mutations in a study of African American breast cancer patients. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(11 Pt 1):1794-1799, [PMID: 15533909]
Exclusion code: 3

Paley PJ, Swisher EM, Garcia RL, et al. Occult cancer of the fallopian tube in BRCA-1 germline mutation carriers at prophylactic oophorectomy: a case for recommending hysterectomy at surgical prophylaxis. *Gynecol. Oncol.* 2001;80:176-180, [PMID: 11161856]
Exclusion code: 2

Palli D, Falchetti M, Masala G, et al. Association between the BRCA2 N372H variant and male breast cancer risk: a population-based case-control study in Tuscany, Central Italy. *BMC Cancer.* 2007;7:170, [PMID: 17767707]
Exclusion code: 3

Palma MD, Domchek SM, Stopfer J, et al. The relative contribution of point mutations and genomic rearrangements in BRCA1 and BRCA2 in high-risk breast cancer families. *Cancer Res.* 2008;68(17):7006-7014, [PMID: 18703817]
Exclusion code: 3

Palmer MK, Ribeiro GG. Thirty-four year follow up of patients with breast cancer in clinical trial of postoperative radiotherapy. *Br. Med. J. (Clin. Res. Ed).* 1985;291(6502):1088-1091, [PMID: 3931806]
Exclusion code: 3

Palmero EI, Ashton-Prolla P, da Rocha JCC, et al. Clinical characterization and risk profile of individuals seeking genetic counseling for hereditary breast cancer in Brazil. *J Genet Couns.* 2007;16(3):363-371, [PMID: 17318454]
Exclusion code: 4

Palmero EI, Caleffi M, Schüler-Faccini L, et al. Population prevalence of hereditary breast

cancer phenotypes and implementation of a genetic cancer risk assessment program in southern Brazil. *Genet Mol Biol.* 2009;32(3):447-455, [PMID: 21637504]
Exclusion code: 8

Palmieri G, Palomba G, Cossu A, et al. BRCA1 and BRCA2 germline mutations in Sardinian breast cancer families and their implications for genetic counseling. *Ann. Oncol.* 2002;13(12):1899-1907, [PMID: 12453858]
Exclusion code: 3

Paradise J, Andrews L, Holbrook T. Intellectual property. Patents on human genes: an analysis of scope and claims. *Science.* 2005;307(5715):1566-1567, [PMID: 15761140]
Exclusion code: 5

Paradiso A, Bruno M, Cicoria O, et al. Analysis of the reasons for accepting or declining participation in genetic research for breast cancer: a hospital-based population study. *Tumori.* 2004;90(4):435-436, [PMID: 15510991]
Exclusion code: 8

Paradiso A, Muggia F. Familial breast cancer screening: ethical and social implications. *Ann. Oncol.* 2004;15 Suppl 1:I5-I6, [PMID: 15280180]
Exclusion code: 5

Park N-J, Kang D-H, Weaver MT. Objective and subjective breast cancer risk: Relationships with natural killer cell activity and psychological distress in healthy women. *Cancer Nurs.* 2010;33(6):411-420, [PMID: 20562616]
Exclusion code: 4

Parker WH, Broder MS, Chang E, et al. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. *Obstet. Gynecol.* 2009;113(5):1027-1037, [PMID: 19384117]
Exclusion code: 4

Parkerson GR, Broadhead WE, Tse CKJ. Original contributions. Validation of the Duke Social Support and Stress Scale. *Fam. Med.* 1991;23(5):357-360, [PMID: 1884930]
Exclusion code: 2

Parmigiani G, Chen S, Iversen ES, Jr., et al. Validity of models for predicting BRCA1 and BRCA2 mutations. *Ann. Intern. Med.* 2007;147(7):441-450, [PMID: 17909205]
Exclusion code: 5

Parthasarathy S. Architectures of genetic medicine: comparing genetic testing for breast cancer in the USA and the UK. *Soc Stud Sci.* 2005;35(1):5-40, [PMID: 15991444]
Exclusion code: 4

Passaperuma K, Warner E, Causer PA, et al. Long-term results of screening with magnetic resonance imaging in women with BRCA mutations. *Br. J. Cancer.* 2012;107(1):24-30, [PMID: 22588560]
Exclusion code: 3

Patenaude AF. Helping your patients to deal with a predisposition to genetic disease. *JAAPA.* 2009;22(11):68-69, [PMID: 19999182]
Exclusion code: 5

Patenaude AF. *Prophylactic mastectomy: Insights from women how chose to reduce their risk*. Santa Barbara, CA: Praeger/ABC-CLIO; US; 2012, [PMID: Book: 2012-03561-000]
Exclusion code: 5

Patenaude AF, Dorval M, DiGianni LS, Schneider KA, Chittenden A, Garber JE. Sharing BRCA1/2 test results with first-degree relatives: factors predicting who women tell. *J. Clin. Oncol.* 2006;24(4):700-706, [PMID: 16446344]
Exclusion code: 8

Patenaude AF, Julian-Reynier C. Cancer genetic testing: Current and emerging issues. *Psychooncology.* 2008;17(8):733-736, [PMID: 18688786]
Exclusion code: 5

Patenaude AF, Orozco S, Li X, et al. Support needs and acceptability of psychological and peer consultation: attitudes of 108 women who had undergone or were considering prophylactic

mastectomy. *Psychooncology.* 2008;17(8):831-843, [PMID: 18636423]
Exclusion code: 4

Patmasiriwat P, Bhothisuwan K, Sinilnikova OM, et al. Analysis of breast cancer susceptibility genes BRCA1 and BRCA2 in Thai familial and isolated early-onset breast and ovarian cancer. *Hum. Mutat.* 2002;20(3):230, [PMID: 12203997]
Exclusion code: 8

Peacock S, Apicella C, Andrews L, et al. A discrete choice experiment of preferences for genetic counselling among Jewish women seeking cancer genetics services. *Br. J. Cancer.* 2006;95(10):1448-1453, [PMID: 17102813]
Exclusion code: 8

Peelen T, van Vliet M, Petrij-Bosch A, et al. A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. *Am J Hum Genet.* 1997;60(5):1041-1049, [PMID: 9150151]
Exclusion code: 2

Pellegrini I, Rapti M, Extra JM, et al. Tailored chemotherapy based on tumour gene expression analysis: breast cancer patients' misinterpretations and positive attitudes. *Eur. J Cancer Care.* 2012;21(2):242-250, [PMID: 22070677]
Exclusion code: 3

Peng S, Lü B, Ruan W, Zhu Y, Sheng H, Lai M. Genetic polymorphisms and breast cancer risk: Evidence from meta-analyses, pooled analyses, and genome-wide association studies. *Breast Cancer Res. Treat.* 2011;127(2):309-324, [PMID: 21445572]
Exclusion code: 2

Pennisi VR, Capozzi A. Subcutaneous mastectomy data. *Aesthetic Plast. Surg.* 1989;13:15-21, [PMID: 2728994]
Exclusion code: 2

Perez GK, Cruess DG, Cruess S, et al. Attitudes toward direct-to-consumer advertisements and online genetic testing among high-risk women participating in a hereditary cancer clinic. *J Health Commun.* 2011;16(6):607-628, [PMID: 21432710]
Exclusion code: 2

Perez Segura P, Jimenez P, Olivera H, et al. Risk-reduction surgery in BRCA mutation carriers in a Spanish population: adherence and results. *Clin Transl Oncol.* 2008;10(10):660-664, [PMID: 18940747]
Exclusion code: 3

Perry CE. *Managing susceptibility to hereditary breast and ovarian cancer*, Perry, Cynthia E : U San Diego, US; 2006
Exclusion code: 5

Peshkin BN. Breast cancer risk assessment and genetic testing: complexities, conundrums, and community. *Breast Dis.* 2006;27:1-3, [PMID: 17917137]
Exclusion code: 5

Peshkin BN, Demarco TA, Tercyak KP. On the development of a decision support intervention for mothers undergoing BRCA1/2 cancer genetic testing regarding communicating test results to their children. *Fam Cancer.* 2010;9(1):89-97, [PMID: 19609726]
Exclusion code: 5

Peshkin BN, Isaacs C. Evaluation and management of women with BRCA1/2 mutations. *Oncology (Williston Park).* 2005;19(11):1451-1459; discussion 1459-1468 1474, [PMID: 16370446]
Exclusion code: 5

Peshkin BN, Nusbaum RH, DeMarco TA. Genetic counseling about reproductive options for hereditary cancer: what is the standard of care? *J. Clin. Oncol.* 2007;25(7):911-912; author reply 913, [PMID: 17327618]
Exclusion code: 5

Petersen RW, Quinlivan JA. Preventing anxiety and depression in gynaecological cancer: a randomised controlled trial. *Bjog.* 2002;109(4):386-394, [PMID: 12013159]
Exclusion code: 3

Peto J, Collins N, Barfoot R, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J. Natl. Cancer Inst.* 1999;91(11):943-949, [PMID: 10359546]
Exclusion code: 2

Petracci E, Decarli A, Schairer C, et al. Risk factor modification and projections of absolute breast cancer risk. *J. Natl. Cancer Inst.* 2011;103(13):1037-1048, [PMID: 21705679]
Exclusion code: 2

Petrucelli N, Daly MB, Feldman GL. Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2. *Genet Med.* 2010;12(5):245-259, [PMID: 20216074]
Exclusion code: 5

False-negative results in screening programmes: systematic review of impact and implications (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32000008231
Exclusion code: 5

Petticrew MP, Sowden AJ, Lister Sharp D, Wright K. False-negative results in screening programmes: systematic review of impact and implications. *Health Technol. Assess.* 2000;4(5):1-120, [PMID: 10859208]
Exclusion code: 5

Pharoah PD, Day NE, Duffy S, Easton DF, Ponder BA. Family history and the risk of breast cancer: a systematic review and meta-analysis. *Int. J. Cancer.* 1997;71(5):800-809, [PMID: 9180149]
Exclusion code: 2

Phelps C, Wood F, Bennett P, Brain K, Gray J. Knowledge and expectations of women undergoing cancer genetic risk assessment: a qualitative analysis of free-text questionnaire comments. *J Genet Couns.* 2007;16(4):505-514, [PMID: 17318449]
Exclusion code: 4

Phillips KA, Jenkins MA, Lindeman GJ, et al. Risk-reducing surgery, screening and chemoprevention practices of BRCA1 and BRCA2 mutation carriers: a prospective cohort study. *Clin. Genet.* 2006;70(3):198-206, [PMID: 16922722]
Exclusion code: 8

Pichert G, Stahel RA. Organizing cancer genetics programs: the Swiss model. *J. Clin. Oncol.* 2000;18(21 Suppl):65S-69S, [PMID: 11060330]
Exclusion code: 2

Pierce BL, Carlson CS, Kuszler PC, Stanford JL, Austin MA. The impact of patents on the development of genome-based clinical diagnostics: an analysis of case studies. *Genet Med.* 2009;11(3):202-209, [PMID: 19367193]
Exclusion code: 2

Pieterse AH, van Dulmen AM, Beemer FA, Bensing JM, Ausems MG. Cancer genetic counseling: Communication and counselees' post-visit satisfaction, cognitions, anxiety, and needs fulfillment. *J Genet Couns.* 2007;16(1):85-96, [PMID: 17295054]
Exclusion code: 3

Pieterse AH, van Dulmen S, van Dijk S, Bensing JM, Ausems MGEM. Risk communication in completed series of breast cancer genetic counseling visits. *Genet Med.* 2006;8(11):688-696, [PMID: 17108760]
Exclusion code: 3

Pieterse K, van Dooren S, Seynaeve C, et al. Passive coping and psychological distress in women adhering to regular breast cancer surveillance. *Psychooncology.* 2007;16(9):851-858, [PMID: 17219399]
Exclusion code: 8

Pietschmann A, Mehdipour P, Atri M, et al. Mutation analysis of BRCA1 and BRCA2 genes in Iranian high risk breast cancer families. *J. Cancer Res. Clin. Oncol.* 2005;131(8):552-558, [PMID: 15918047]
Exclusion code: 8

Pijpe A, Andrieu N, Easton DF, et al. Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations:

retrospective cohort study (GENE-RAD-RISK). *BMJ*. 2012;345, [PMID: 22956590]
Exclusion code: 3

Piver MS, Jishi MF, Tsukada Y, Nava G. Primary peritoneal carcinoma after prophylactic oophorectomy in women with a family history of ovarian cancer. A report of the Gilda Radner Familial Ovarian Cancer Registry. *Cancer.* 1993;71(9):2751-2755, [PMID: 8467455]
Exclusion code: 2

Pizzichetta MA, Giuliotto N, Calzavara GI, et al. Study of chemoprevention in breast carcinoma with tamoxifen. *Tumori.* 1994;80(4):110.
Exclusion code: 5

Plon SE, Cooper HP, Parks B, et al. Genetic testing and cancer risk management recommendations by physicians for at-risk relatives. *Genet Med.* 2011;13(2):148-154, [PMID: 21224735]
Exclusion code: 8

Plon SE, Peterson LE, Friedman LC, Richards CS. Mammography behavior after receiving a negative BRCA1 mutation test result in the Ashkenazim: A community-based study. *Genet Med.* 2000;2(6):307-311, [PMID: 11339650]
Exclusion code: 6

Posluszny DM, McFeeley S, Hall L, Baum A. Stress, breast cancer risk, and breast self-examination: Chronic effects of risk and worry. *Journal of Applied Biobehavioral Research.* 2004;9(2):91-105.
Exclusion code: 2

Power TE. *The decision to undergo genetic testing for BRCA1/2 in a community sample of Ashkenazi Jewish women: Coping with the risk of cancer or coping with anticipated emotion?*, Power, Tara E : U Western Ontario, Canada; 2006
Exclusion code: 8

Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet.* 1998;352(9122):98-101, [PMID: 9672274]
Exclusion code: 2

Powles TJ. Chemoprevention of breast cancer using tamoxifen. *Endocr Relat Cancer.* 1997;4:255-260.
Exclusion code: 5

Powles TJ, Davey JB, McKinna A. A feasibility trial of tamoxifen chemoprevention of breast cancer in Great Britain. *Cancer Invest.* 1988;6(5):621-624, [PMID: 3063341]
Exclusion code: 5

Powles TJ, Hickish TF, Kedar R. Update of the Royal Marsden Hospital tamoxifen prevention programme in healthy women at increased risk of breast cancer. *Proceedings of American Society of Clincial Oncology.* Vol 131994
Exclusion code: 5

Powles TJ, Jones AL, Ashley SE, et al. The Royal Marsden Hospital pilot tamoxifen chemoprevention trial. *Breast Cancer Res. Treat.* 1994;31(1):73-82, [PMID: 7981459]
Exclusion code: 5

Powles TJ, McKinna A, Davey J. Chemoprevention of breast cancer. *J. Endocrinol.* 1993;137(3 suppl):S32.
Exclusion code: 5

Press N, Reynolds S, Pinsky L, Murthy V, Leo M, Burke W. 'That's like chopping off a finger because you're afraid it might get broken': disease and illness in women's views of prophylactic mastectomy. *Soc. Sci. Med.* 2005;61(5):1106-1117, [PMID: 15955410]
Exclusion code: 8

Pruthi S, Gostout BS, Lindor NM. Identification and Management of Women With BRCA Mutations or Hereditary Predisposition for Breast and Ovarian Cancer. *Mayo Clin. Proc.* 2010;85(12):1111-1120, [PMID: 21123638]
Exclusion code: 5

Qiu L-X, Yao L, Xue K, et al. BRCA2 N372H polymorphism and breast cancer susceptibility: a meta-analysis involving 44,903 subjects. *Breast Cancer Res. Treat.* 2010;123(2):487-490, [PMID: 20135345]

Exclusion code: 5

Quach J, Porter K, Leventhal H, Kelly KM. Health behaviors among Ashkenazi Jewish individuals receiving counseling for BRCA1 and BRCA2 mutations. *Fam Cancer.* 2009;8(3):241-250, [PMID: 19184534]
Exclusion code: 8

Quillin JM, Bodurtha JN, McClish D, Wilson DB. Genetic risk, perceived risk, and cancer worry in daughters of breast cancer patients. *J Genet Couns.* 2011;20(2):157-164, [PMID: 21132457]
Exclusion code: 4

Quillin JM, Fries E, McClish D, deParedes ES, Bodurtha J. Gail Model Risk Assessment and Risk Perceptions. *J. Behav. Med.* 2004;27(2):205-214, [PMID: 15171107]
Exclusion code: 8

Quillin JM, Lyckholm LJ. A principle-based approach to ethical issues in predictive genetic testing for breast cancer. *Breast Dis.* 2007;27:137-148, [PMID: 17917145]
Exclusion code: 5

Quillin JM, McClish DK, Jones RM, Burruss K, Bodurtha JN. Spiritual coping, family history, and perceived risk for breast cancer--can we make sense of it? *J Genet Couns.* 2006;15(6):449-460, [PMID: 17013546]
Exclusion code: 4

Quinn GP, Vadaparampil ST, Bower B, Friedman S, Keefe DL. Decisions and ethical issues among BRCA carriers and the use of preimplantation genetic diagnosis. *Minerva Med.* 2009;100(5):371-383, [PMID: 19910890]
Exclusion code: 5

Radner LL. *Cancer-free women living with the breast cancer gene mutation: A narrative investigation*, Radner, Lori L : Michigan School of Professional Psychology, US; 2012
Exclusion code: 4

Radtke JV, Terhorst L, Cohen SM. The menopause-specific quality of life questionnaire: Psychometric evaluation among breast cancer survivors. *Menopause.* 2011;18(3):289-295, [PMID: 20881889]
Exclusion code: 2

Rafnar T, Benediktsdottir KR, Eldon BJ, et al. BRCA2, but not BRCA1, mutations account for familial ovarian cancer in Iceland: a population-based study. *Eur. J. Cancer.* 2004;40(18):2788-2793, [PMID: 15571962]
Exclusion code: 3

Rahm AK. *Direct-to-consumer genetics: Media messages and public perceptions*. Denver: Health and Bahvioral Science, University of Colorado Denver; 2010
Exclusion code: 2

Rahman N, Stratton MR. The genetics of breast cancer susceptibility. *Annu. Rev. Genet.* 1998;32:95-121, [PMID: 9928476]
Exclusion code: 2

Ramus SJ, Antoniou AC, Kuchenbaecker KB, et al. Ovarian cancer susceptibility alleles and risk of ovarian cancer in BRCA1 and BRCA2 mutation carriers. *Hum. Mutat.* 2012;33(4):690-702, [PMID: 22253144]
Exclusion code: 8

Ratajska M, Brozek I, Senkus-Konefka E, et al. BRCA1 and BRCA2 point mutations and large rearrangements in breast and ovarian cancer families in Northern Poland. *Oncol. Rep.* 2008;19(1):263-268, [PMID: 18097605]
Exclusion code: 8

Ray JA, Loescher LJ, Brewer M. Risk-reduction surgery decisions in high-risk women seen for genetic counseling. *J Genet Couns.* 2005;14(6):473-484, [PMID: 16388328]
Exclusion code: 8

Ready KJ, Vogel KJ, Atchley DP, et al. Accuracy of the BRCAPRO model among women with bilateral breast cancer. *Cancer.* 2009;115(4):725-730, [PMID: 19127556]
Exclusion code: 3

Rebbeck TR. Prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers. *Eur. J.*

Cancer. 2002;38(Suppl 6):S15-S17, [PMID: 12409058]
Exclusion code: 5

Rebbeck TR. Inherited predisposition and breast cancer: Modifiers of BRCA1/2-associated breast cancer risk. *Environ. Mol. Mutagen.* 2002;39(2-3):228-234, [PMID: 11921193]
Exclusion code: 5

Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral Prophylactic Mastectomy Reduces Breast Cancer Risk in BRCA1 and BRCA2 Mutation Carriers: the PROSE Study Group. *J. Clin. Oncol.* 2004;22(6):1055-1062, [PMID: 14981104]
Exclusion code: 5

Rebbeck TR, Friebel T, Wagner T, et al. Effect of short-term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. *J. Clin. Oncol.* 2005;23(31):7804-7810, [PMID: 16219936]
Exclusion code: 4

Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. *J. Natl. Cancer Inst.* 2009;101(2):80-87, [PMID: 19141781]
Exclusion code: 5

Rebbeck TR, Levin AM, Eisen A, et al. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. *J. Natl. Cancer Inst.* 1999;91(17):1475-1479, [PMID: 10469748]
Exclusion code: 5

Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. *N. Engl. J. Med.* 2002;346(21):1616-1622, [PMID: 12023993]
Exclusion code: 5

Rebbeck TR, Mitra N, Domchek SM, et al. Modification of BRCA1-Associated Breast and Ovarian Cancer Risk by BRCA1-Interacting Genes. *Cancer Res.* 2011;71(17):5792-5805, [PMID: 21799032]
Exclusion code: 4

Rebbeck TR, Wang Y, Kantoff PW, et al. Modification of BRCA1- and BRCA2-associated breast cancer risk by AIB1 genotype and reproductive history. *Cancer Res.* 2001;61(14):5420-5424, [PMID: 11454686]
Exclusion code: 8

Rees G, Fry A, Cull A, Sutton S. Illness perceptions and distress in women at increased risk of breast cancer. *Psychol Health.* 2004;19(6):749-765.
Exclusion code: 4

Rees G, Gaff C, Young M-A, Martin PR. Health beliefs and behaviors of women who have received genetic counseling for breast cancer. *J Genet Couns.* 2007;16(4):457-468, [PMID: 17619128]
Exclusion code: 5

Reis MM, Tavakoli M, Dewar J, et al. Evaluation of a surveillance programme for women with a family history of breast cancer. *J. Med. Genet.* 2009;46(5):319-323, [PMID: 19279022]
Exclusion code: 3

Rennert G, Bisland-Naggan S, Barnett-Griness O, et al. Clinical outcomes of breast cancer in carriers of BRCA1 and BRCA2 mutations. *N. Engl. J. Med.* 2007;357(2):115-123, [PMID: 17625123]
Exclusion code: 3

Rennert G, Dishon S, Rennert HS, Fares F. Differences in the characteristics of families with BRCA1 and BRCA2 mutations in Israel. *Eur. J. Cancer Prev.* 2005;14(4):357-361, [PMID: 16030426]
Exclusion code: 8

Rhiem K, Foth D, Wappenschmidt B, et al. Risk-reducing salpingo-oophorectomy in BRCA1 and BRCA2 mutation carriers. *Arch. Gynecol. Obstet.* 2011;283(3):623-627, [PMID: 20428881]
Exclusion code: 3

Richardson H, Johnston D, Pater J, Goss P. The National Cancer Institute of Canada Clinical Trials Group MAP.3 trial: An international breast cancer prevention trial. *Curr Oncol.* 2007;14(3):89-95, [PMID: 17593981]
Exclusion code: 5

Ridge Y, Panabaker K, McCullum M, Portigal-Todd C, Scott J, McGillivray B. Evaluation of group genetic counseling for hereditary breast and ovarian cancer. *J Genet Couns.* 2009;18(1):87-100, [PMID: 19127417]
Exclusion code: 8

Riedl CC, Ponhold L, Flöry D, et al. Magnetic resonance imaging of the breast improves detection of invasive cancer, preinvasive cancer, and premalignant lesions during surveillance of women at high risk for breast cancer. *Clin. Cancer Res.* 2007;13(20):6144-6152, [PMID: 17947480]
Exclusion code: 3

Riley BD, Culver JO, Skrzynia C, et al. Essential elements of genetic cancer risk assessment, counseling, and testing: updated recommendations of the National Society of Genetic Counselors. *J Genet Couns.* 2012;21(2):151-161, [PMID: 22134580]
Exclusion code: 2

Riman T, Persson I, Nilsson S. Hormonal aspects of epithelial ovarian cancer: Review of epidemiological evidence. *Clin. Endocrinol. (Oxf).* 1998;49(6):695-707, [PMID: 10209555]
Exclusion code: 5

Rimer BK, Sugarman J, Winer E, Bluman LG, Lerman C. Informed consent for BRCA1 and BRCA2 testing. *Breast Dis.* 1998;10(1-2):99-114, [PMID: 15687553]
Exclusion code: 6

Rimes KA, Salkovskis PM, Jones L, Lucassen AM. Applying a cognitive-behavioral model of health anxiety in a cancer genetics service. *Health Psychol.* 2006;25(2):171-180, [PMID: 16569108]
Exclusion code: 3

Rini C, O'Neill SC, Valdimarsdottir H, et al. Cognitive and emotional factors predicting decisional conflict among high-risk breast cancer survivors who receive uninformative BRCA1/2 results. *Health Psychol.* 2009;28(5):569-578, [PMID: 19751083]
Exclusion code: 3

Ripperger T, Gadzicki D, Meindl A, Schlegelberger B. Breast cancer susceptibility: current knowledge and implications for genetic counselling. *Eur. J. Hum. Genet.* 2009;17(6):722-731, [PMID: 19092773]
Exclusion code: 5

Robertson C, Arcot Ragupathy SK, Boachie C, et al. The clinical effectiveness and costeffectiveness of different surveillance mammography regimens after the treatment for primary breast cancer: Systematic reviews, registry database analyses and economic evaluation. *Health Technol. Assess.* 2011;15(34):V-136, [PMID: 21951942]
Exclusion code: 8

Robertson L, Hanson H, Seal S, et al. BRCA1 testing should be offered to individuals with triple-negative breast cancer diagnosed below 50 years. *Br. J. Cancer.* 2012;106(6):1234-1238, [PMID: 22333603]
Exclusion code: 2

Robles-Diaz L, Goldfrank DJ, Kauff ND, Robson M, Offit K. Hereditary ovarian cancer in Ashkenazi Jews. *Fam Cancer.* 2004;3(3-4):259-264, [PMID: 15516850]
Exclusion code: 5

Robson M. Do women remain at risk even if they do not inherit a familial BRCA1/2 mutation? *J. Clin. Oncol.* 2011;29(34):4477-4478, [PMID: 22042956]
Exclusion code: 5

Robson M, Gilewski T, al. e. BRCA-associated breast cancer in young women. *J. Clin. Oncol.* 1998;16:1642-1649, [PMID: 9586873]
Exclusion code: 2

Robson M, Offit K. Clinical practice. Management of an inherited predisposition to

breast cancer. *N. Engl. J. Med.* 2007;357(2):154-162, [PMID: 17625127]
Exclusion code: 5

Robson ME, Offit K. Breast MRI for women with hereditary cancer risk. *JAMA.* 2004;292(11):1368-1370, [PMID: 15367560]
Exclusion code: 8

Robson ME, Storm CD, Weitzel J, Wollins DS, Offit K. American Society of Clinical Oncology Policy Statement update: Genetic and genomic testing for cancer susceptibility. *J. Clin. Oncol.* 2010;28(5):893-901, [PMID: 20065170]
Exclusion code: 2

Rockhill B, Byrne C, Rosner B, Louie MM, Colditz G. Breast cancer risk prediction with a log-incidence model: evaluation of accuracy. *J. Clin. Epidemiol.* 2003;56(9):856-861, [PMID: 14505770]
Exclusion code: 2

Rockhill B, Spiegelman D, Byrne C, al. e. Validation of the Gail, et al model of breast cancer risk prediction and implications for chemoprevention. *J. Natl. Cancer Inst.* 2001;93:358-366, [PMID: 11238697]
Exclusion code: 2

Rodriguez SR, Osborne D, Jacobellis J. Health Plan Implementation of U.S. Preventive Services Task Force A and B Recommendations -- Colorado, 2010. *MMWR.* 2011;60(39):1348-1350, [PMID: 67199559]
Exclusion code: 5

Rolnick SJ, Altschuler A, Nekhlyudov L, et al. What women wish they knew before prophylactic mastectomy. *Cancer Nurs.* 2007;30(4):285-293, [PMID: 17666977]
Exclusion code: 8

Ronckers CM, Erdmann CA, Land CE. Radiation and breast cancer: A review of current evidence. *Breast Cancer Res.* 2005;7(1):21-32, [PMID: 15642178]
Exclusion code: 8

Rosen B, Kwon J, Fung MFK, Gagliardi A, Chambers A. Systematic review of management options for women with a hereditary predisposition to ovarian cancer. *Gynecol. Oncol.* 2004;93(2):280-286, [PMID: 15099934]
Exclusion code: 6

Roshanai AH, Rosenquist R, Lampic C, Nordin K. Cancer genetic counselees' self-reported psychological distress, changes in life, and adherence to recommended surveillance programs 3-7 years post counseling. *J Genet Couns.* 2009;18(2):185-194, [PMID: 19212811]
Exclusion code: 3

Rothemund Y, Paepke S, Flor H. Perception of risk, anxiety, and health behaviors in women at high risk for breast cancer. *International Journal of Behavioral Medicine.* 2001;8(3):230-239.
Exclusion code: 6

Rothwell E, Kohlmann W, Jasperson K, Gammon A, Wong B, Kinney A. Patient outcomes associated with group and individual genetic counseling formats. *Fam Cancer.* 2012;11(1):97-106, [PMID: 22057473]
Exclusion code: 3

Roudgari H, Miedzybrodzka ZH, Haites NE. Probability estimation models for prediction of BRCA1 and BRCA2 mutation carriers: COS compares favourably with other models. *Fam Cancer.* 2008;7(3):199-212, [PMID: 18097771]
Exclusion code: 8

Roussi P, Miller SM. Genetic testing for breast and ovarian cancer: A review of psychological and behavioral outcomes. *Hellenic Journal of Psychology.* 2005;2(2):135-158.
Exclusion code: 5

Roussi P, Sherman KA, Miller S, et al. Enhanced counselling for women undergoing BRCA1/2 testing: Impact on knowledge and psychological distress-results from a randomised clinical trial. *Psychol Health.* 2010;25(4):401-415, [PMID: 20204945]
Exclusion code: 3

Roussi P, Sherman KA, Miller SM, et al. Identification of cognitive profiles among women considering BRCA1/2 testing through the utilisation of cluster analytic techniques.

Psychol Health. 2011;26(10):1327-1343, [PMID: 21756124]
Exclusion code: 3

Rowan K. Beyond the Gail model: lobular involution may help refine breast cancer risk assessment. *J. Natl. Cancer Inst.* 2009;101(3):134-135, [PMID: 19176450]
Exclusion code: 5

Rowe J. *Health locus of control expectancies and perceived risk for breast cancer in women*, Rowe, Jennifer: Yeshiva U , US; 2004
Exclusion code: 8

Rowe JL, Montgomery GH, Duberstein PR, Bovbjerg DH. Health locus of control and perceived risk for breast cancer in healthy women. *Behav. Med.* 2005;31(1):33-40, [PMID: 16078524]
Exclusion code: 4

Rubinstein WS, Acheson LS, O'Neill SM, et al. Clinical utility of family history for cancer screening and referral in primary care: a report from the Family Healthware Impact Trial. *Genet Med.* 2011;13(11):956-965, [PMID: 22075527]
Exclusion code: 3

Rueth NM, McMahon M, Arrington AK, Swenson K, Leach J, Tuttle TM. Preoperative risk assessment among women undergoing bilateral prophylactic mastectomy for cancer risk reduction. *Ann. Surg. Oncol.* 2011;18(9):2515-2520, [PMID: 21424371]
Exclusion code: 8

Rumiantseva UV, Rumiantsev PO, Kiseleva MV. [Peculiarities of diagnosis, treatment and prevention of hereditary cancer of the breast and ovary. Clinical and genetic screening among BRCA1/2 mutation carriers and individuals with high family risk of cancer]. *Vopr. Onkol.* 2008;54(3):251-259, [PMID: 18652227]
Exclusion code: 7

Rutter JL, Wacholder S, Chetrit A, et al. Gynecologic surgeries and risk of ovarian cancer in women with BRCA1 and BRCA2 Ashkenazi founder mutations: An Israeli population-based case-control study. *J. Natl. Cancer Inst.* 2003;95(14):1072-1078, [PMID: 12865453]
Exclusion code: 6

Saetersdal A, Dorum A, Heimdal K, et al. Inherited predisposition to breast carcinoma. Results of first round examination of 537 women at risk. *Anticancer Res.* 1996;16(4A):1989-1992, [PMID: 8712731]
Exclusion code: 8

Salhab M, Al Sarakbi W, Joseph A, Sheards S, Travers J, Mokbel K. Skin-sparing mastectomy and immediate breast reconstruction: patient satisfaction and clinical outcome. *Int J Clin Oncol.* 2006;11(1):51-54, [PMID: 16508729]
Exclusion code: 3

Salhab M, Bismohun S, Mokbel K. Risk-reducing strategies for women carrying BRCA1/2 mutations with a focus on prophylactic surgery. *BMC Womens Health.* 2010;10:28, [PMID: 20961453]
Exclusion code: 5

Salsman JM, Pavlik E, Boerner LM, Andrykowski MA. Clinical, demographic, and psychological characteristics of new, asymptomatic participants in a transvaginal ultrasound screening program for ovarian cancer. *Prev. Med.* 2004;39(2):315-322, [PMID: 15226040]
Exclusion code: 4

Salzberg SL, Pertea M. Do-it-yourself genetic testing. *Genome Biol.* 2010;11(10):404, [PMID: 20932271]
Exclusion code: 5

Samphao S, Wheeler AJ, Rafferty E, et al. Diagnosis of breast cancer in women age 40 and younger: delays in diagnosis result from underuse of genetic testing and breast imaging. *Am. J. Surg.* 2009;198(4):538-543, [PMID: 19800464]
Exclusion code: 3

Samuel JC, Ollila DW. Prophylaxis and screening options: recommendations for young women with BRCA mutations. *Breast Dis.* 2005;23:31-35, [PMID: 16823164]

Exclusion code: 5

Sardanelli F, Podo F, D'Agnolo G, et al. Multicenter comparative multimodality surveillance of women at genetic-familial high risk for breast cancer (HIBCRIT study): interim results. *Radiology.* 2007;242(3):698-715, [PMID: 17244718]
Exclusion code: 3

Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. *CA. Cancer J. Clin.* 2007;57(2):75-89, [PMID: 17392385]
Exclusion code: 5

Satagopan JM, Offit K, Foulkes W, et al. The lifetime risks of breast cancer in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. *Cancer Epidemiology, Biomarkers & Prevention.* 2001;10(5):467-473, [PMID: 11352856]
Exclusion code: 2

Sattin RW, Rubin GL, Webster LA. Family history and the risk of breast cancer. *JAMA.* 1985;253(13):1908-1913, [PMID: 3974080]
Exclusion code: 6

Saunders KH, Nazareth S, Pressman PI. Case report: BRCA in the Ashkenazi population: Are current testing guidelines too exclusive? *Hered Cancer Clin Pract.* 2011;9(1), [PMID: 21711529]
Exclusion code: 5

Scheuer L, Kauff N, Robson M, et al. Outcome of preventive surgery and screening for breast and ovarian cancer in BRCA mutation carriers. *J. Clin. Oncol.* 2002;20(5):1260-1268, [PMID: 11870168]
Exclusion code: 3

Schiffman SC, Chagpar AB. Does a family history of male breast cancer influence risk perception and use of genetic testing? *Am. Surg.* 2010;76(8):879-882, [PMID: 20726421]
Exclusion code: 8

Schlebusch CM, Dreyer G, Sluiter MD, Yawitch TM, van den Berg HJ, van Rensburg EJ. Cancer prevalence in 129 breast-ovarian cancer families tested for BRCA1 and BRCA2 mutations. *S. Afr. Med. J.* 2010;100(2):113-117, [PMID: 20459916]
Exclusion code: 3

Schlich-Bakker KJ, ten Kroode HFJ, Ausems MGEM. A literature review of the psychological impact of genetic testing on breast cancer patients. *Patient Educ. Couns.* 2006;62(1):13-20, [PMID: 16242293]
Exclusion code: 3

Schmeler KM, Sun CC, Bodurka DC, et al. Prophylactic bilateral salpingo-oophorectomy compared with surveillance in women with BRCA mutations. *Obstet. Gynecol.* 2006;108(3 Pt 1):515-520, [PMID: 16946209]
Exclusion code: 8

Schmitz N, Hartkamp N, Kiuse J, Franke GH, Reister G, Tress W. The Symptom Check-List-90-R (SCL-90-R): A German validation study. *Qual. Life Res.* 2000;9(2):185-193, [PMID: 10983482]
Exclusion code: 2

Schneegans SM, Rosenberger A, Engel U, Sander M, Emons G, Shoukier M. Validation of three BRCA1/2 mutation-carrier probability models Myriad, BRCAPRO and BOADICEA in a population-based series of 183 German families. *Fam Cancer.* 2012;11(2):181-188, [PMID: 22160602]
Exclusion code: 3

Schneider KA, Chittenden AB, Branda KJ, et al. Ethical issues in cancer genetics: I 1) whose information is it? *J Genet Couns.* 2006;15(6):491-503, [PMID: 17106632]
Exclusion code: 8

Schonfeld SJ, Pee D, Greenlee RT, et al. Effect of changing breast cancer incidence rates on the calibration of the Gail model. *J. Clin. Oncol.* 2010;28(14):2411-2417, [PMID: 20368565]
Exclusion code: 2

Schrading S, Kuhl CK. Mammographic, US, and MR imaging phenotypes of familial breast cancer. *Radiology.* 2008;246(1):58-70, [PMID: 18096529]
Exclusion code: 8

Schroevers MJ, Sanderman R, Van Sonderen E, Ranchor AV. The evaluation of the Center for Epidemiologic Studies Depression (CES-D) scale: Depressed and Positive Affect in cancer patients and healthy reference subjects. *Qual. Life Res.* 2000;9(9):1015-1029, [PMID: 11332223]
Exclusion code: 2

Schwartz MD, Isaacs C, Graves KD, et al. Long-term outcomes of BRCA1/BRCA2 testing: risk reduction and surveillance. *Cancer.* 2012;118(2):510-517, [PMID: 21717445]
Exclusion code: 8

Segal J, Esplen MJ, Toner B, Baedorf S, Narod S, Butler K. An investigation of the disclosure process and support needs of BRCA1 and BRCA2 carriers. *Am J Med Genet A.* 2004;125A(3):267-272, [PMID: 14994235]
Exclusion code: 2(MW used in her draft)

Seyednoori T, Pakseresht S, Roushan Z. Risk of developing breast cancer by utilizing Gail model. *Women Health.* 2012;52(4):391-402, [PMID: 22591234]
Exclusion code: 8

Seymour IJ, Casadei S, Zampiga V, et al. Disease family history and modification of breast cancer risk in common BRCA2 variants. *Oncol. Rep.* 2008;19(3):783-786, [PMID: 18288416]
Exclusion code: 3

Shah P, Rosen M, Stopfer J, et al. Prospective study of breast MRI in BRCA1 and BRCA2 mutation carriers: effect of mutation status on cancer incidence. *Breast Cancer Res. Treat.* 2009;118(3):539-546, [PMID: 19609668]
Exclusion code: 3

Shannon KM, Rodgers LH, Chan-Smutko G, Patel D, Gabree M, Ryan PD. Which individuals undergoing BRACAnalysis need BART testing? *Cancer Genet.* 2011;204(8):416-422, [PMID: 21962891]
Exclusion code: 8

Shapiro DE, Rodrigue JR, Boggs SR, Robinson ME. Cluster analysis of the medical coping modes questionnaire: Evidence for coping with cancer styles? *J. Psychosom. Res.* 1994;38(2):151-159, [PMID: 8189404]
Exclusion code: 2

Shapiro SP, Nunez C. Psammoma bodies in the cervicovaginal smear in association with a papillary tumor of the peritoneum. *Obstet. Gynecol.* 1983;61(1):130-134, [PMID: 6823343]
Exclusion code: 6

Sharff ME, DeMarco TA, Mays D, et al. Parenting through genetic uncertainty: themes in the disclosure of breast cancer risk information to children. *Genet Test Mol Biomarkers.* 2012;16(5):376-382, [PMID: 22085394]
Exclusion code: 2

Shattuck-Eidens D, Oliphant A, McClure M, et al. BRCA1 sequence analysis in women at high risk for susceptibility mutations: Risk factor analysis and implications for genetic testing. *JAMA.* 1997;278(15):1242-1250, [PMID: 9333265]
Exclusion code: 2

Shedlosky-Shoemaker R, Ngo TL, Ferketich AK, Porter K, Leventhal H, Kelly KM. Exploring perceptions of genetic testing: An examination of perceived accuracy over time. *Patient Educ. Couns.* 2010;78(1):34-39, [PMID: 19553058]
Exclusion code: 3

Sheehan J, Sherman KA, Lam T, Boyages J. Association of information satisfaction, psychological distress and monitoring coping style with post-decision regret following breast reconstruction. *Psychooncology.* 2007;16(4):342-351, [PMID: 16874745]
Exclusion code: 3

Sheehan J, Sherman KA, Lam T, Boyages J. Regret associated with the decision for breast reconstruction: The association of negative body

image, distress and surgery characteristics with decision regret. *Psychol Health.* 2008;23(2):207-219.
Exclusion code: 3

Sherwin S. BRCA testing: ethics lessons for the new genetics. *Clin Invest Med.* 2004;27(1):19-22, [PMID: 15061582]
Exclusion code: 5

Shibata A, Hayashi Y, Imai T, Funahashi H, Nakao A, Seo H. Somatic gene alteration of AIB1 gene in patients with breast cancer. *Endocr. J.* 2001;48(2):199-204, [PMID: 11456268]
Exclusion code: 3

Shiloh S, Avdor O, Goodman RM. Satisfaction with genetic counseling: Dimensions and measurement. *Am. J. Med. Genet.* 1990;37(4):522-529, [PMID: 2260600]
Exclusion code: 2

Shkedi-Rafid S, Gabai-Kapara E, Grinshpun-Cohen J, Levy-Lahad E. BRCA genetic testing of individuals from families with low prevalence of cancer: experiences of carriers and implications for population screening. *Genet Med.* 2012;14(7):688-694, [PMID: 22481128]
Exclusion code: 3

Sidon L, Ingham S, Clancy T, et al. Uptake of risk-reducing salpingo-oophorectomy in women carrying a BRCA1 or BRCA2 mutation: evidence for lower uptake in women affected by breast cancer and older women. *Br. J. Cancer.* 2012;106(4):775-779, [PMID: 22187038]
Exclusion code: 8

Sim LSJ, Hendriks JHCL, Fook-Chong SMC. Breast ultrasound in women with familial risk of breast cancer. *Ann. Acad. Med. Singapore.* 2004;33(5):600-606, [PMID: 15531956]
Exclusion code: 3

Simard J, Dumont M, Moisan A-M, et al. Evaluation of BRCA1 and BRCA2 mutation prevalence, risk prediction models and a multistep testing approach in French-Canadian families with high risk of breast and ovarian cancer. *J. Med. Genet.* 2007;44(2):107-121, [PMID: 16905680]
Exclusion code: 8

Simon MS, Petrucelli N. Hereditary breast and ovarian cancer syndrome : the impact of race on uptake of genetic counseling and testing. *Methods Mol. Biol.* 2009;471:487-500, [PMID: 19109796]
Exclusion code: 5

Sinicrope PS, Brockman TA, Patten CA, et al. Factors associated with breast cancer prevention communication between mothers and daughters. *J Womens Health.* 2008;17(6):1017-1023, [PMID: 18554093]
Exclusion code: 8

Sirgo A, Rubio B, Torres A, Salvat M, Brunet J. Psychosocial impact of genetic testing in patients diagnosed with breast or colorectal cancer and relatives of high risk: The Genetic Counselling Unit in the University Hospital Saint Joan. *Psicooncologia.* 2005;2(2-3):369-382.
Exclusion code: 7

Siskind V, Green A, Bain C, Purdie D. Beyond ovulation: Oral contraceptives and epithelial ovarian cancer. *Epidemiology.* 2000;11(2):106-110, [PMID: 11021605]
Exclusion code: 6

Cancer genetic risk assessment for individuals at risk of familial breast cancer. 2010. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=coch&AN=00075320-100000000-02764.
Exclusion code: 5

Skinner CS, Rawl SM, Moser BK, et al. Impact of the Cancer Risk Intake System on patient-clinician discussions of tamoxifen, genetic counseling, and colonoscopy. *J. Gen. Intern. Med.* 2005;20(4):360-365, [PMID: 15857495]
Exclusion code: 4

Slattery ML, Baumgartner KB, Giuliano AR, Byers T, Herrick JS, Wolff RK. Replication of five GWAS-identified loci and breast cancer risk among Hispanic and non-Hispanic white women

living in the Southwestern United States. *Breast Cancer Res. Treat.* 2011;129(2):531-539, [PMID: 21475998]
Exclusion code: 8

Slattery ML, Kerber RA. A comprehensive evaluation of family history and breast cancer risk. The Utah Population Database. *JAMA.* 1993;270(13):1563-1568, [PMID: 8371466]
Exclusion code: 2

A comparison of gene expression profiling tests for breast cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32010001703
Exclusion code: 5

Smith AW, Dougall AL, Posluszny DM, Somers TJ, Rubinstein WS, Baum A. Psychological distress and quality of life associated with genetic testing for breast cancer risk. *Psychooncology.* 2008;17(8):767-773, [PMID: 17992698]
Exclusion code: 3

Smith KR, Ellington L, Chan AY, Croyle RT, Botkin JR. Fertility intentions following testing for a BRCA1 gene mutation. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(5):733-740, [PMID: 15159303]
Exclusion code: 3

Smith KR, West JA, Croyle RT, Botkin JR. Familial context of genetic testing for cancer susceptibility: Moderating effect of siblings' test results on psychological distress one to two weeks after BRCA1 mutation testing. *Cancer Epidemiol. Biomarkers Prev.* 1999;8(4 II):385-392, [PMID: 10207644]
Exclusion code: 3

Smith LH, Ol RH. Detection of malignant ovarian neoplasms: A review of the literature. I. Detection of the patient at risk; Clinical, radiological and cytological detection. *Obstet. Gynecol. Surv.* 1984;39(6):313-328, [PMID: 6374536]
Exclusion code: 6

Snyderman R, Langheier J. Prospective health care: The second transformation of medicine. *Genome Biol.* 2006;7(2), [PMID: 16522218]
Exclusion code: 5

Soegaard M, Kjaer SK, Cox M, et al. BRCA1 and BRCA2 Mutation Prevalence and Clinical Characteristics of a Population-Based Series of Ovarian Cancer Cases from Denmark. *Clin. Cancer Res.* 2008(14):3761 - 3767, [PMID: 18559594]
Exclusion code: 3

Somers TJ. *Risk reducing behaviors in a community sample of women with a family history of breast cancer*, Somers, Tamara J : U Pittsburgh, US; 2007
Exclusion code: 4

Son BH, Ahn SH, Kim S-W, et al. Prevalence of BRCA1 and BRCA2 mutations in non-familial breast cancer patients with high risks in Korea: the Korean Hereditary Breast Cancer (KOHBRA) Study. *Breast Cancer Res. Treat.* 2012;133(3):1143-1152, [PMID: 22382806]
Exclusion code: 3

Song CG, Hu Z, Wu J, et al. The prevalence of BRCA1 and BRCA2 mutations in eastern Chinese women with breast cancer. *J. Cancer Res. Clin. Oncol.* 2006;132(10):617-626, [PMID: 16835750]
Exclusion code: 3

Soucek P, Borovanova T, Pohlreich P, Kleibl Z, Novotny J. Role of single nucleotide polymorphisms and haplotypes in BRCA1 in breast cancer: Czech case-control study. *Breast Cancer Res. Treat.* 2007;103(2):219-224, [PMID: 17039264]
Exclusion code: 8

Spear SL, Carter ME, Schwarz K. Prophylactic mastectomy: indications, options, and reconstructive alternatives. *Plast. Reconstr. Surg.* 2005;115(3):891-909, [PMID: 15731693]
Exclusion code: 8

Spector DJ. *Breast cancer risk, risk perception and lifestyle behaviors among women with a family history of the disease: A mixed-method*

approach, Spector, Denise Jean: U North Carolina at Chapel Hill, US; 2009
Exclusion code: 8

Spiegel TN, Hill KA, Warner E. The attitudes of women with BRCA1 and BRCA2 mutations toward clinical breast examinations and breast self-examinations. *J Womens Health.* 2009;18(7):1019-1024, [PMID: 20377375]
Exclusion code: 8

Spitzer E, Abbaszadegan MR, Schmidt F, et al. Detection of BRCA1 and BRCA2 mutations in breast cancer families by a comprehensive two-stage screening procedure. *Int. J. Cancer.* 2000;85(4):474-481, [PMID: 10699917]
Exclusion code: 8

Spurdle AB, Marquart L, McGuffog L, et al. Common genetic variation at BARD1 is not associated with breast cancer risk in BRCA1 or BRCA2 mutation carriers. *Cancer Epidemiol. Biomarkers Prev.* 2011;20(5):1032-1038, [PMID: 21393566]
Exclusion code: 8

Spurna Z, Drazan L, Foretova L, Dvorska L. [The effect of prophylactic mastectomy with recontruction on quality of life in BRCA positive women]. *Klin.* 2012;25 Suppl:S74-77, [PMID: 22920211]
Exclusion code: 7

Srivastava A, McKinnon W, Wood ME. Risk of breast and ovarian cancer in women with strong family histories. *Oncology (Williston Park).* 2001;15(7):889-902, [PMID: 11499690]
Exclusion code: 2

Stadler ZK, Salo-Mullen E, Patil SM, et al. Prevalence of BRCA1 and BRCA2 mutations in Ashkenazi Jewish families with breast and pancreatic cancer. *Cancer.* 2012;118(2):493-499, [PMID: 21598239]
Exclusion code: 3

Stanton AL, Kirk SB, Cameron CL, Danoff-Burg S. Coping through emotional approach: Scale construction and validation. *J. Pers. Soc. Psychol.* 2000;78(6):1150-1169, [PMID: 10870915]
Exclusion code: 2

Staton AD, Kurian AW, Cobb K, Mills MA, Ford JM. Cancer risk reduction and reproductive concerns in female BRCA1/2 mutation carriers. *Fam Cancer.* 2008;7(2):179-186, [PMID: 18026853]
Exclusion code: 8

Steed L. Further validity and reliability evidence for Beck Hopelessness Scale scores in a nonclinical sample. *Educational and Psychological Measurement.* 2001;61(2):303-316.
Exclusion code: 2

Steele SL. *Psychological distress, executive cognitive function and mammography utilization among a high-risk African-American sample*, Steele, Sharon Lee: Howard U , US; 2007
Exclusion code: 4

Stefanek ME. Bilateral prophylactic mastectomy: issues and concerns. *J Natl Cancer Inst Monogr.* 1995(17):37-42, [PMID: 8573451]
Exclusion code: 5

Stein KD, Jacobsen PB, Blanchard CM, Thors C. Further validation of the multidimensional fatigue symptom inventory-short form. *J. Pain Symptom Manage.* 2004;27(1):14-23, [PMID: 14711465]
Exclusion code: 2

Stirling D, Evans DGR, Pichert G, et al. Screening for familial ovarian cancer: Failure of current protocols to detect ovarian cancer at an early stage according to the International Federation of Gynecology and Obstetrics System. *J. Clin. Oncol.* 2005;23(24):5589-5596, [PMID: 16110018]
Exclusion code: 3

Stolier AJ, Corsetti RL. Newly diagnosed breast cancer patients choose bilateral mastectomy over breast-conserving surgery when testing positive for a BRCA1/2 mutation. *Am. Surg.* 2005;71(12):1031-1033, [PMID: 16447474]
Exclusion code: 3

Stoppa-Lyonnet D, Ansquer Y, Dreyfus H, et al. Familial invasive breast cancers: Worse outcome related to BRCA1 mutations. *J. Clin. Oncol.* 2000;18(24):4053-4059, [PMID: 11118466]
Exclusion code: 6

Stratton JF, Pharoah P, Smith SK, Easton D, Ponder BA. A systematic review and meta-analysis of family history and risk of ovarian cancer. *Br. J. Obstet. Gynaecol.* 1998;105(5):493-499, [PMID: 9637117]
Exclusion code: 2

Stroup AM, Smith KR. Familial effects of BRCA1 genetic mutation testing: changes in perceived family functioning. *Cancer Epidemiol. Biomarkers Prev.* 2007;16(1):135-141, [PMID: 17220342]
Exclusion code: 3

Stuckey A, Dizon D, Scalia Wilbur J, et al. Clinical characteristics and choices regarding risk-reducing surgery in BRCA mutation carriers. *Gynecol. Obstet. Invest.* 2010;69(4):270-273, [PMID: 20090358]
Exclusion code: 8

Stuppia L, Di Fulvio P, Aceto G, et al. BRCA1 and BRCA2 mutations in breast/ovarian cancer patients from central Italy. *Hum. Mutat.* 2003;22(2):178-179, [PMID: 12872265]
Exclusion code: 5

Sueta A, Ito H, Kawase T, et al. A genetic risk predictor for breast cancer using a combination of low-penetrance polymorphisms in a Japanese population. *Breast Cancer Res. Treat.* 2012;132(2):711-721, [PMID: 22160591]
Exclusion code: 3

Sullivan M, Karlsson J, Ware Jr JE. The Swedish SF-36 Health Survey - I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. *Soc. Sci. Med.* 1995;41(10):1349-1358, [PMID: 8560302]
Exclusion code: 2

Surbone A. Social and ethical implications of BRCA testing. *Ann. Oncol.* 2011;22 Suppl 1:i60-66, [PMID: 21285154]
Exclusion code: 5

Sussner KM, Edwards TA, Thompson HS, et al. Ethnic, racial and cultural identity and perceived benefits and barriers related to genetic testing for breast cancer among at-risk women of African descent in New York City. *Public Health Genomics.* 2011;14(6):356-370, [PMID: 21540561]
Exclusion code: 3

Sussner KM, Jandorf L, Thompson HS, Valdimarsdottir HB. Interest and beliefs about BRCA genetic counseling among at-risk Latinas in New York City. *J Genet Couns.* 2010;19(3):255-268, [PMID: 20151317]
Exclusion code: 3

Sussner KM, Thompson HS, Jandorf L, et al. The influence of acculturation and breast cancer-specific distress on perceived barriers to genetic testing for breast cancer among women of African descent. *Psychooncology.* 2009;18(9):945-955, [PMID: 19090507]
Exclusion code: 3

Sussner KM, Thompson HS, Valdimarsdottir HB, Redd WH, Jandorf L. Acculturation and familiarity with, attitudes towards and beliefs about genetic testing for cancer risk within Latinas in East Harlem, New York City. *J Genet Couns.* 2009;18(1):60-71, [PMID: 18686019]
Exclusion code: 8

Suthers GK, Armstrong J, McCormack J, Trott D. Letting the family know: balancing ethics and effectiveness when notifying relatives about genetic testing for a familial disorder. *J. Med. Genet.* 2006;43(8):665-670, [PMID: 16371501]
Exclusion code: 8

Sutton M, Elliott RL. Genetic diseases: is there a duty to a patient's family members? *J. Med. Assoc. Ga.* 2011;100(3):28-29, [PMID: 22164651]
Exclusion code: 5

Presymptomatic diagnosis of hereditary breast cancer - early assessment briefs (ALERT) (Brief record). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC

=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32000001833
Exclusion code: 5

Syamala V, Syamala VS, Sreeja L, et al. Hereditary breast/ovarian cancer: clinicopathological characteristics and survival of BRCA2 positive and negative cases. *J. Exp. Ther. Oncol.* 2008;7(3):227-236, [PMID: 19066131]
Exclusion code: 3

Tailor A, Bourne TH, Campbell S, Okokon E, Dew T, Collins WP. Results from an ultrasound-based familial ovarian cancer screening clinic: A 10-year observational study. *Ultrasound Obstet. Gynecol.* 2003;21(4):378-385, [PMID: 12704748]
Exclusion code: 6

Tamimi RM, Rosner B, Colditz GA. Evaluation of a breast cancer risk prediction model expanded to include category of prior benign breast disease lesion. *Cancer.* 2010;116(21):4944-4953, [PMID: 20645399]
Exclusion code: 2

Teixeira L, Julien C, Guimiot F. Polygenes, risk prediction, and targeted prevention of breast cancer. *N. Engl. J. Med.* 2008;359(13):1406-1407, [PMID: 18822456]
Exclusion code: 5

Teixeira RJ, Pereira MdG. Impact of parental cancer in offspring's psychological development: Literature review. *Psicologia: Reflexao e Critica.* 2011;24(3):513-522.
Exclusion code: 7

Genetic counselling in breast cancer and colon cancer (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32008000097
Exclusion code: 5

Teraoka SN, Bernstein JL, Reiner AS, et al. Single nucleotide polymorphisms associated with risk for contralateral breast cancer in the Women's Environment, Cancer, and Radiation Epidemiology (WECARE) Study. *Breast Cancer Res.* 2011;13(6):R114, [PMID: 22087758]
Exclusion code: 3

Tercyak KP, Demarco TA, Mars BD, Peshkin BN. Women's satisfaction with genetic counseling for hereditary breast-ovarian cancer: psychological aspects. *Am J Med Genet A.* 2004;131(1):36-41, [PMID: 15389697]
Exclusion code: 3

Tercyak KP, Johnson SB, Roberts SF, Cruz AC. Psychological response to prenatal genetic counseling and amniocentesis. *Patient Educ. Couns.* 2001;43(1):73-84, [PMID: 11311841]
Exclusion code: 3

Tercyak KP, Lerman C, Peshkin BN, et al. Effects of coping style and BRCA1 and BRCA2 test results on anxiety among women participating in genetic counseling and testing for breast and ovarian cancer risk. *Health Psychol.* 2001;20(3):217-222, [PMID: 11403219]
Exclusion code: 6

Tercyak KP, Peshkin BN, DeMarco TA, Brogan BM, Lerman C. Parent-child factors and their effect on communicating BRCA1/2 test results to children. *Patient Educ. Couns.* 2002;47(2):145-153, [PMID: 12191538]
Exclusion code: 8

Tercyak KP, Peshkin BN, Demarco TA, et al. Information needs of mothers regarding communicating BRCA1/2 cancer genetic test results to their children. *Genet Test.* 2007;11(3):249-255, [PMID: 17949286]
Exclusion code: 8

Tercyak KP, Peshkin BN, Streisand R, Lerman C. Psychological issues among children of hereditary breast cancer gene (BRCA1/2) testing participants. *Psychooncology.* 2001;10(4):336-346, [PMID: 11462232]
Exclusion code: 2

Tereschenko IV, Basham VM, Ponder BA, Pharoah PD. BRCA1 and BRCA2 mutations in Russian familial breast cancer. *Hum. Mutat.* 2002;19(2):184, [PMID: 11793480]

Exclusion code: 3

Teutsch S, Bradley LA, Palomaki GE, et al. Evaluation of Genomic Applications in Practice and Prevention [EGAPP] Working Group for tests of risk assessment/susceptibility. *Genet Med.* 2009;11(1):3-14, [PMID: 18813139]
Exclusion code: 2

The 104th Congress. Health Insurance Portability and Accountability Act of 1996. *Public Law 104-191*1996
Exclusion code: 5

The Lancet. Control of direct-to-consumer genetic testing. *Lancet.* 2008;372(9647):1360, [PMID: 18940452]
Exclusion code: 5

Thirlaway K, Fallowfield L, Cuzick J. The sexual activity questionnaire: A measure of women's sexual functioning. *Qual. Life Res.* 1996;5(1):81-90, [PMID: 8901370]
Exclusion code: 2

Thomassen M, Hansen TVO, Borg Å, et al. BRCA1 and BRCA2 mutations in Danish families with hereditary breast and/or ovarian cancer. *Acta Oncol.* 2008;47(4):772-777, [PMID: 18465347]
Exclusion code: 8

Thombs BD, Arthurs E, El-Baalbaki G, Meijer A, Ziegelstein RC, Steele RJ. Risk of bias from inclusion of patients who already have diagnosis of or are undergoing treatment for depression in diagnostic accuracy studies of screening tools for depression: systematic review. *BMJ (Clinical research ed.).* 2011;343, [PMID: 21852353]
Exclusion code: 3

Thompson D, Easton DF, The Breast Cancer Linkage Consortium. Cancer Incidence in BRCA1 Mutation Carriers. *J. Natl. Cancer Inst.* 2002;94(18):1358-1365, [PMID: 12237281]
Exclusion code: 2

Thomsen A, Kolesar JM. Chemoprevention of breast cancer. *Am. J. Health. Syst. Pharm.* 2008;65(23):2221-2228, [PMID: 19020189]
Exclusion code: 5

Thorlacius S, Olafsdottir G, Tryggvadottir L, et al. A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat. Genet.* 1996;13(1):117-119, [PMID: 8673089]
Exclusion code: 2

Tice JA, Cummings SR, Smith-Bindman R, Ichikawa L, Barlow WE, Kerlikowske K. Using clinical factors and mammographic breast density to estimate breast cancer risk: development and validation of a new predictive model. *Ann. Intern. Med.* 2008;148(5):337-347, [PMID: 18316752]
Exclusion code: 2 (included later)

Tice JA, Cummings SR, Ziv E, Kerlikowske K. Mammographic breast density and the Gail model for breast cancer risk prediction in a screening population. *Breast Cancer Res. Treat.* 2005;94(2):115-122, [PMID: 16261410]
Exclusion code: 2

Tice JA, Miike R, Adduci K, Petrakis NL, King E, Wrensch MR. Nipple aspirate fluid cytology and the Gail model for breast cancer risk assessment in a screening population. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(2):324-328, [PMID: 15734953]
Exclusion code: 4

Tilanus-Linthorst MM, Bartels CC, Obdeijn IM, Oudkerk M. Earlier detection of breast cancer by surveillance of women at familiar risk. *Eur. J. Cancer.* 2000;36:514-519.
Exclusion code: 8

Tilanus-Linthorst MM, Obdeijn IM, Bartels KCM. MARIBS study. *Lancet.* 2005;366(9482):291-292, [PMID: 16039329]
Exclusion code: 8

Tilanus-Linthorst MMA, Kriege M, Boetes C, et al. Hereditary breast cancer growth rates and its impact on screening policy. *Eur. J. Cancer.* 2005;41(11):1610-1617, [PMID: 15978801]
Exclusion code: 3

Tilburt JC, James KM, Sinicrope PS, et al. Factors influencing cancer risk perception in

high risk populations: A systematic review. *Hered Cancer Clin Pract.* 2011;9(1), [PMID: 21595959]
Exclusion code: 3

Tiller K, Meiser B, Butow P, et al. Psychological impact of prophylactic oophorectomy in women at increased risk of developing ovarian cancer: a prospective study. *Gynecol. Oncol.* 2002;86(2):212-219, [PMID: 12144830]
Exclusion code: 5

Tinley ST, Houfek J, Watson P, et al. Screening Adherence in BRCA1/2 Families Is Associated with Primary Physicians' Behavior. *Am. J. Med. Genet.* 2004;125 A(1):5-11, [PMID: 14755459]
Exclusion code: 8

Tobacman JK, Tucker MA, Kase RG, M. H., Costa J, Fraumeni JF, JR. Intra-abdominal carcinomatosis after prophylactic oophorectomy in ovarian-cancer-prone families. *Lancet.* 1982;2(8302):795-797, [PMID: 6126666]
Exclusion code: 2

Tone A, Begley H, Sharma M, et al. ene expression profiles of luteal phase fallopian tube epithelium from BRCA mutation carriers resemble high-grade serous carcinoma. *Clin. Cancer Res.* 2008;14(13):4067-4078, [PMID: 18593983]
Exclusion code: 2

Tonin P, Maugard C, Perret C. A review of histopathological subtypes of ovarian cancer in BRCA-related French Canadian cancer families. *Fam Cancer.* 2007;6(4):491-497, [PMID: 17636423]
Exclusion code: 2

Torrance N, Mollison J, Wordsworth S, et al. Genetic nurse counsellors can be an acceptable and cost-effective alternative to clinical geneticists for breast cancer risk genetic counselling. Evidence from two parallel randomised controlled equivalence trials. *Br. J. Cancer.* 2006;95(4):435-444, [PMID: 16832415]
Exclusion code: 3

Touboul C, Uzan C, Ichante JL, et al. Factors Associated with Altered Long-Term Well-Being After Prophylactic Salpingo-Oophorectomy Among Women at Increased Hereditary Risk for Breast and Ovarian Cancer. *Oncologist.* 2011;16:1250-1257, [PMID: 21765195]
Exclusion code: 3

Trainer AH, Thompson E, James PA. BRCA and beyond: a genome-first approach to familial breast cancer risk assessment. *Discov Med.* 2011;12(66):433-443, [PMID: 22127114]
Exclusion code: 8

Contribution of BRCA1/2 mutation testing to risk assessment for susceptibility to breast and ovarian cancer. Summary report (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32006000246
Exclusion code: 5

Trecate G, Vergnaghi D, Manoukian S, et al. MRI in the early detection of breast cancer in women with high genetic risk. *Tumori.* 2006;92(6):517-523, [PMID: 17260493]
Exclusion code: 3

Trepanier A, Ahrens M, McKinnon W, et al. Genetic cancer risk assessment and counseling: Recommendations of the national society of genetic counselors. *J Genet Couns.* 2004;13(2):83-114, [PMID: 15604628]
Exclusion code: 5

Trivers KF, Baldwin L-M, Miller JW, et al. Reported referral for genetic counseling or BRCA 1/2 testing among United States physicians. *Cancer.* 2011;117(23):5334-5343, [PMID: 21792861]
Exclusion code: 2

Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. *Stat. Med.* 2004;23(7):1111-1130, [PMID: 15057881]
Exclusion code: 6

U. S. Preventive Services Task Force. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: Recommendation Statement. *Ann. Intern. Med.* 2005;143(5):355-361, [PMID: 16144894]
Exclusion code: 2

U.S. Cancer Statistics Working Group. United States Cancer Statistics: 1999–2007 Incidence and Mortality Web-based Report. 2010; http://apps.nccd.cdc.gov/uscs/toptencancers.aspx . Accessed 10 October 2012 Exclusion code: 2.

U.S. Preventive Services Task Force. Chemoprevention of Breast Cancer: Recommendations and Rationale. *Ann. Intern. Med.* 2002;137(1):56-58, [PMID: 12093249]
Exclusion code: 2

U.S. Preventive Services Task Force. Screening for Ovarian Cancer Recommendation Statement. 2004; http://www.uspreventiveservicestaskforce.org/3rduspstf/ovariancan/ovcanrs.htm. Accessed 25 Oct, 2011
Exclusion code: 2

Ugalde A, Martin P, Rees G. Psychological impact of receiving genetic risk information for breast cancer, with and without lifestyle information. *Australian Journal of Psychology.* 2008;60(1):1-9.
Exclusion code: 3

Ulusoy C, Kepenekci I, Kose K, Aydintug S, Cam R. Applicability of the Gail model for breast cancer risk assessment in Turkish female population and evaluation of breastfeeding as a risk factor. *Breast Cancer Res. Treat.* 2010;120(2):419-424, [PMID: 19760030]
Exclusion code: 2

Underhill ML, Dickerson SS. Engaging in medical vigilance: understanding the personal meaning of breast surveillance. *Oncol. Nurs. Forum.* 2011;38(6):686-694, [PMID: 22037331]
Exclusion code: 5

Unger MA, Nathanson KL, Calzone KA, et al. Screening for genomic rearrangements in families with breast and ovarian cancer identifies BRCA1 mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. *Am J Hum Genet.* 2000;67(4):841-850, [PMID: 10978226]
Exclusion code: 2

Ustun C, Ceber E. Ethical issues for cancer screenings. Five countries--four types of cancer. *Prev. Med.* 2004;39(2):223-229, [PMID: 15226029]
Exclusion code: 5

Vacek PM, Skelly JM, Geller BM. Breast cancer risk assessment in women aged 70 and older. *Breast Cancer Res. Treat.* 2011;130(1):291-299, [PMID: 21604157]
Exclusion code: 2

Vadaparampil S, Quinn G, Dutil J, et al. A pilot study of knowledge and interest of genetic counseling and testing for hereditary breast and ovarian cancer syndrome among Puerto Rican women. *J Community Genet.* 2011;2(4):211-221, [PMID: 22109874]
Exclusion code: 4

Vadaparampil ST, Malo T, De La Cruz C, Christie J. Do breast cancer patients tested in the oncology care setting share BRCA mutation results with family members and health care providers? *J Cancer Epidemiol.* 2012, [PMID: 2848222]
Exclusion code: 3

Vadaparampil ST, Miree CA, Wilson C, Jacobsen PB. Psychosocial and behavioral impact of genetic counseling and testing. *Breast Dis.* 2006;27:97-108, [PMID: 17917142]
Exclusion code: 5

Vadaparampil ST, Quinn GP, Miree CA, Brzosowicz J, Carter B, Laronga C. Recall of and reactions to a surgeon referral letter for BRCA genetic counseling among high-risk breast cancer patients. *Ann. Surg. Oncol.* 2009;16(7):1973-1981, [PMID: 19408048]
Exclusion code: 8

Vadaparampil ST, Ropka M, Stefanek ME. Measurement of psychological factors associated with genetic testing for hereditary

breast, ovarian and colon cancers. *Fam Cancer.* 2005;4(2):195-206, [PMID: 15951974]
Exclusion code: 6

Vadaparampil ST, Wey JP, Kinney AY. Psychosocial aspects of genetic counseling and testing. *Semin. Oncol. Nurs.* 2004;20(3):186-195, [PMID: 15491028]
Exclusion code: 5

Vaidyanathan K, Lakhotia S, Ravishankar HM, Tabassum U, Mukherjee G, Somasundaram K. BRCA1 and BRCA2 germline mutation analysis among Indian women from south India: identification of four novel mutations and high-frequency occurrence of 185delAG mutation. *J Biosci.* 2009;34(3):415-422, [PMID: 19805903]
Exclusion code: 8

van der Groep P, van der Wall E, van Diest PJ. Pathology of hereditary breast cancer. *Cellular oncology (Dordrecht).* 2011;34(2):71-88, [PMID: 21336636]
Exclusion code: 2

Van Der Hout AH, Van Den Ouweland AMW, Van Der Luijt RB, et al. A DGGE system for comprehensive mutation screening of BRCA1 and BRCA2: Application in a Dutch cancer clinic setting. *Hum. Mutat.* 2006;27(7):654-666, [PMID: 16683254]
Exclusion code: 8

van Der Looij M, Wysocka B, Brozek I, Jassem J, Limon J, Olah E. Founder BRCA1 mutations and two novel germline BRCA2 mutations in breast and/or ovarian cancer families from North-Eastern Poland. *Hum. Mutat.* 2000;15(5):480-481, [PMID: 10790213]
Exclusion code: 8

van der Velde NM, Mourits MJE, Arts HJG, et al. Time to stop ovarian cancer screening in BRCA1/2 mutation carriers? *Int. J. Cancer.* 2009;124(4):919-923, [PMID: 19035463]
Exclusion code: 3

van Dijk S, Otten W, Tollenaar RAEM, van Asperen CJ, Tibben A. Putting it all behind: long-term psychological impact of an inconclusive DNA test result for breast cancer. *Genet Med.* 2008;10(10):745-750, [PMID: 18813137]
Exclusion code: 3

van Dijk S, Otten W, van Asperen CJ, et al. Feeling at risk: how women interpret their familial breast cancer risk. *Am J Med Genet A.* 2004;131(1):42-49, [PMID: 15382029]
Exclusion code: 3

van Dijk S, van Asperen CJ, Jacobi CE, et al. Variants of uncertain clinical significance as a result of BRCA1/2 testing: impact of an ambiguous breast cancer risk message. *Genet Test.* 2004;8(3):235-239, [PMID: 15727245]
Exclusion code: 6

van Dijk S, van Roosmalen MS, Otten W, Stalmeier PFM. Decision making regarding prophylactic mastectomy: stability of preferences and the impact of anticipated feelings of regret. *J. Clin. Oncol.* 2008;26(14):2358-2363, [PMID: 18467728]
Exclusion code: 8

van Dooren S, Rijnsburger AJ, Seynaeve C, et al. Psychological distress in women at increased risk for breast cancer: the role of risk perception. *Eur. J. Cancer.* 2004;40(14):2056-2063, [PMID: 15341979]
Exclusion code: 6

Van Nagell Jr JR, DePriest PD, Puls LE, et al. Ovarian cancer screening in asymptomatic postmenopausal women by transvaginal sonography. *Cancer.* 1991;68(3):458-462, [PMID: 2065264]
Exclusion code: 6

van Oostrom I, Meijers-Heijboer H, Duivenvoorden HJ, et al. A prospective study of the impact of genetic susceptibility testing for BRCA1/2 or HNPCC on family relationships. *Psychooncology.* 2007;16(4):320-328, [PMID: 16909428]
Exclusion code: 3

van Oostrom I, Meijers-Heijboer H, Duivenvoorden HJ, et al. Experience of parental cancer in childhood is a risk factor for psychological distress during genetic cancer

susceptibility testing. *Ann. Oncol.* 2006;17(7):1090-1095, [PMID: 16600981]
Exclusion code: 8

van Oostrom I, Meijers-Heijboer H, Duivenvoorden HJ, et al. Family system characteristics and psychological adjustment to cancer susceptibility genetic testing: a prospective study. *Clin. Genet.* 2007;71(1):35-42, [PMID: 17204044]
Exclusion code: 3

Van Riper M. Genetic testing and the family. *J Midwifery Womens Health.* 2005;50(3):227-233, [PMID: 15895001]
Exclusion code: 5

Van Roosmalen MS, Stalmeier PFM, Verhoef LCG, et al. Impact of BRCA1/2 Testing and Disclosure of a Positive Test Result on Women Affected and Unaffected with Breast or Ovarian Cancer. *Am. J. Med. Genet.* 2004;124 A(4):346-355, [PMID: 14735581]
Exclusion code: 6

Vanchieri C. Risk reduction works for BRCA mutation carriers--with heavy costs. *J. Natl. Cancer Inst.* 2005;97(14):1032-1033, [PMID: 16030299]
Exclusion code: 5

Vargas AC, Silva LD, Lakhani SR. The contribution of breast cancer pathology to statistical models to predict mutation risk in BRCA carriers. *Fam Cancer.* 2010;9(4):545-553, [PMID: 20577821]
Exclusion code: 5

Vasen HFA, Tesfay E, Boonstra H, et al. Early detection of breast and ovarian cancer in families with BRCA mutations. *Eur. J. Cancer.* 2005;41(4):549-554, [PMID: 15737559]
Exclusion code: 3

Venne VL, Hamann HA. Successful use of peer educators for sharing genetic information. *J Genet Couns.* 2007;16(4):515-525, [PMID: 17597387]
Exclusion code: 3

Veronesi U, Costa A. Prevention of breast cancer with tamoxifen: The Italian study in hysterectomized women. *Breast.* 1995;4(4):267-272.
Exclusion code: 5

Veronesi U, Maisonneuve P, Costa A, et al. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among hysterectomised women. *Lancet.* 1998;352(9122):93-97, [PMID: 9672273]
Exclusion code: 2

Veronesi U, Maisonneuve P, Rotmensz N, et al. Italian randomized trial among women with hysterectomy: tamoxifen and hormone-dependent breast cancer in high-risk women. *J. Natl. Cancer Inst.* 2003;95(2):160-165, [PMID: 12529349]
Exclusion code: 5

Veschi S, Aceto G, Scioletti AP, et al. High prevalence of BRCA1 deletions in BRCAPRO-positive patients with high carrier probability. *Ann. Oncol.* 2007;18 (Suppl 6):vi86-92, [PMID: 17591842]
Exclusion code: 8

Visser A, Prins JB, Hoogerbrugge N, van Laarhoven HWM. Group medical visits in the follow-up of women with a BRCA mutation: design of a randomized controlled trial. *BMC Womens Health.* 2011;11:39, [PMID: 21864353]
Exclusion code: 5

Vogel KJ, Atchley DP, Erlichman J, et al. BRCA1 and BRCA2 genetic testing in Hispanic patients: mutation prevalence and evaluation of the BRCAPRO risk assessment model. *J. Clin. Oncol.* 2007;25(29):4635-4641, [PMID: 17925560]
Exclusion code: 2

Vogel VG, Costantino JP, Wickerham DL, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: The NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. *JAMA.* 2006;295(23):2727-2741, [PMID: 16754727]
Exclusion code: 2

Vogl FD, Badzioch MD, Steele L, Neuhausen SL, Goldgar DE. Risks of cancer due to a single BRCA1 mutation in an extended Utah kindred. *Fam Cancer.* 2007;6(1):63-71, [PMID: 17051349]
Exclusion code: 8

Von Minckwitz G, Loibl S, Jackisch C, et al. The GISS trial: A phase II prevention trial of screening plus goserelin, ibandronate, versus screening alone in premenopausal women at increased risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 2011;20(10):2141-2149, [PMID: 21795500]
Exclusion code: 4

Vos J, Gomez-Garcia E, Oosterwijk JC, et al. Opening the psychological black box in genetic counseling. The psychological impact of DNA testing is predicted by the counselees' perception, the medical impact by the pathogenic or uninformative BRCA 1/2-result. *Psychooncology.* 2012;21(1):29-42, [PMID: 21072753]
Exclusion code: 3

Vos J, Oosterwijk JC, Gomez-Garcia E, et al. Exploring the short-term impact of DNA-testing in breast cancer patients: The counselees' perception matters, but the actual BRCA1/2 result does not. *Patient Educ. Couns.* 2012;86(2):239-251, [PMID: 21684708]
Exclusion code: 3

Vos J, Otten W, van Asperen C, Jansen A, Menko F, Tibben A. The counsellees' view of an unclassified variant in BRCA1/2: recall, interpretation, and impact on life. *Psychooncology.* 2008;17(8):822-830, [PMID: 18157792]
Exclusion code: 3

Vos J, Stiggelbout AM, Oosterwijk J, et al. A counselee-oriented perspective on risk communication in genetic counseling: explaining the inaccuracy of the counselees' risk perception shortly after BRCA1/2 test result disclosure. *Genet Med.* 2011;13(9):800-811, [PMID: 21885922]
Exclusion code: 3

Wacholder S, Struewing JP, Hartge P, Greene MH, Tucker MA. Breast cancer risks for BRCA1/2 carriers. *Science.* 2004;306(5705):2187-2191; author reply 2187-2191, [PMID: 15622558]
Exclusion code: 2

Wagner Costalas J, Itzen M, Malick J, et al. Communication of BRCA1 and BRCA2 results to at-risk relatives: a cancer risk assessment program's experience. *Am J Med Genet C Semin Med Genet.* 2003;119C(1):11-18, [PMID: 12704633]
Exclusion code: 2

Wainberg S, Husted J. Utilization of screening and preventive surgery among unaffected carriers of a BRCA1 or BRCA2 gene mutation. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(12):1989-1995, [PMID: 15598752]
Exclusion code: 8

Walker MJ, Chiarelli AM, Mirea L, et al. Validity of self-reported mammography use among women with familial history of breast cancer [abstract]. *Am. J. Epidemiol.* 2011;173(11 Suppl).
Exclusion code: 8

Walsh T, Casadei S, Coats KH, et al. Spectrum of Mutations in BRCA1, BRCA2, CHEK2, and TP53 in Families at High Risk of Breast Cancer. *JAMA.* 2006;295(12):1379-1388, [PMID: 16551709]
Exclusion code: 8

Wang C, Gonzalez R, Janz NK, Milliron KJ, Merajver SD. The role of cognitive appraisal and worry in BRCA1/2 testing decisions among a clinic population. *Psychol Health.* 2007;22(6):719-736.
Exclusion code: 3

Wang C, Gonzalez R, Milliron KJ, Strecher VJ, Merajver SD. Genetic counseling for BRCA1/2: a randomized controlled trial of two strategies to facilitate the education and counseling process. *Am J Med Genet A.* 2005;134A(1):66-73, [PMID: 15690408]
Exclusion code: 3

Wang F, Fang Q, Ge Z, Yu N, Xu S, Fan X. Common BRCA1 and BRCA2 mutations in breast cancer families: a meta-analysis from systematic review. *Mol. Biol. Rep.* 2012;39(3):2109-2118, [PMID: 21643751]
Exclusion code: 5

Wapnir IL, Rabinowitz B, Greco RS. A reappraisal of prophylactic mastectomy. *Surg. Gynecol. Obstet.* 1990;171(2):171-184, [PMID: 2200150]
Exclusion code: 2

Warner E. Intensive radiologic surveillance: a focus on the psychological issues. *Ann. Oncol.* 2004;15 (Suppl 1):I43-I47, [PMID: 15280187]
Exclusion code: 5

Warner E. The role of magnetic resonance imaging in screening women at high risk of breast cancer. *Top. Magn. Reson. Imaging.* 2008;19(3):163-169, [PMID: 18941396]
Exclusion code: 5

Warner E, Foulkes W, Goodwin P, et al. Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. *J. Natl. Cancer Inst.* 1999;91(14):1241-1247, [PMID: 10413426]
Exclusion code: 2

Warner E, Hill K, Causer P, et al. Prospective study of breast cancer incidence in women with a BRCA1 or BRCA2 mutation under surveillance with and without magnetic resonance imaging. *J. Clin. Oncol.* 2011;29(13):1664-1669, [PMID: 21444874]
Exclusion code: 3

Warner E, Messersmith H, Causer P, Eisen A, Shumak R, Plewes D. Systematic review: using magnetic resonance imaging to screen women at high risk for breast cancer. *Ann. Intern. Med.* 2008;148(9):671-679, [PMID: 18458280]
Exclusion code: 3

Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. *JAMA.* 2004;292(11):1317-1325, [PMID: 15367553]
Exclusion code: 3

Warren RML, Thompson D, Pointon LJ, et al. Evaluation of a prospective scoring system designed for a multicenter breast MR imaging screening study. *Radiology.* 2006;239(3):677-685, [PMID: 16714457]
Exclusion code: 8

Waters E, McNeel T, Stevens W, Freedman A. Use of tamoxifen and raloxifene for breast cancer chemoprevention in 2010. *Breast Cancer Res. Treat.* 2012;134:875-880, [PMID: 22622807]
Exclusion code: 2

Watson M, Foster C, Eeles R, et al. Psychosocial impact of breast/ovarian (BRCA1/2) cancer-predictive genetic testing in a UK multi-centre clinical cohort. *Br. J. Cancer.* 2004;91(10):1787-1794, [PMID: 15505627]
Exclusion code: 6

Watson M, Kash KM, Homewood J, Ebbs S, Murday V, Eeles R. Does genetic counseling have any impact on management of breast cancer risk? *Genet Test.* 2005;9(2):167-174, [PMID: 15943558]
Exclusion code: 8

Weber BL. Familial breast cancer. *Recent Results Cancer Res.* 1996;140:5-16, [PMID: 8787076]
Exclusion code: 5

Weitzel J, Lagos V, Blazer K, al e. Evidence for common ancestral origin of a recurring BRCA1 genomic rearrangement identified in high-risk Hispanic families. *Cancer Epidemiol. Biomarkers Prev.* 2007;16(8):1615-1620, [PMID: 17646271]
Exclusion code: 2

Weitzel JN, Blazer KR, MacDonald DJ, Culver JO, Offit K. Genetics, genomics, and cancer risk assessment. *CA. Cancer J. Clin.* 2011;61(5):327-359, [PMID: 21858794]
Exclusion code: 2

Weitzel JN, Robson M, Pasini B, et al. A comparison of bilateral breast cancers in BRCA carriers. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(6):1534-1538, [PMID: 15941968]
Exclusion code: 2

Wellisch DK, Cohen MM. The special case of complicated grief in women at high risk for breast cancer. *Palliat Support Care.* 2010;8(1):7-15, [PMID: 20163755]
Exclusion code: 3

Wellisch DK, Gritz ER, Schain W, Wang HJ, Siau J. Psychological functioning of daughters of breast cancer patients - Part II: Characterizing the distressed daughter of the breast cancer patient. *Psychosomatics.* 1992;33(2):171-179, [PMID: 1557482]
Exclusion code: 3

Werner-Lin A. Beating the biological clock: the compressed family life cycle of young women with BRCA gene alterations. *Soc. Work Health Care.* 2008;47(4):416-437, [PMID: 19042494]
Exclusion code: 5

Werner-Lin A. Formal and informal support needs of young women with BRCA mutations. *J Psychosoc Oncol.* 2008;26(4):111-133, [PMID: 19042275]
Exclusion code: 8

Werner-Lin A. Building the cancer family: Family planning in the context of inherited breast and ovarian cancer risk. *Journal of the Society for Social Work and Research.* 2010;1(1):14-27.
Exclusion code: 2

Werner-Lin A, Rubin LR, Doyle M, et al. "My funky genetics": BRCA1/2 mutation carriers' understanding of genetic inheritance and reproductive merger in the context of new reprogenetic technologies. *Fam Syst Health.* 2012;30(2):166-180, [PMID: 22709328]
Exclusion code: 8

Werner-Lin AV. Danger zones: Risk perceptions of young women from families with hereditary breast and ovarian cancer. *Fam. Process.* 2007;46(3):335-349, [PMID: 17899857]
Exclusion code: 8

West CN, Barton MB, Liu AI, Geiger AM. Bilateral prophylactic mastectomy: Complications and subsequent procedures. *Journal of Clinical Oncology : ASCO annual meeting proceedings.* Vol 222004:6041
Exclusion code: 5

Wevers MR, Ausems MGEM, Verhoef S, et al. Behavioral and psychosocial effects of rapid genetic counseling and testing in newly diagnosed breast cancer patients: design of a multicenter randomized clinical trial. *BMC Cancer.* 2011;11:6, [PMID: 21219598]
Exclusion code: 3

Wewers ME, Lowe NK. A critical review of visual analogue scales in the measurement of clinical phenomena. *Res. Nurs. Health.* 1990;13(4):227-236, [PMID: 2197679]
Exclusion code: 2

White DB, Bonham VL, Jenkins J, Stevens N, McBride CM. Too many referrals of low-risk women for BRCA1/2 genetic services by family physicians. *Cancer Epidemiol. Biomarkers Prev.* 2008;17(11):2980-2986, [PMID: 18990739]
Exclusion code: 8

Whittemore AS, Balise RR, Pharoah PDP, et al. Oral contraceptive use and ovarian cancer risk among carriers of BRCA1 or BRCA2 mutations. *Br. J. Cancer.* 2004;91(11):1911-1915, [PMID: 15545966]
Exclusion code: 3

Whittemore AS, Gong G, Itnyre J. Prevalence and contribution of BRCA1 mutations in breast cancer and ovarian cancer: Results from three U.S. population-based case-control studies of ovarian cancer. *Am. J. Hum. Genet.* 1997;60(3):496-504, [PMID: 9042908]
Exclusion code: 3

Whittemore AS, Gong G, John EM, et al. Prevalence of BRCA1 mutation carriers among U.S. non-Hispanic Whites. *Cancer Epidemiol. Biomarkers Prev.* 2004;13(12):2078-2083, [PMID: 15598764]
Exclusion code: 2

Wickerham DL, Costantino JP, Vogel V, et al. The study of tamoxifen and raloxifene (STAR): Initial findings from the NSABP P-2 breast cancer prevention study. *Journal of Clinical Oncology: ASCO annual meeting proceedings.* Vol 242006
Exclusion code: 5

Wickerham DL, Costantino JP, Vogel VG, et al. The use of tamoxifen and raloxifene for the prevention of breast cancer. *Recent Results Cancer Res.* 2009;181:113-119, [PMID: 19213563]
Exclusion code: 5

Wilkening S, Burwinkel B, Grzybowska E, et al. Polyglutamine repeat length in the NCOA3 does not affect risk in familial breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(1):291-292, [PMID: 15668512]
Exclusion code: 8

Improving the referral process for familial breast cancer genetic counselling: findings of three randomised controlled trials of two interventions (Structured abstract). 2012. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=clhta&AN=HTA-32005000090
Exclusion code: 5

Wilson BJ, Torrance N, Mollison J, et al. Improving the referral process for familial breast cancer genetic counselling: findings of three randomised controlled trials of two interventions. *Health Technol. Assess.* 2005;9(3):iii-iv, 1-126, [PMID: 15694064]
Exclusion code: 3

Wilson DB, Quillin J, Bodurtha JN, McClish D. Comparing screening and preventive health behaviors in two study populations: Daughters of mothers with breast cancer and women responding to the Behavioral Risk Factor Surveillance System Survey. *J Womens Health.* 2011;20(8):1201-1206, [PMID: 21671767]
Exclusion code: 8

Wong N. Investigating the effects of cancer risk and efficacy perceptions on cancer prevention adherence and intentions. *Health Commun.* 2009;24(2):95-105, [PMID: 19280453]
Exclusion code: 4

Wood ME, Stockdale A, Flynn BS. Interviews with primary care physicians regarding taking and interpreting the cancer family history. *Fam. Pract.* 2008;25(5):334-340, [PMID: 18765407]
Exclusion code: 3

Woods JE. Breast reconstruction: current state of the art. *Mayo Clin. Proc.* 1986;61:579-585, [PMID: 3713262]
Exclusion code: 2

Wooster R, Weber BL. Breast and ovarian cancer. *N. Engl. J. Med.* 2003;348(23):2339-2347, [PMID: 12788999]
Exclusion code: 2

Yang Q, Khoury MJ, Rodriguez C, Calle EE, Tatham LM, Flanders WD. Family history score as a predictor of breast cancer mortality: prospective data from the Cancer Prevention Study II, United States, 1982-1991. *Am. J. Epidemiol.* 1998;147(7):652-659, [PMID: 9554604]
Exclusion code: 2

Yip C-H, Taib NA, Choo WY, Rampal S, Thong MK, Teo SH. Clinical and pathologic differences between BRCA1-, BRCA2-, and non-BRCA-associated breast cancers in a multiracial developing country. *World J. Surg.* 2009;33(10):2077-2081, [PMID: 19649760]
Exclusion code: 3

Yoon S-Y, Thong M-K, Taib NAM, Yip C-H, Teo S-H. Genetic counseling for patients and families with hereditary breast and ovarian cancer in a developing Asian country: an observational descriptive study. *Fam Cancer.* 2011;10(2):199-205, [PMID: 21318382]
Exclusion code: 8

You M. *Determinants of risk perception among women with a family history of breast cancer*, You, Myoungsoon: U California, Berkeley, US; 2009
Exclusion code: 8

Young D, McLeish L, Sullivan F, et al. Familial breast cancer: Management of 'lower risk' referrals. *Br. J. Cancer.* 2006;95(8):974-978, [PMID: 17047645]
Exclusion code: 8

Zakaria S, Degnim AC. Prophylactic mastectomy. *Surg. Clin. North Am.* 2007;87(2):317-331, viii, [PMID: 17498529]
Exclusion code: 5

Zapka J, Fisher G, Lemon S, Clemow L, Fletcher K. Relationship and distress in relatives of breast cancer patients. *Fam Syst Health.* 2006;24(2):198-212.
Exclusion code: 4

Zelada-Hedman M, Arver BW, Claro A, et al. A screening for BRCA1 mutations in breast and breast-ovarian cancer families from the Stockholm region. *Cancer Res.* 1997;57(12):2474-2477, [PMID: 9192828]
Exclusion code: 6

Zhang Y, Huang M, Zhu Z. AIB1 polymorphisms with breast cancer susceptibility: a pooled analysis of variation in BRCA1/2 mutation carriers and non-carriers. *Mol. Biol. Rep.* 2012;39(6):6881-6886, [PMID: 22307791]
Exclusion code: 5

Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* 1983;67(6):361-370, [PMID: 6880820]
Exclusion code: 6

Zilberg NJ, Weiss DS, Horowitz MJ. Impact of Event Scale: A cross-validation study and some empirical evidence supporting a conceptual model of stress response syndromes. *J. Consult. Clin. Psychol.* 1982;50(3):407-414, [PMID: 7096742]
Exclusion code: 2

Zilliacus EM, Meiser B, Lobb EA, et al. Are videoconferenced consultations as effective as face-to-face consultations for hereditary breast and ovarian cancer genetic counseling? *Genet Med.* 2011;13(11):933-941, [PMID: 21799430]
Exclusion code: 3

Ziv E. Genetics of breast cancer: applications to the Mexican population. *Salud Publica Mex.* 2011;53(5):415-419, [PMID: 22218795]
Exclusion code: 5

Zorn KK, Gardner G, Birrer MJ. Epithelial ovarian cancer. *Cancer Chemoprevention: Strategies for Cancer Chemoprevention.* Vol 2. 1 ed. 2005.
Exclusion code: 5

Zweemer R, van Diest P, Verheijen R, et al. Molecular evidence linking primary cancer of the fallopian tube to BRCA1 germline mutations. *Gynecol. Oncol.* 2000;76(1):45-50, [PMID: 10620440]
Exclusion code: 2

Appendix C1. Quality Ratings for Randomized, Controlled Trials

Author, Year	Randomization adequate?	Allocation concealment adequate?	Groups similar at baseline?	Maintain Comparable Groups?	Eligibility criteria specified?	Outcome assessors masked?	Care provider masked?	Patient masked?	Reporting of attrition, crossovers, adherence, and contamination
Bloom et al, 2006[151]	Unclear	NR	NR	NR	No	NR	NR	No	Yes
Bowen et al, 2002[57]	Yes	NR	Yes	Yes	Yes	No	No	No	Yes
Bowen et al, 2004[62]	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Bowen et al, 2006[152]	NR	NR	Yes	Yes	Yes	No	No	No	Yes
Brain et al, 2002[166]	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes
Braithwaite et al, 2005[154]	NR	NR	Yes	Yes	Yes	NR	Yes	No	Yes
Burke et al, 2000[58]	Yes	NR	Yes	Yes	Yes	No	No	No	Yes
Cull et al, 1998[59]	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Cuzick et al, 2007[288] IBIS-I Trial See also Cuzick, 2002[328]	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Fisher et al, 2005[284] NSABP P-1 Trial See also Fisher et al, 1998[71]	Yes	Yes	Yes	No	Yes	Unclear	Yes: intervention No: followup	Yes: intervention No: followup	Yes; after unblinding, 32% crossover from placebo to medication
Fry et al, 2003[155]	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Grady et al, 2008[73] RUTH Trial See also Barrett-Connor et al, 2006[299]	NR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Helmes et al, 2006[157]	NR	NR	Yes	Yes	Yes	NR	No	No	Yes
Lerman et al, 1996[168]	Yes	NR	Yes	Yes	Yes	Yes	No	No	Yes
Lerman et al, 1999[60]	Yes	NR	Yes	No	Yes	Yes	No	No	Yes
Lippman et al, 2006[288] MORE/CORE Trials See also Cummings et al, 1999[74]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Matloff et al, 2006[160]	No	No	Yes	Yes	Yes	NR	No	No	Yes
Powles et al, 2007[285] Royal Marsden Trial See also Powles et al, 1998[70]	NR	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes; after median of 70 months, 58% still on treatment
Roshanai et al, 2009[164]	Unclear	Yes	Yes	Yes	Yes	NR	Yes	No	Yes

Appendix C1. Quality Ratings for Randomized, Controlled Trials

Author, Year	Randomization adequate?	Allocation concealment adequate?	Groups similar at baseline?	Maintain Comparable Groups?	Eligibility criteria specified?	Outcome assessors masked?	Care provider masked?	Patient masked?	Reporting of attrition, crossovers, adherence, and contamination
Veronesi et al, 2007[287] Italian Randomized Tamoxifen Prevention Trial See also Veronosi et al, 1998[72]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes; 61% completed treatment period
Vogel et al, 2010[289] See also Vogel et al, 2006[329]	Yes	Unclear	Yes	Yes	Yes	Yes	Yes: intervention No: followup	Yes: intervention No: followup	Yes
Watson et al, 1998[170]	Yes	YEs	Yes	Yes	Yes	No	No	No	Yes

Author, Year	Loss to followup differential/high	Intention-to-treat analysis	Post-randomization exclusions	Outcomes Prespecified	Funding source	External validity	Quality rating
Bloom et al, 2006[151]	No	Yes	No	Yes	Grant 4EB-5800, California Breast Cancer Research Program	Population-based from San Francisco area	Poor
Bowen et al, 2002[57]	No	No	No	Yes	National Cancer Institute and National Human Genome Institute (HG01190)	Women in general public with breast cancer	Fair
Bowen et al, 2004[62]	NR	No	No	Yes	National Human Genome Institute, National Cancer Institute, and National Office for Research on Women's Health (HG/CA01190)	Women in Seattle area with lower risk of breast cancer	Fair
Bowen et al, 2006[152]	No	Yes	No	Yes	National Human Genome Research Institute (HG01190)	Ashkenazi Jewish women from large metropolitian area	Fair
Brain et al, 2002[166]	No	Yes	No	Yes	Medical Research Council, National Assembly for Wales, NHS R&D (Wales), and Imperial Cancer Research Fund (Dr. Gray is supported by Tenovus, a cancer charity)	Cancer clinics, Wales	Good
Braithwaite et al, 2005[154]	No	No	No	Yes	CUK (Cancer Research U.K.) (CI345/A169)	Greater London area	Fair

Appendix C1. Quality Ratings for Randomized, Controlled Trials

Author, Year	Loss to followup differential/high	Intention-to-treat analysis	Post-randomization exclusions	Outcomes Prespecified	Funding source	External validity	Quality rating
Burke et al, 2000[58]	No	NR	No	Yes	The National Institutes of Health (HGO1190)	Women in Seattle area with intermediate family history of breast cancer	Fair
Cull et al, 1998[59]	No/Yes	NR	No	Yes	NHS R&D (Cancer) Programme and Imperial Cancer Research Fund	Women from 4 Scottish cancer family clinics	Good
Cuzick et al, 2007[288] IBIS-I Trial See also Cuzick, 2002[328]	Unclear	Yes	No	Yes	CUK; National Health and Medical Research Council Australia	Women at increased risk for breast cancer; general population and clinic recruitment; United Kingdom, Europe, Australia, New Zealand	Fair
Fisher et al, 2005[284] NSABP P-1 Trial See also Fisher et al, 1998[71]	No/Unclear	Yes	No	Yes	National Cancer Institute; U.S. Department of Health and Human Services	Women at increased risk for breast cancer; clinical centers; United States and Canada	Fair
Fry et al, 2003[155]	No/Yes	No	No	Yes	Chief Scientists's Office and Cancer Research U.K.	General population recruitment	Fair
Grady et al, 2008[73] RUTH Trial See also Barrett-Connor et al, 2006[299]	No	Yes	No	Yes	Eli Lilly and Company	Postmenopausal women with history of heart disease or at increased risk of coronary events; multinational sites, including United States	Good
Helmes et al, 2006[157]	No	Yes	No	Yes	National Human Genome Research Institute	Large network of PCPs	Fair
Lerman et al, 1996[168]	No	NR	No	Yes	Public Health Service grants ROICA57767 and K07CAOI604 from the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services	Georgetown University Medical Center and Washington Hospital Center	Fair
Lerman et al, 1999[60]	No/Yes	NR	No	Yes	National Institutes of Mental Health and National Human Genome Research Institute (MH/HG54435)	Cancer treatment centers	Fair
Lippman et al, 2006[288] MORE/CORE Trials See also Cummings et al, 1999[74]	Unclear	Yes	No	Yes	Costs of publication of this article defrayed in part by payment of page charges; funding source NR	Postmenopausal women with osteoporosis; clinical centers; multinational, including United States	Good

Appendix C1. Quality Ratings for Randomized, Controlled Trials

Author, Year	Loss to followup differential/high	Intention-to-treat analysis	Post-randomization exclusions	Outcomes Prespecified	Funding source	External validity	Quality rating
Matloff et al, 2006[160]	No	No	No	Yes	Susan G. Komen Foundation	General population recruitment	Fair
Powles et al, 2007[285] Royal Marsden Trial See also Powles et al, 1998[70]	Unclear	Yes	No	Yes	National Health Service; CUK	Breast cancer clinics in United Kingdom	Fair
Roshanai et al, 2009[164]	No	No	No	Yes	Swedish Cancer Society	Cancer genetic clinics	Fair
Veronesi et al, 2007[287] Italian Randomized Tamoxifen Prevention Trial See also Veronosi et al, 1998[72]	No	Yes	No	Yes	Italian National Research Council; Italian Foundation for Cancer Research; American-Italian Cancer Foundation; Italian League Against Cancer	Hysterectomized women; general population and clinic recruitment; Italy	Fair
Vogel et al, 2010[289] See also Vogel et al, 2006[329]	No	Yes	No	Yes	National Cancer Institute; U.S. Department of Health and Human Services	Postmenopausal women with increased risk of breast cancer; multiple clinical centers; United States and Canada	Good
Watson et al, 1998[170]	No	Yes	No	Yes	Cancer Research Campaign (Project CP1026)	Women with a family history of breast cancer attending two London genetic clinics	Good

Abbreviations: CORE = Continuing Outcomes Relevant to Evista; CRC = Cancer Research Campaign; CUK = Cancer Research United Kingdom; IBIS-I = International Breast Cancer Intervention Study; MORE = Multiple Outcomes of Raloxifene Evaluation; NHS = National Health Service; NR = not reported; NSABP = National Surgical Adjuvant Breast and Bowel Project P-1; PCPs = primary care physicians; R&D = research and design; RUTH = Raloxifene Use for the Heart; STAR = Study of Tamoxifen and Raloxifene.

Appendix C2. Quality Ratings for Cohort Studies

Author, Year	Attempt to enroll a random sample or consecutive patients meeting inclusion criteria	Groups comparable at baseline	Used accurate methods for ascertaining exposures, potential confounders, and outcomes	Outcome assessors and/or data analysts blinded to treatment	Report attrition	Appropriate statistical analyses on potential confounders	Important differential or overall high loss to followup	Outcomes prespecified, defined, and ascertained using accurate methods	Quality rating
Domchek et al, 2010[292]	Yes	Not reported	Yes	No	No	Yes	Not reported	Yes	Fair
Foster et al, 2007[238]	Unclear	Not reported	Yes	No	Yes	Yes	No	Yes	Fair
Geirdal et al, 2005[240]	Yes	Yes	Yes	No	Yes	Unclear	No	Yes	Good
Geirdal and Dahl, 2008[239]	Yes	No	Yes	No	Yes	Yes	No	Yes	Good
Hopwood et al, 1998[167]	Unclear	Yes	Yes	No	Yes	Yes	No	Yes	Fair
Julian-Reynier et al, 2011[242]	Unclear	Yes	Yes	No	Yes	Yes	No	Yes	Good
Kinney et al, 2005[243]	No	Not reported	Yes	No	No	Yes	Not reported	Yes	Poor
Kramer et al, 2005[185]	Yes	Not reported	Yes	No	No	Yes	Not reported	Yes	Fair
Lobb et al, 2004[169]	Unclear	Yes	Yes	No	Yes	Yes	No	Yes	Good
Low et al, 2008[244]	Unclear	No	Yes	No	Yes	Yes	Yes	Yes	Fair
Meiser et al, 2002[250]	Unclear	Yes	Yes	No	Yes	Yes	No	Yes	Good
Mikkelsen et al, 2007[161]	Yes	No	Yes	No	Yes	Yes	No	Yes	Fair
Mikkelsen et al, 2009[162]	Yes	No	Yes	No	Yes	Yes	No	Yes	Fair
Reichelt et al, 2004[245]	Yes	Not reported	Yes	No	Yes	Yes	No	Yes	Good
Rijnsburger et al, 2004[275]	No	No	Yes	Unclear: not reported	Yes	Yes	No	Yes	Fair
Skytte et al, 2011[294]	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Good
Struewing et al, 1995[229]	Yes	Not reported	Not reported	No	No	No	Not reported	Yes	Poor
van Dijk et al, 2006[248]	Yes	Not reported	Yes	No	Yes	Yes	No	Yes	Good
Watson et al, 1999[170]	Unclear	Yes	Yes	No	Yes	Yes	No	Yes	Good

Appendix C3. Quality Ratings for Case-Control Studies

Author, year	Did study attempt to enroll all or random sample of cases using predefined criteria?	Were controls derived from the same population as the cases?	Were groups comparable at baseline on key prognostic factors?	Were enrollment rates similar in cases and controls invited to participate?	Did study use accurate methods for identifying outcomes?	Did study use accurate methods for ascertaining exposures and potential confounders?	Did study perform appropriate statistical analyses on potential confounders?	Quality
Armstrong et al, 2005[148]	Yes	No	No	No	Yes	Yes	Yes	Good
Dagan and Shochat, 2009[237] Shochat and Dagan, 2010[248]	Yes	Unclear	Matched	No	Yes	Yes	Yes	Fair

Appendix C4. Quality Rating for Systematic Review

Author, year	Search dates	Search methods reported	Comprehensive search	Inclusion criteria reported	Selection bias avoided	Validity criteria reported	Validity assessed appropriately	Methods used to combine studies reported	Findings combined appropriately	Conclusions supported by data	Quality rating
Smerecnik et al, 2009[165]	2000 to 2007	Yes	Yes	Yes	Yes	No	Not reported	No	Not reported	Yes	Fair

Appendix C5. Familial Risk Assessment Models

Ontario Family History Assessment Tool (FHAT)[142]

Risk Factor		Points
Breast and ovarian cancer	Mother	10
	Sibling	7
	2nd/3rd degree relative	5
Breast cancer relatives	Parent	4
	Sibling	3
	2nd/3rd degree relative	2
	Male relative (add to above)	2
Breast cancer characteristics	Onset age 20-29	6
	Onset age 30-39	4
	Onset age 40-49	2
	Pre (peri) menopausal	2
	Bilateral/multifocal	3
Ovarian cancer relatives	Mother	7
	Sibling	4
	2nd/3rd degree relative	3
Ovarian cancer onset age	<40	6
	40-60	4
	>60	2
Prostate cancer onset	Age <50	1
Colon cancer onset	Age <50	1
Family Total	**Referral**	**≥10**

Referral with score ≥10 corresponds to doubling of lifetime risk for breast cancer (22%).

Manchester Scoring System[141]

Risk Factor (age of onset for relative in direct lineage)	*BRCA1* Score	*BRCA2* Score
Female breast cancer		
<30	6	5
30-39	4	4
40-49	3	3
50-59	2	2
≥60	1	1
Male breast cancer		
<60	5*	8†
≥60	5*	5†
Ovarian cancer		
<60	8	5
≥60	5	5
Pancreatic cancer	0	1
Prostate cancer		
<60	0	2
≥60	0	1
Total individual genes	**10**	**10**
Total for combined=15		

Probability of ≥10% chance of *BRCA1* or *BRCA2* mutation individually or combined.
*If *BRCA2* tested.
†If *BRCA1* tested.

Appendix C5. Familial Risk Assessment Models

Referral Screening Tool (RST)[143]

History of breast or ovarian cancer in the family? If yes, complete checklist.		
Risk Factor	**Breast cancer age ≤50**	**Ovarian cancer at any age**
Yourself		
Mother		
Sister		
Daughter		
Mother's side		
Grandmother		
Aunt		
Father's side		
Grandmother		
Aunt		
≥2 cases of breast cancer after age 50 on the same side of the family		
Male breast cancer at any age in any relative		
Jewish ancestry		

Referral if ≥2 checks in table.

Pedigree Assessment Tool (PAT)[144]

Risk Factor	**Score for every family member with breast or ovarian cancer diagnosis, including 2nd/3rd degree**
Breast cancer at age ≥50	3
Breast cancer at age <50	4
Ovarian cancer at any age	5
Male breast cancer at any age	8
Ashkenazi Jewish heritage	4
Total	

Score ≥8 is the optimal referral threshold.

FHS-7[145]

1. Did any of your 1st degree relatives have breast *or* ovarian cancer?
2. Did any of your relatives have bilateral breast cancer?
3. Did any man in your family have breast cancer?
4. Did any woman in your family have breast *and* ovarian cancer?
5. Did any woman in your family have breast cancer before the age of 50 years?
6. Do you have 2 or more relatives with breast *and/or* ovarian cancer?
7. Do you have 2 or more relatives with breast *and/or* bowel cancer?

One positive response initiates referral.

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
Current report						
Armstrong et al, 2005[148] Good	Cancer worry Attitudes	To assess the association between race and use of genetic counseling for *BRCA1/2* testing in women at risk of carrying a *BRCA1/2* mutation and to evaluate the potential contributions of socioeconomic characteristics about genetic testing, and interactions with primary care physicians to this association	Case-control	Eligible: NR Enrolled: NR Randomized: NR Analyzed: 408 (217 cases, 191 controls)	U.S.	Visit to University of Pennsylvania Health System Cases: women from reference population who presented for genetic counseling, mean age 42.5 years, 29% Jewish Controls: random sample of women from reference population, mean age 53.1 years, 11% Jewish
Bennett et al, 2008[150] NA	Psychological	To examine the relationship between measures of anxiety and depression and a number of variables identified to be associated with distress	Before and after	Eligible: 367 Enrolled: 319 Analyzed: 128	U.K.	Women referred for genetic risk assessment to a large Cancer Genetics Service for Wales (CGSW) center
Bennett et al, 2009[149] NA	Cancer worry Psychological	To explore the relationship between a number of factors hypothesized to be associated with the frequency of intrusive worries close to the time women were informed of their genetic risk for developing breast and/or ovarian cancer	Before and after	Eligible: 221 Enrolled: 221 Analyzed: 128	U.K.	Women referred for genetic risk assessment to a large Cancer Genetics Service for Wales (CGSW) center
Bloom et al, 2006[151] Poor	Risk perception Cancer worry Health behaviors	To compare women in a telephone counseling intervention to controls and determine whether perceived risk would be more consistent with objective risk and whether there would be reduction in breast cancer worries, improvement in health protective behaviors, and an increase in breast cancer screening	RCT	Eligible: NR Enrolled: 163 Randomized: 163 (80 in intervention, 83 in control) Analyzed: 149 (71 in intervention, 78 in control)	U.S.	Sisters of women diagnosed with breast cancer at age ≤50; predominantly Euro-American and well educated; substantial majority receive regular breast cancer screening
Bowen et al, 2006[152] Fair	Risk perception Cancer worry Interest in genetic testing	To test the efficacy of 2 counseling methods in Ashkenazi Jewish women with average or moderately increased risk of breast cancer	RCT	Eligible: 347 Enrolled: 221 Randomized: 221 (68 to psychosocial counseling, 77 to genetic counseling, 75 to control) Analyzed: 96% followup rate	U.S.	Ashkenazi Jewish women from the greater Seattle area; mean age of 47 years; 100% Ashkenazi Jewish

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
Brain et al, 2011[153] NA	Cancer worry	To provide 6-year followup on women in TRACE study and the predictors of long-term cancer worry, perceived risk, and health behaviors	Before and after	Eligible: 545 Enrolled: 384 Analyzed: 263	U.K.	Women who took part in the TRACE study
Braithwaite et al, 2005[154] Fair	Risk perception	To examine the acceptability of the GRACE prototype to women with a family history of breast cancer and test the hypothesis that GRACE would perform as well as the nurse counselor at improving women's risk perceptions without causing adverse emotional reactions	RCT	Eligible: 89 Enrolled: 72 Randomized: 72 (38 to GRACE, 34 to clinical nurse specialist) Analyzed: 58	U.K.	Women with a family history of breast cancer recruited through newspaper ads and posters
Fry et al, 2003[155] Fair	Perceived risk Cancer worry	To compare the psychological outcomes of 2 models of breast cancer genetics services	RCT	Eligible:574 Enrolled:373 Analyzed: 244	Scotland	Women referred by GP for breast cancer genetic risk counseling
Gurmankin et al, 2005[156] NA	Risk perception	To examine the risk perception derived from a risk communication with a health care provider during genetic counseling for breast cancer and *BRCA1/2* mutation risks	Before and after	Eligible: NR Enrolled: 58 Analyzed: NR	U.S.	New patients at university cancer evaluation program; mean age of 46 years; most were white and had some college education or more
Helmes et al, 2006[157] Fair	Cancer worry Risk perception	To assess whether women participating in either in-person or telephone counseling sessions would have a more accurate perception of their personal breast cancer risk, increase their intentions for breast screening, have reduced levels of cancer worry, and have less interest in genetic testing	RCT	Eligible: 898 Enrolled: 340 Randomized: 340 (104 to the in-person arm, 121 to the telephone arm, 115 to control) Analyzed: 335 (102 in the in-person arm, 119 in the telephone arm, 114 control arm)	U.S.	Physicians network in Washington Mean age, 40.7 years
Hopwood et al, 2004[158] NA	Cancer worry Psychological factors	To assess changes in risk perception, psychological distress, health care behaviors, and use of health care resources; to assess satisfaction with services, to describe regional variations in outcomes	Before and after	Eligible: 271 Enrolled: 256 Analyzed: 234 (1 month), 202 (12 month), 192 (precounsel, 1 and 12 months)	U.K.	Cancer genetic services centers Age range, 49-52 years

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
Kelly et al, 2008[159] NA	Risk perception	To examine change in subjective risk of ovarian cancer over time in response to genetic counseling and testing in the short- and long-term; discrepancy between subjective and objective estimates of ovarian cancer risk; and new methods for conceptualizing subjective risk derived from the Common Sense Model	Before and after	Eligible: 78 Enrolled: 78 (40 to no personal history of breast cancer, 38 to personal history) Analyzed: NR	U.S.	Women were recruited from the community Mean age, 48.64 years
Matloff et al, 2006[160] Fair	Risk perception	To examine if a personalized risk assessment and genetic counseling intervention would affect knowledge, risk perception, and decisionmaking in a group of women who had 1 FDR with breast cancer compared with a control group	RCT	Eligible: NR Enrolled: NR Randomized: 64 (32 in each group) Analyzed: 54 completed 1 month followup (28 control and 26 intervention), 48 completed 6 month followup (25 control and 23 intervention)	U.S.	Women recruited through advertisements in New Haven, CT
Mikkelsen et al, 2007[161] Fair	Risk perception	To explore the impact of genetic counseling on perceived personal lifetime risk of breast cancer, the accuracy of risk perception, and possible predictors of inaccurate risk perception 1 year following counseling	Prospective cohort	Eligible: 3257 (568 in counseling group, 689 in reference group 1, 2000 in reference group 2) Enrolled: 1971 (319 in counseling group, 381 in comparison group 1, and 1271 in group 2) Analyzed: 1602 (213 in counseling group, 319 in comparison group 1, and 1070 in group 2)	Denmark	Danish women at risk of hereditary breast and ovarian cancer
Mikkelsen et al, 2009[162] Fair	Psychological factors Cancer worry Quality of life changes	To clarify the psychosocial impact of genetic counseling for hereditary breast and ovarian cancer	Prospective cohort	Eligible: 3257 (568 in counseling group, 689 in reference group 1, 2000 in reference group 2) Enrolled: 1971 (319 in counseling group, 381 in comparison group 1, and 1271 in group 2) Analyzed: 1602 (213 in counseling group, 319 in comparison group 1,	Denmark 2007	Danish women at risk of hereditary breast and ovarian cancer

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
				and 1070 in group 2)		
Pieterse et al, 2011[163] NA	Risk perception accuracy, correct knowledge, perceived personal control, generalized state anxiety, and cancer-related distress	To assess changes in cognitions (accurate risk perception, correct knowledge, perceived personal control) and distress (state anxiety, cancer-related stress reactions) from before to immediately and 6 months after concluding breast cancer genetic counseling in female counselees, and whether changes in cognitions and distress were similar in affected versus unaffected women	Before and after	Eligible: 204 Enrolled: 77 Randomized: N/A Analyzed: 77	The Netherlands	Women seeking counseling for hereditary cancer at University Medical Center in the Netherlands, surveys exchanged through the mail
Roshanai et al, 2009[164] Fair	Risk perception Psychological factors	To investigate the effect of an informational intervention on counselees' knowledge, risk perception, communication of information to at-risk relatives and satisfaction with the service	RCT	Eligible: 210 Randomized: 163 (85 in intervention, 78 in control group) Analyzed: 147 at precounseling (73 in intervention, 74 in control); 144 for risk perception (71 in intervention, 73 in control); 147 2 weeks postcounseling (73 in intervention, 74 in control); 139 at 8 months postcounseling (68 in intervention, 71 in control)	Sweden	Swedish women visiting a university cancer genetic clinic, mainly referred due to breast cancer or family history of breast, ovarian or colorectal cancer (groups separated for analysis)

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
Prior report						
Bowen et al, 2002[57] Fair	Interest in genetic testing	To test the effects of breast cancer risk on interest in genetic testing in women who have a family history of breast cancer	RCT	Eligible: 561 Enrolled: 357 Randomized: 357 (120 to genetic counseling, 114 to psychosocial group, 123 to delayed counseling) Analyzed: 317 (105 to genetic counseling, 103 to psychosocial, 109 to delayed counseling)	U.S.	Women recruited from the Seattle area; see Bowen et al, 1999. All volunteered after seeing a notice, hearing about the study from a network, or through a relative with cancer
Bowen et al, 2004[62] Fair	Cancer worry Psychological factors Risk perception	To test the effects of 2 types of breast cancer risk counseling (group psychosocial or individual genetic) on perceived risk, negative affect, and worry about breast cancer	RCT	Eligible: 561 Enrolled: 354 Randomized: 354 (118 genetic counseling arm, 114 psychosocial counseling arm, 122 delayed intervention arm) Analyzed: 348 (117 genetic counseling arm, 110 psychosocial counseling arm, 121 delayed intervention arm)	U.S.	Recruitment from among family members with breast cancer and through notices in local electronic and print outlets. Recruitment completed in 8 months. Women with a range of actual breast cancer risk levels were included
Brain et al, 2002[166] Good	Psychological factors	To compare the psychological impact of a multidisciplinary specialist genetics service with surgical provision in women at high risk and lower risk of familial breast cancer	RCT	Eligible: 1,000 Enrolled: 740 Randomized: 735 (369 control, 366 trial) Analyzed: 653 (315 control, 338 trial)	Wales	Welsh women with family history of breast cancer referred to breast cancer clinic by doctor in 18-month trial period (1996 to 1997). Randomized to trial (n=366) or control group (n=369)
Burke et al, 2000[58] Fair	Cancer worry Risk perception	To assess whether modified traditional genetic counseling causes women with an intermediate risk of breast cancer to have a more realistic view of their risk, of genetic testing, and to decrease breast cancer worry	RCT	Eligible: 793 Enrolled: 356 Randomized: 243 (120 to genetic counseling, 123 to control group) Analyzed: 237 (116 to genetic counseling, 121 to control group)	U.S.	Sources for solicitation include women who live within 60 miles of Seattle: 2 studies at Fred Hutchinson Cancer Research Center, an oncologist's practice at University of Washington, mass media announcements

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
Cull et al, 1998[59] Good	Psychological factors Risk perception	To evaluate use of video for education on the genetic basis of breast cancer and on strategies for breast cancer risk management in a breast cancer family clinic	RCT	Eligible: 159 Enrolled: 144 Randomized: 128 (66 to video before group, 62 to video after) Analyzed: 95 (53 to video before group, 42 to video after group)	U.K.	A consecutive series of women newly referred to the breast cancer family clinic were invited by mail to participate; 24% of the video before and 30% of the video after group were referred by another hospital clinic; 1 subject in each group had been referred from another genetic clinic. The remaining were referred by GPs
Hopwood et al, 1998[167] Fair	Psychological factors	To understand psychological support needs for women at high genetic risk for breast cancer	Cohort	Eligible: 176 Enrolled: 174 Analyzed: 158	England	All were consecutive first-time attendees at the Family History Clinics (Manchester, U.K.)
Lerman et al, 1996[168] Fair	Cancer worry Risk perception	To study effect of individualized breast cancer risk counseling	RCT	Eligible: 438 Enrolled: 227 Randomized: 227 (group randomization NR) Analyzed: 200 (90 to risk counseling, 110 to control group)	U.S.	Subjects identified by relatives under treatment for breast cancer at either Fox Chase Cancer Center or Duke Comprehensive Cancer Center
Lerman et al, 1999[60] Fair	Cancer worry Interest in genetic testing	To investigate racial differences in response to 2 alternate pretest education strategies for *BRCA1* genetic testing: a standard education model and an education plus counseling model	RCT	Eligible: 581 Enrolled: 364 Randomized: 364 (group randomization NR) Analyzed: 298 (157 to education only, 141 to education plus counseling)	U.S.	Subjects were recruited from 2 cancer centers (Georgetown University Medical Center or Washington Hospital Center)
Lobb et al, 2004[169] Good	Psychological factors	To examine the effect of different consultant communication styles on a variety of outcomes	Longitudinal	Eligible: NR for unaffected group Enrolled: NR for unaffected group Analyzed: 89	Australia	Women from high-risk breast cancer families attending their first consultation before genetic testing
Watson et al, 1998[171] Good	Cancer worry Psychological factors Risk perception	To look at recall of risk information after genetic counseling and to determine impact of receiving an audiotape of the genetic consultation on level of recall, cancer-related worry, and uptake of risk management methods	RCT	Eligible: 135 Enrolled: 115 Randomized: 115 (60 cases, 55 controls) Analyzed: 107 (56 cases, 51 controls)	U.K.	First-time attendees at the cancer family clinics of 2 London hospitals: Royal Marsden, Sutton and London, and St. George's Hospitals

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/setting
Watson et al, 1999[170] Good	Psychological factors	To investigate perception of genetic risk and the psychological effects of genetic counseling in women with a family history of breast cancer	Prospective cohort	Eligible: 303 Enrolled: 282 Analyzed: 282	England	First-time genetic clinic attendees recruited from 4 South London genetic counseling centers (Royal Marsden NHS Trust Hospital [2 separate clinics], Mayday University Hospital, and St. Georges' Hospital)

Author, year Quality	Demographics	Inclusion/exclusion criteria	Risk level definition
Current report			
Armstrong et al, 2005[148] Good	Cases vs. controls Mean age (years): 42.5 (range, 19-66) vs. 53.1 (range, 20-89) Race/ethnicity: African American: 7.4% vs. 29% Asian American: 3.3% vs. 3.2% White: 85% vs. 66% Hispanic: 0% vs. 2.1% Other: 4.6% vs. 0% Religious heritage: Jewish: 29% vs. 11% Christian: 52% vs. 60% Other: 13% vs. 13% NR: 5.9% vs. 16%	Inclusion: Women ages 18-80 years seeing a primary care physician within the University of Pennsylvania Health System in the 3 years prior to the start of the study, with FDR or SDR with a breast or ovarian cancer diagnosis Exclusion: Personal diagnosis of breast or ovarian cancer, identified as being unable to participate because of illness or mental incapacity by their primary care physician Controls: Previously participated in *BRCA1/2* genetic counseling	FDR or SDR with a breast or ovarian cancer diagnosis
Bennett et al, 2008[150] NA	Mean age, 43.3 years	Inclusion: Women undergoing assessment for risk of breast/ovarian cancer at the CGSW and who completed followup questionnaires Exclusion: Did not complete risk assessment process before the end of the study	23% low risk 45% moderate risk 31% high risk
Bennett et al, 2009[149] NA	Mean age, 44.3 years (SD, 10.81; range, 18-76)	Inclusion: Women undergoing assessment for risk of breast/ovarian cancer at the CGSW and who completed followup questionnaires Exclusion: Did not complete risk assessment process before the end of the study	30/128 (23.4%) at population risk 61/128 (47.7%) at moderate risk 37/128 (28.9%) at high risk
Bloom et al, 2006[151] Poor	Mean age, 47.4 years (SD, 7.2) 77% Euro-American 6.1% African American 9.2% Latina 8.0% Asian/Other	Inclusion: Not reported Exclusion: Prior breast cancer	All had ≥1 FDR (sister) with breast cancer diagnosis at age ≤50

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Demographics	Inclusion/exclusion criteria	Risk level definition
Bowen et al, 2006[152] Fair	Mean age, 47 years	Inclusion: Women ages 18-74 years with ≥1 Ashkenazi Jewish ancestor who lived within 60 miles of Seattle Exclusion: Personal history of breast or ovarian cancer, family history consistent with an autosomal dominant inheritance of breast cancer predisposition	≥1 Ashkenazi Jewish ancestor
Brain et al, 2011[153] NA	Mean age, 42.3 years (SD, 8.22)	Inclusion: Women who took part in TRACE study and were approved by physician to be contacted Exclusion: NR	Moderate risk not otherwise described
Braithwaite et al, 2005[154] Fair	GRACE (n=37) vs. counseling (n=34) Age (years): 18-34: 62.2% vs. 67.6% 35-49: 27% vs. 20.6% ≥50: 10.8% vs. 11.8% Ethnicity: White: 91.9% vs. 94.1% Other: 8.1% vs. 5.8%	Inclusion: Having ≥1 FDR or SDR with breast cancer Exclusion: Personal history of breast cancer	All had ≥1 FDR or SDR with breast cancer
Fry et al, 2003[155] Fair	Mean age (SD) Standard service: 37.3 (9.4) Novel service: 39.1 (9.6)	Inclusion: Women who lived in the region and were able to give informed consent and complete a baseline questionnaire Exclusion: Women who were symptomatic or diagnosed with breast and/or ovarian cancer, or women who had previously consulted with another clinic about their family history of cancer	Criteria for significantly increased risk: Having a FDR with breast cancer diagnosis before age 40; having 2 FDRs or SDRs on the same side of the family with breast cancer diagnosis before age 60 or with ovarian cancer; having 3 FDRs or SDRs on the same side of the family with breast or ovarian cancer; having a FDR with breast cancer in both breasts; and having a male relative with breast cancer
Gurmankin et al, 2005[156] NA	Mean age of 45.9 years (SD, 10.5) 88% White 10% Black 2% Other 42% Ashkenazi Jewish	Inclusion: Females only Exclusion: Health care provider indicated they were too ill to participate	NR

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Demographics	Inclusion/exclusion criteria	Risk level definition
Helmes et al, 2006[157] Fair	Mean age (years): In-person counseling: 39.9 (SD, 9.2) Telephone counseling: 40.4 (SD, 9.7) Delayed counseling: 41.8 (SD, 10.1)	Inclusion: Women ages 18-64 years within 60 miles of research institute, planning to live in area for 1 year, spoke English, telephone in home, covered by commercial health insurance plan Exclusion: Women with personal history of breast/ovarian cancer, personal history of genetic counseling or testing for cancer risk	14.7% had family history of breast cancer
Hopwood et al, 2004[158] NA	Average across all 5 cancer genetics services: Mean age, 41 years (range, 22-72) 94% Female 2% Ethnic minority	Inclusion: Women seen at a cancer genetics services center Exclusion: Women who had been diagnosed with cancer, age <18 years	NR
Kelly et al, 2008[159] NA	Mean age, 48.64 years (SD, 12.69) 100% Ashkenazi Jewish women	Inclusion: Ashkenazi Jewish women with personal or family histories suggestive of an inherited predisposition to breast and/or ovarian cancer Exclusion: Prior history of ovarian cancer, men, women having prophylactic oophorectomies	≥1 Ashkenazi Jewish grandparent
Matloff et al, 2006[160] Fair	Mean age, 49 years (range, 41-55) 21% Ashkenazi Jewish	Inclusion: Women age ≥40 years with ≥1 FDR with breast cancer, had gone through natural menopause Exclusion: Taking menopausal therapy, having had cancer, atypical hyperplasia, or LCIS, being a known carrier of a *BRCA1/2* mutation, having heart disease, women with family history that placed them at >10% risk of carrying a mutation	≥1 FDR with breast cancer
Mikkelsen et al, 2007[161] Fair	Median age (years): Counseling: 39 (range, 18-72) Group 1: 56 (range, 28-76) Group 2: 45 (range, 18-75)	Inclusion: Women age ≥18 years who attended an initial genetic counseling session for breast or ovarian cancer Exclusion: Women affected with breast or ovarian cancer at baseline or who developed cancer during the followup period	NR
Mikkelsen et al, 2009[162] Fair	Median age (years): Counseling: 39 (range, 18-72) Group 1: 56 (range, 28-76) Group 2: 45 (range, 18-75)	Inclusion: Women age >18 years who attended an initial genetic counseling session for breast or ovarian cancer Exclusion: Women affected with breast or ovarian cancer at baseline or who developed cancer during the followup period	NR

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Demographics	Inclusion/exclusion criteria	Risk level definition
Pieterse et al, 2011[163] NA	Age ≥18 years	Inclusion: Patients sought counseling for hereditary cancer; were first among their 1st- and 2nd-degree relatives to request counseling; were first-time attendees; and age >18 years Exclusion: NR	Seeking counseling for hereditary cancer
Roshanai et al, 2009[164] Fair	Female: 90.5% (n=133) Male: 9.5% (n=14) Median age, females (years): 56 (range, 23-84)	Inclusion: Women age ≥18 years; able to read, write, and speak Swedish Exclusion: Suffered from any mental illness	Risk estimated by geneticist: Intervention n (%) vs. control n (%) ≤20%: 5 (15) vs. 3 (23) 21%-40%: 29 (72.5) vs. 37 (77) >40%: 3 (9) vs. 1 (4)
Prior report			
Bowen et al, 2002[57] Fair	Psychological counseling arm: Mean age, 41.9 years (SD, 11.3) 90% White, nonHispanic 3.5 % White, Hispanic 0.9% African American 2.6% Asian or Pacific Islander 1.8% Native American 0.9% Multiracial Genetic counseling arm: Mean age, 42.8 years (SD, 11.8) 94% White, nonHispanic 0.0% White, Hispanic 0.8% African American 1.7% Asian or Pacific Islander 1.7% Native American 1.7% Multiracial Control arm: Mean age, 42.4 years (SD, 11.5) 93% White, nonHispanic 0.0% White, Hispanic 2.5% African American 3.3% Asian or Pacific Islander 0.0% Native American 0.8% Multiracial	Inclusion: Women ages 18-74, lived within 60 miles of research center, agreed to participate in counseling and complete questionnaires, and had ≥1 relative affected by breast cancer Exclusion: Lack of family history of breast cancer, age outside the 18-74 range, >1 close relative affected by breast cancer, living outside the catchment area and lack of interest in completing the study	Family history: Close relatives affected by breast cancer included grandmothers, mothers, sisters, and aunts Risk level: Gail and Claus scores, along with population data
Bowen et al, 2004[62] Fair	Mean age, years (SD) Genetic counseling: 42.6 (11.8) Psychosocial counseling: 42.1 (11.4) Delayed intervention: 42.5 (11.5)	Inclusion: Women ages 18-74 with ≥1 relative with breast cancer, no personal history of breast or ovarian cancer, no family history consistent with a BRCA mutation for breast cancer risk, living within 60 mile radius of research center, willingness to complete research activities and completed and returned baseline questionnaire Exclusion: NR	Family history: Self-report of any family history of breast cancer Risk level: Calculated by use of Gail and Claus models, along with population data

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Demographics	Inclusion/exclusion criteria	Risk level definition
Brain et al, 2002[166] Good	Mean age, years (SD) Low vs. moderate vs. high risk Control group: 48.6 (10.25) vs. 40.5 (9.13) vs. 39.2 (7.33) Trial group: 52.9 (7.75) vs. 41.6 (8.52) vs. 33.7 (8.19)	Inclusion: Women with a 1st-degree female relative diagnosed with breast cancer before age 50 years or with bilateral breast cancer diagnosed at any age, ≥2 FDRs with breast cancer, or a FDR and SDR with breast cancer Exclusion: Personal history of breast cancer, previously received genetic counseling, or was not a resident of Wales	Family history risk definition: 1st-degree female relative diagnosed with breast cancer before age 50; 1st-degree female relative with bilateral breast cancer at any age; ≥2 FDRs with breast cancer; or a FDR and SDR with breast cancer. Risk definition: In trial group, risk was assessed on detailed pedigree data collected and analyzed by geneticist using Claus model. In control group, surgical assessment of risk was based on info collected on age, reproductive history, and minimal family history
Burke et al, 2000[58] Fair	Genetic counseling arm: Average age, 43 years (SD, 12) 94% White Control group arm: Average age, 42 years (SD, 12) 93% White	Inclusion: Women ages 18-74, lived within 60 miles of Seattle, and had ≥1 biological relative who has been diagnosed with breast cancer Exclusion: A personal history of breast or ovarian cancer and a family history indicative of autosomal dominant inheritance of breast cancer	Intermediate family history of breast cancer: ≥1 biological relative with breast cancer but whose pedigree suggests a low likelihood of autosomal dominant transmission. Family history indicative of autosomal dominant inheritance of breast cancer: ≥2 1st-degree or 1 1st- and 1 2nd-degree relative with either breast cancer before age 50 or ovarian cancer at any age, or ≥2 paternal 2nd-degree relatives with either breast cancer before age 50 or ovarian cancer at any age. The Claus model showed that these women would have ≥20% breast cancer risk by age 79
Cull et al, 1998[59] Good	Mean age, 39 years (SD, 8)	NR	NR
Hopwood et al, 1998[167] Fair	Mean age, 36.19 years (range, 22.63-46.35)	Inclusion: Women ages 18-45 living within a 25 mile radius of the FHC with risk ≥2-fold greater than the population for breast cancer Exclusion: NR	Risk was ≥2-fold greater than the population for breast cancer (i.e., 1:6 lifetime risk or greater as assessed using the Claus model)

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Demographics	Inclusion/exclusion criteria	Risk level definition
Lerman et al, 1996[168] Fair	18% ages 35-40 years 41% ages 41-49 years 42% age ≥50 years 90% White 10% Black	Inclusion: Women age ≥35 years and a family history of breast cancer Exclusion: A personal history of cancer and younger than age 35 years	≥1 FDR with breast cancer; breast cancer risk estimates for individual women were calculated using subject's Gail model variables and estimated the lifetime probability of developing breast cancer, 95% CIs, and the estimated lifetime risk for a woman of the same age with the lowest risk of disease
Lerman et al, 1999[60] Fair	24% Black 34% age <40 years 66% age ≥40 years 76% White 41% age <40 years 59% age ≥40 years	Inclusion: Caucasian and African American women with a family history of breast cancer or ovarian cancer Exclusion: Personal history of cancer (except basal cell or squamous cell skin cancer)	≥1 FDR affected with breast cancer and/or ovarian cancer
Lobb et al, 2004[169] Good	Mean age, 38.7 years (range, 19-60)	Inclusion: Women attending their first consultation before genetic testing with no prior testing for or carrier of *BRCA1* or *BRCA2* Exclusion: Unable to give informed consent, age <18 years, showed evidence of severe mental illness, and nonfluent in English	NR
Watson et al, 1998[171] Good	Median age, 37 years (range, 28-56) for participants from the Royal Marsden Hospital Median age, 41 years (range, 23-71) for participants from St. George's Hospital	Inclusion: Women with a family history of breast cancer, first visit to genetic clinic, never having been clinically affected with cancer, no known mental illness, and age ≥18 years Exclusion: NR	NR
Watson et al, 1999[170] Good	Median age, 37 years (range, 19-76)	Inclusion: Women with a family history of breast cancer, never clinically affected by cancer, no known serious mental illness, age ≥18 years, and able to complete a questionnaire Exclusion: NR	Breast cancer risk calculated using CASH model based on the number of breast cancer cases in 1st- and 2nd-degree relatives, age of family members at disease onset, and age of woman presenting for genetic counseling

Author, year Quality	Interventions	Measures	Duration of followup
Current report			
Armstrong et al, 2005[148] Good	A) Genetic counseling prior to testing, otherwise not described B) Controls		1999-2003 Not applicable

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Bennett et al, 2008[150] NA	CGSW referral guidelines and BRCAPRO risk calculation model	Medical Coping Modes Questionnaire (MCMQ, scale NR) Impact of Events Scale (IES, subscales 0 to 28) DUKE Social Support Questionnaire (DUKE-SSQ, scale 1 to 5) Hospital Anxiety and Depression Scale (HADS, subscales 0 to 21) Perceived health Quality of Life	Year NR 1 week following risk notification
Bennett et al, 2009[149] NA	CGSW referral guidelines and BRCAPRO risk calculation model	Impact of Events Scale (IES, subscales 0 to 28) Medical Coping Modes Questionnaire (MCMQ, scale NR) DUKE Social Support Questionnaire (DUKE-SSQ, scale 1 to 5)	Year NR Approximately 5 to 7 weeks
Bloom et al, 2006[151] Poor	A) Telephone counseling from a master's level counselor within 2 weeks; breast cancer worries measured by 4-point Likert scale; perceived risk measured on 5-point scale; rating chances of diagnosis (0%-100%). Telephone counseling session included establishment of rapport and determination of special concerns, emotional readiness; risk notification by providing modified Gail model lifetime risk estimate and discussing in terms of her pretest self-assessment of risk; deescalation of tension regarding breast cancer checkup; evaluation of coping skills, reinforcement of problem solving and coping skills; information on health protective behaviors; early detection through American Cancer Society screening; and information on genetic testing when requested. B) Delayed telephone counseling following the posttest	NSI: 3-item measure of breast cancer worry: perceived risk of breast cancer, health behaviors, and breast cancer sreening	1999-2002 6 months
Bowen et al, 2006[152] Fair	A) Group psychosocial counseling: psychologist led four 2-hour, weekly sessions of 5 to 6 women per group. Each session included 20-minute group cohesion activities followed by 1 of 4 major intervention components: risk assessment and perception, education, stress management, and problem solving and social support. B) Individual genetic counseling: genetic counselor provided 1-hour counseling sessions, individually. Sessions covered several topics, including participant's family background, breast cancer risk assessment, *BRCA1/2* mutations in the Ashkenazi Jewish population, nongenetic risk factors for breast cancer, and breast screening. C) Delayed counseling: no counseling, served as control	NSI: Continuous scale of 0-100 to assess risk perception BSI: 53-item self-reported psychological symptom scale	Year NR 6 months
Brain et al, 2011[153] NA	A) Claus model B) Generalized risk level based on age, reproductive history, and minimal family history	Cancer Worry Scale-Revised (CWS-R, scale 6 to 24) Perceived risk (single item scale, 1 to 5)	Year NR 6 years

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Braithwaite et al, 2005[154] Fair	Both interventions were 1 session; cognitive outcomes assessed at baseline, postclinic, and at 3 months A) Risk counseling arm: Clinical nurse specialist undertook counseling sessions and drew pedigree with information from family history and assessed risk as low, moderate, or high based on GRACE guidelines. Participants were mailed letters summarizing content afterward B) GRACE: Participants completed their pedigrees in GRACE and assessed their risk, learning their risk assessment and how to manage their risk. They received a numerical estimate of lifetime risk; a visual display of cumulative risk with general population as comparator; and a qualitative description. Clinical nurse specialists then offered to book mammography and arrange meetings with geneticists, where appropriate	NSI: Measured attitude, perceived benefit, risk perception, and satisfaction and risk communication on a likert scale STAI: Measures an individual's current anxiety feelings HADS: 14-item self-report scale for the detection of depression and anxiety in hospitalized patients	Year NR 3 months
Fry et al, 2003[155] Fair	Standard (regional) service: Self-report family history and baseline questionnaire; genetics consultant and genetics nurse specialist assigned categorical risk via Cancer Research Campaign. Women at low risk receive infomative letter; women at moderate/high risk offered appointment at familial breast cancer clinic where a genetics consutant discusses risk status and breast surgeon discusses risk management. Where appropriate, clinical exams and mammography included. Patients' GPs receive summary data, and patients receive followup questionnaires 4 weeks and 6 months later Novel (community-based) service: Women sent an appointment for a community-based clinic near their residence. Meetings run by genetics nurse specialist where family history collected and compared to published criteria (Cancer Research Campaign) to determine risk. Women at low risk offered information, reassurance, and discharged. Women at moderate/high risk offered appointment at a regional center with a geneticist and genetics nurse specialist, and asked to complete followup questoinnaires at 4 weeks and 6 months	Cancer Worry Scale (scale 5 to 24) GHQ-30	6 months
Gurmankin et al, 2005[156] NA	A) Precounseling interview assessed patient's breast cancer risk perception, *BRCA1/2* mutation risk perception, worry about breast cancer, family history of cancer, breast cancer risk reduction behaviors, and demographic information B) Postcounseling interview assessed patient's breast cancer risk, *BRCA1/2* mutation risk, recall of actual risk information, worry about breast cancer, completion of the Spielberger Trait Anxiety Inventory (20-80 score range) and	STAI: Measures an individual's current anxiety feelings NSI: Scale of 0 to 100 to assess risk perception Scale of 1 to 7 to asses cancer worry	October 2002 to February 2004 1 week

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
	the Life Orientation Test-Revised (0-32 measure of optimism)		
Helmes et al, 2006[157] Fair	A) In-person counseling: board-certified genetic counselor conducted counseling consisting of a review of family history, discussion of breast cancer risk, and education about breast cancer genes. Also discussed genetic testing considerations, including implications of results, testing strategies, potential risks and benefits of test, cost of test, and psychological effects of test. Information packet was provided that contained personal risk information comparing the woman's risk with average woman's risk; personal computer-drawn 3-generation pedigree; brochures on self-breast exams, Pap smear, and mammography; genetics visual aids; list of community resources; and cover letter B) Telephone counseling: information packet was sent in the mail with instructions to open at the beginning of the telephone counseling, which was identical in content and structure to in-person counseling. C) Control group did not receive counseling	NSI: Scale of 0 to 100 to assess risk perception Scale of 1-4 to measure intention to obtain breast cancer screening 4-item questionnaire to assess interest in genetic testing	Year NR 3 months
Hopwood et al, 2004[158] NA	A) Genetic counseling, otherwise not described	NSI: 5-response category assement of perceived cancer risk GHQ: 60-item questionnaire to screen individuals for psychiatric disorders	Year NR At 1 month and 1 year following precounseling
Kelly et al, 2008[159] NA	A) Genetic counseling included review of family cancer history, personal risk factors for breast and ovarian cancer, mechanisms of cancer inheritance, meaning of a positive and negative test result, and risks and benefits associated with testing	CWS: 3-item questionnaire to measure how frequently an individual worries about getting breast cancer	Year NR 6 months
Matloff et al, 2006[160] Fair	A) Counseling session with personalized letter summarizing patient data B) Controls who received no counseling	NSI: Reviewed detailed information about menopause, the risks and benefits of each menopasue therapy option, and a disease risk factor assessment	August 2002 to January 2004 6 months
Mikkelsen et al, 2007[161] Fair	A) Genetic counseling: information on incidence of sporadic breast cancer, genetics, inheritance patterns, and estimated personal lifetime risk of inherited cancer B) Comparison group 1: women referred for mammography C) Comparison group 2: random sample of women	IES: 17-item questionnaire to measure an individual's level of distress in relation to a specific event or condition	2003-2004 1 year
Mikkelsen et al, 2009[162] Fair	A) Genetic counseling: information on incidence of sporadic breast cancer, genetics, inheritance patterns, and estimated personal lifetime risk of inherited cancer B) Comparison group 1: women referred for mammography C) Comparison group 2: random sample of women	HADS: 14-item self-report scale for the detection of depression and anxiety in hospitalized patients	2003-2004 1 year

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Pieterse et al, 2011[163] NA	A) First session topics included family's occurrence of breast and other cancers, inheritance, and criteria on probability of inherited breast cancer. Likelihood of hereditary breast cancer running in family was estimated. Genetic testing was offered to counselees or affected relatives when they had an a priori chance (≥10%) of carrying BRCA gene. Counselees eligible for testing informed of medical consequences and options. Periodic surveillance recommended to all counselees at increased risk (>20%). Counselees and referring physician receive summary letter about genetic and risk information. Counselors distributed postcounseling questionnaire after last session and asked participants to complete it within a day. 6 months later, counselees were sent a followup questionnaire. All 3 of these questionnaires assessed cognitions and distress. Counselors completed a questionnaire after counselee's last visit. Counseling spanned 1 to 4 visits over 6 to 24 months; STAI, IES, and VAS were used to measure anxiety levels	VAS: Any of a number of pain self-assessment tools where subjects indicate their level of pain in response to a continuous visual scale NSI: Scale of 0 to 100 to assess risk perception Scale of 0 to 7 to assess hereditary breast cancer knowledge PPC: Construct reflecting the degree to which a person believes that a situation is under their control STAI: Measures an individual's current anxiety feelings IES: 17-item questionnaire to measure an individual's level of distress in relation to a specific event or condition	24 months (6 months after last counseling session)
Roshanai et al, 2009[164] Fair	A) Genetic counseling from specialist nurse: pedigree explanation; Buckman's Breaking Bad News model to inform at-risk relatives; pamphlet, videotape, copies of pedigree and medical records B) Control group received standard care given at the clinic: genetic counseling from a specialist nurse, no additional information, and no help in identifying at-risk relatives	SPIKES: A 6-step protocol for delivering bad news HADS: 14-item self-report scale for the detection of depression and anxiety in hospitalized patients	2003-2005 At 2 weeks and at 8 months postcounseling
Prior report			
Bowen et al, 2002[57] Fair	A) IGC: Phone call to review pedigree information followed by a single 2-hour counseling session. Subject given information on her own risk for breast cancer using Gail and Claus scores along with population data. Information given on genetic testing, current knowledge about nonhereditary risk factors, and current screening techniques. Summary letter provided B) PGC: Four 2-hour group meetings with 4 to 6 women led by a health counselor. Included: risk assessment and perception, education, stress management, problem solving and social support. Personal risk for breast cancer, interpretation, and appropriate screening provided privately to subjects. C) CG: Offered choice of counseling modality after the final followup	NSI: 3-item questionnaire to assess awareness, candidacy, and interest in genetic testing Tolerance for ambiguity assessed using a questionnaire derived from previous research 5-point response scale to beliefs about genetic testing	Years: 6 months

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Bowen et al, 2004[62] Fair	Telephone screening survey to determine eligibility followed by mailed baseline survey. Those who returned completed surveys were randomized to individual genetic counseling (IGC), group psychosocial counseling (PC), or a delayed intervention control group (CG) A) IGC: Telephone contact with genetic counselor to review pedigree information. One 2-hour session following protocol based on standard genetic practice. Letter sent to participant within 2 weeks summarizing the session B) PC: Group of 4 to 6 participants met for four 2-hour sessions with trained health counselor. Each participant received her own risk assessment sheet, personalizing the group discussion to her own risk status. Main topics: risk assessment and perception, screening, stress management and problem solving, and social support C) CG: Offered counseling following study completion. For ICG and PC, brief survey on reactions to counseling within 4 weeks of last counseling contact. Mailed second assessment 6 months after randomization, with a reminder call and offer of phone completion to those who did not return survey after 2 weeks	NSI: 4-item questionnaire to assess risk perception Survey to assess reactions to counseling	Years: 6 months
Brain et al, 2002[166] Good	A) Control group: 1) breast cancer surveillance; 2) surgical assessment of individual breast cancer risk; 3) option to enter U.K. Tamoxifen Prevention Trial; and 40 annual surgical followup with surveillance and advice B) Trial group: components 1, 3, and 4 of control group with genetic risk assessment and counseling	STAI: Measures an individual's current anxiety feelings NSI: 3-item scale to assess interest in genetic testing	Years: Immediately
Burke et al, 2000[58] Fair	Random assignment to 3 groups: individual genetic counseling (120 women), psychosocial group counseling (113 women, reported elsewhere [Bowen 1999]), control (123 women) A) Adapted genetic counseling protocol for women with intermediate risk included precounseling telephone call, baseline questionnaire, individual genetic counseling session, immediate followup questionnaire, 6 month followup questionnaire, mailed summary letter B) Control group was offered group counseling following completion of the study	NSI: Questionnaire to assess breast cancer worry, opinions on genetic testing, and risk perception	Year NR 6 months

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Cull et al, 1998[59] Good	A) Subjects sent information about study with initial clinic appointment 4 weeks before the appointment. They were asked to return baseline questionnaires and forms within 2 weeks if wanting to participate. Those who did so were randomized either to the Video Before group, and were sent a copy of the educational video about 10 days before the clinic consultation, or to the Video After group, taking the video home after the postclinic assessment. B) Clinic consultation: individual meeting with geneticist to discuss individual risk and with breast surgeon to discuss risk management. Clinicians noted session length and rated assessment of it. Postclinic assessment included completion of instruments. Followup assessment by mail 4 weeks later	NSI: 12-response category assessment of risk perception 4-point scale to assess genetic risk Multiple choice questionnaire to assess objective risk STAI: Measures an individual's current anxiety feelings GHQ: 30-item questionnaire to screen individuals for psychiatric disorders	Year NR 1 month following clinic consultation
Hopwood et al, 1998[167] Fair	A) Postal questionnaire prior to counseling B) At attendance for risk counseling, women were asked to complete GHQ together with several other self-report measures C) Questionnaires completed again at 3, 6, 9, and 12 months later D) Three months after Family History Consultation, home visit conducted with research interviews, including administration of the Psychiatric Assessment Schedule. Additional structured questions assessed attitude to risk information, reaction, and concerns about cancer	NSI: 5-item questionnaire to assess risk perception GHQ: 60-item questionnaire to screen individuals for psychiatric disorders PAS: Semistructured clinical interview designed for use with respondents who have learning disability	3, 6, 9, and 12 months following genetic counseling
Lerman et al, 1996[168] Fair	A) Study group: 1) discussion of individual factors contributing to elevated risk, 2) presentation of individualized risk data, 3) recommendations for annual mammography and clinical breast exams, 4) instruction in breast self-exam B) Control group: 1) interview assessment of current health practices, 2) age-specific recommendations for variety of cancer screening tests, 3) encouragement to quit smoking, 4) suggestions for reducing dietary fat to ≤30%, 5) recommendations for regular aerobic exercise	IES: 17-item questionnaire to measure an individual's level of distress in relation to a specific event or condition	Year NR 3 months

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Lerman et al, 1999[60] Fair	A) Education only: topics discussed included individual risk factors for breast and ovarian cancer and patterns of inheritance for breast and ovarian cancer susceptibility. Subjects given qualitative estimates of their risk of developing breast and ovarian cancer. Pedigrees were reviewed. Potential benefits, limitations, and risks of genetic testing for inherited breast and ovarian cancer susceptibility also reviewed. B) Education plus counseling: provided the same education and materials described above. Subjects guided through a set of questions that explored personal issues related to cancer and genetic testing. Subjects discussed the emotional impact of having a family history of cancer, psychosocial implications of genetic testing for inherited breast and ovarian cancer susceptibility, anticipated reactions to a positive and negative test result, and intentions to communicate test results to family members and friends	IES: 17-item questionnaire to measure an individual's level of distress in relation to a specific event or condition	Year NR 1 month
Lobb et al, 2004[169] Good	A) Self-administered questionnaires were mailed 2 weeks before and 4 weeks after their genetic consultation. Consultations were taped and retained for analysis. Questionnaires included Breast Cancer Genetics Knowledge, Expectations, Perceived Risk, IES, HADS, and Satisfaction with Genetic Counseling Scale B) Women came to the center for their genetic consultation. The consultation was recorded, analyzed, and coded to capture 10 aspects of genetic counseling. Not all counselors incorporated all aspects, and this was the basis for the study	NSI: Scale of 0 to 7 to assess genetic clinic expectations Scale of 0 to 9 to assess information sought Scale of 0 to 100 to assess risk perception IES: 15-item scale measuring intrusion and avoidance responses in relation to a specific stressor HADS: 14-item self-report scale for the detection of depression and anxiety in hospitalized patients	4 weeks
Watson et al, 1998[171] Good	All subjects were referred for genetic counseling with a clinical geneticist who provided a consultation (randomized at clinic immediately after consultation to minimize bias), including pedigree based on risk calculation and information regarding management options based on risk level. All were part of consultation A) Consultation plus audiotape group offered instructions on self-exam and clinical exam and received an audiotape of the consultation B) Consultation-only group offered instructions on self-exam and clinical exam	GHQ-12: 12-item questionnaire to screen individuals for psychiatric disorders CWS: 3-item questionnaire to measure how frequently an individual worries about getting breast cancer VAS: Any of a number of pain self-assessment tools where subjects indicate their level of pain in response to a continuous visual scale	Year NR 6 months

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Interventions	Measures	Duration of followup
Watson et al, 1999[170] Good	A) Self-administered questionnaires given at genetic clinic immediately, pre-, and post-genetic consultation, and by postal survey at 1-, 6-, and 12-month followup	NSI: Lifetime risk perception assess as a 1 in x odds ratio Relative risk assessed on a 5-point scale Breast cancer incidence assessed as 1 in x GHQ: 12-item questionnaire to screen individuals for psychiatric disorders IES: 17-item questionnaire to measure an individual's level of distress in relation to a specific event or condition STAI: Measures an individual's current anxiety feelings	Years: 12 months

Author, year Quality	Results	Conclusions	Funding source
Current report			
Armstrong et al, 2005[148] Good	<u>Logistic regression model of association between race and use of genetic counseling: OR (95% CI)</u> African American (vs. white): 0.28 (0.09 to 0.89) Increased age: 0.97 (0.93 to 0.99) Increased probability of BRCA mutation: 1.25 (1.10 to 1.42) Increased risk perception for breast cancer: 2.88 (1.98 to 4.21) Increased risk perception for ovarian cancer: 1.56 (1.02 to 2.38) Increased ovarian cancer worry: 1.56 (1.02 to 2.38) Belief that testing leads to discrimination: 0.74 (0.57 to 0.96) Increased belief that testing provides reassurance: 1.60 (1.15 to 2.23) Gynecologist discussed BRCA testing: 1.79 (1.02 to 3.13) PCP discussed BRCA testing: 1.93 (1.00 to 3.74) NS associations: marital status, education, income, health insurance, increased breast cancer worry, belief that testing provides information, belief that testing creates anxiety, and number of visits to gynecologist or PCP	African Americans are less likely to undergo genetic counseling than whites. Women who believe testing is likely to lead to discrimination were not likely to undergo genetic counseling. Older women were less likely to undergo genetic counseling than younger women. Women with an increased risk perception for either breast or ovarian cancer were likely to undergo genetic counseling.	The American Cancer Clinical Research Training Grant and the Robert Wood Johnson Generalist Physician Faculty Scholar Award

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Results	Conclusions	Funding source
Bennett et al, 2008[150] NA	**Baseline vs. followup after risk assessment** Mean scores (SE) HADS-D: 4.44 (3.77) vs. 4.05 (3.85); NS HADS-A: 8.02 (4.56) vs. 7.03 (4.41); NS IES-I: 13.17 (10.57) vs. 7.76 (8.95); p<0.001 IES-A: 12.19 (10.78) vs. 8.45 (9.61); p<0.01 Perceived health, quality of life (scale, 0 to 100): 76.74 (20.10) vs. 77.96 (17.68); p<0.05 DUKE-SSQ (scale not described): 27.15 (11.93) vs. 24.97 (11.02); p<0.01 **Correlations between key independent variables and HADS-A vs. HADS-D** Age, level or risk assigned, and MCMQ-confrontation were NS IES-I: 0.703 (p<0.01) vs. 0.448 (p<0.01) IES-A: 0.636 (p<0.01) vs. 0.365 (p<0.01) DUKE-SSQ-confidant: 0.364 (p<0.01) vs. 0.493 (p<0.01) DUKE-SSQ-affective: 0.375 (p<0.001) vs. 0.411 (p<0.01 Perceived health: -0.493 (p<0.01) vs. -0.664 (p<0.01) Hopeless about getting cancer: 0.389 (p<0.01) vs. 0.366 (p<0.01) Hopeless about health: 0.374 (p<0.01) vs. 0.197 (p<0.05) Control over getting cancer: -0.372 (p<0.01) vs. 0.175 (NS) MCMQ-avoidance: 0.429 (p<0.001) vs. 0.271 (p<0.01) MCMQ-acceptance-resignation: 0.383 (p<0.01) vs. 0.206 (p<0.05) Neuroticism: 0.265 (p<0.01) vs. 0.193 (p<0.05)	Following risk status disclosure, women did not have changes in their level of anxiety or depression, as measured by the HADS; their intrusive thoughts and avoidance of intrusive thoughts declined after notification, while their perceived quality life of health and satisfaction increased. This indicates the level or risk disclosed does not negatively impact women's psychological well-being.	NR
Bennett et al, 2009[149] NA	**Baseline vs. followup after risk assessment** IES-I (estimated from graph) High risk: 12.5 vs. 7.8 (p<0.001) Moderate risk: 12.5 vs. 7.9 (p<0.001) Low risk: 11.8 vs. 8.2 (p<0.001) Between-group differences were NS (p=0.694) IES-A (estimated from graph) High risk: 13.1 vs. 8.3 (p<0.05) Moderate risk: 10.6 vs. 8.9 (p<0.05) Low risk: 10 vs. 11.3 (p<0.05) Between-group differences for low vs. moderate and high risk was significant (p<0.05) **Key variables associated with IES intrusion scores** Cognitive response Control over risk for cancer: -0.279 (p<0.001) Hopelessness about developing cancer: 0.412 (p<0.001) Emotional response to risk information Hopeful: -0.331 (p<0.001) Relieved: -0.278 (p<0.001) Calm: -0.506 (p<0.001)	Levels of worry fell among all women following risk assessment, regardless of risk status assignment. Only women with low (population) risk had high frequencies of avoidance after risk assessment. Intrusive worries were associated with a lack of confidant support and a confrontive coping response.	NR

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Author, year Quality	Results	Conclusions	Funding source
	Anxious: 0.438 (p<0.001) Social support Confidant support: 0.232 (p<0.01) Affective support: 0.208 (p<0.05) Coping Confrontation: 0.284 (p<0.001) Avoidance: 0.442 (p<0.001) Acceptance-resignation: 0.391 (p<0.001) Variables not associated with IES intrusion scores: age, risk status, and surprised emotional response to risk information Similar results were found for IES avoidance scores		
Bloom et al, 2006[151] Poor	Women overestimated their risk of breast cancer by an average of 25 percentage points; proportion of women underestimating risk was larger in women with perceived lower risk (40%) than those who perceived it as the same (16%), higher (10%), or much higher (5%) than the risk of other women (p=0.009) Women reduced their overestimation more if the initial overestimate was higher (p<0.0001); intervention effect was significant only in women age ≥50 years (p=0.004)	Telephone counseling appears to reduce risk overestimates in women with higher than average risk and to promote healthy behaviors in sisters of women with breast cancer.	Grant 4EB-5800 from the California Breast Cancer Research Program
Bowen et al, 2006[152] Fair	**A vs. B vs. C (results at followup)** Perceived risk (scale, 0% to 100%): 18 (SD, 16) vs. 18 (SD, 16) vs. 32 (SD, 23); p<0.001 for both counseling groups vs. control Cancer worry (scale, 4 to 16): 5.2 (SD, 1.5) vs. 4.9 (SD, 1.1) vs. 6.1 (SD, 1.9); p<0.001 for both counseling groups vs. control Awareness of genetic testing (range from 1=almost nothing to 4=a lot): 2.6 (SD, 0.7) vs. 2.6 (SD, 0.7) vs. 2.2 (SD, 0.7); p<0.001 for both counseling groups vs. control Interest in having genetic testing (range from 1=definitely not to 4=definitely yes): 2.4 (SD, 0.9) vs. 2.4 (SD, 0.9) vs. 2.8 (SD, 0.8); p<0.01 for both counseling groups vs. control Candidacy judgment (range from 1=definitely not to 4=definitely yes): 2.0 (SD, 0.8) vs. 2.0 (SD, 0.8) vs. 2.6 (SD, 0.8); p<0.05 for both counseling groups vs. control Fear of stigma (scale range unclear, higher score indicates higher fear of stigma): 3.4 (SD, 1.1) vs. 3.4 (SD, 1.1) vs. 3.3 (SD, 1.2); no significant difference between groups Access to genetic testing (scale range unclear, higher score indicates more unrestricted access): 3.8 (SD, 1.4) vs. 3.9 (SD, 1.4) vs. 4.3 (SD, 1.4); p<0.05 for both counseling groups vs. control Information flow (scale range unclear, higher score indicates more restrictions on information flow): 2.0 (SD, 1.1) vs. 2.1 (SD, 1.0) vs. 1.9 (SD, 0.9); p<0.05 for both counseling groups vs. control	Counseling, either group or individual, reduced cancer worry, lowered inflated risk perceptions, and decreased interest in genetic testing. Included in Smerecnik 2009 review.	National Human Genome Research Institute grant HG01190

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Author, year Quality	Results	Conclusions	Funding source
Brain et al, 2011[153] NA	**A vs. B** Mean perceived risk after risk assessment: 3.83 (SD, 0.51) vs. 3.97 (SD, 0.38); p=0.01 All other outcomes were NS between groups	Women's cancer worry decreased over time regardless of intervention group, though there was a significant effect immediately after risk assessment, this effect was gone by 9 months followup.	Wales Office for Research and Development in Health and Social Care
Braithwaite et al, 2005[154] Fair	**A vs. B** Mean baseline cancer worry (scale, 1 to 4): 1.92 vs. 1.81 Mean baseline STAI-state anxiety (scale, 20 to 80): 35.73 vs. 40.00 (p<0.01) Perceptions of risk information Participants were positive about risk information from both interventions on credibility, trustworthiness, accuracy, clarity, and helpfulness. Nurse counseling scored significantly higher than GRACE for all; significant differences in participants' satisfaction with risk information. Clinical nurse specialist arm was "very satisfied" with risk information (p<0.01)	No significant differences between GRACE and nurse counseling in risk perception or cancer worry. Nurse counseling was superior to GRACE on patient attitudes and satisfaction indicators.	Cancer Research U.K. (CUK), grant no. C1345/A169
Fry et al, 2003[155] Fair	**A (standard) vs. B (novel)** Cancer worry Baseline: 11.5 (3.2) vs. 11.3 (3.0) 4 weeks: 10.3 (2.4) vs. 10.2 (2.7) 6 months: 9.9 (2.5) vs. 9.7 (2.7) GHQ-30 total score: median (IQR) Baseline: 2 (9) vs. 2 (7.3) 4 weeks: 1 (8) vs. 2 (8.5) 6 months: 0 (4) vs. 0 (5) GHQ-30 case-level distress: n (%) Baseline: 66 (36) vs. 58 (31) 4 weeks: 32 (21) vs. 27 (22) 6 months: 29 (21) vs. 28 (23)	All women experienced a significant reduction in CWS scores, with greatest reductions from baseline to 4 weeks (p<0.000) and a smaller, but still significant, reduction from 4 weeks to 6 months (p=0.003). Women experienced a significant drop in case-level distress from baseline to 4 weeks (p=0.004), but there were no other significant differences in numbers of women with case-level distress between trial arms or time points.	Chief Scientists's Office and Cancer Research U.K.
Gurmankin et al, 2005[156] NA	Mean breast cancer risk perception: 44% Risk perception change from baseline: +17% (p<0.001) Accuracy of recall Risk information patients recalled was higher than risk communicated to them (+6% p=0.02 vs. 8% p=0.001) Patients' belief in recall was positive for breast cancer, showing postcounseling risk perceptions higher than risk information they recalled being told (+9% p=0.001)	Patients' breast cancer risk perceptions following risk communication were higher than corresponding actual risk communicated to them (+19% p<0.001). Inaccurate risk perception (high or low) can lead patients to make different medical decisions than they would with accurate risk perception. They could engage in interventions or experience unnecessary stress if perceived risks are inaccurately high.	The American Cancer Society and a Robert Wood Johnson Faculty Scholar Award

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Author, year Quality	Results	Conclusions	Funding source
Helmes et al, 2006[157] Fair	**A vs. B vs. C (change from baseline to followup)** Mean risk perception (scale, 0 to 100): -10.29 vs. -8.65 vs. +1.14 (p<0.001) Mean cancer worry (scale, 4 to 16): -0.9 vs. -0.82 vs. -0.38 (p=0.002) Breast health intentions (score, 1 to 4): 0 vs. +0.01 vs. +0.02 (NS) Interest in genetic testing (score, 1 to 4): -0.61 vs. -0.52 vs. +0.51 (p<0.001)	There were no differences between in-person and telephone counseling; however, both intervention groups decreased risk perception, cancer worry, and interest in genetic testing compared to the group that did not receive counseling. Counseling and no counseling had no affect on breast health intentions.	National Human Genome Research Institute grant HG01190
Hopwood et al, 2004[158] NA	**Precounseling vs. 1-month followup vs. 12-months followup** Underestimated risk: 49/162 (30%) vs. 37/162 (23%) vs. 36/162 (22%) Mean GHQ (scale, 0 to 28): 3.4 vs. 3.0 vs. 3.4 (NS) Mean CWS (scale, 1 to 16): 11.6 vs. 10.9 vs. 10.8 (p<0.001)	Cancer distress decreased after counseling and continued to be low 1 year later.	NHS Research and Development Directorate, Programme for Cancer; Project NCP/B42
Kelly et al, 2008[15] NA	**Precounseling vs. postcounseling (ovarian cancer)** Accuracy of risk perception (estimated from graph): 1 vs. -5 Mean risk assessment (0% to 100%): 30.81 (SD, 3.84) vs. 25.45 (SD, 3.45) **Postcounseling vs. postresult vs. 6-month followup** Mean risk assessment (0% to 100%) Those with positive result (n=7): 27.86 (SD, 8.01) vs. 31.43 (SD, 7.46) vs. 22.14 (SD, 7.23) Those with informative negative result (n=5): 27.00 (SD, 6.63) vs. 11.00 (SD, 2.45) vs. 15.00 (SD, 5.00) Those with uninformative negative result (n=28): 24.50 (SD, 4.48) vs. 19.76 (SD, 4.29) vs. 17.82 (SD, 3.20)	All women underestimated their risk of developing ovarian cancer.	The New Jersey Commission on Cancer Research and the Mid-Atlantic Region Human Genetics Network
Matloff et al, 2006[160] Fair	**A vs. B** Mean discrepancy between perceived risk for self and average woman Baseline: 16.3 (SD, 17.9) vs. 22.3 (SD, 24.3) 1 month: 0.8 (SD, 22.3) vs. 21.1 (SD, 25.4) 6 months: 3.6 (SD, 20.1) vs. 18.3 (SD, 23.0) **A only** Mean discrepancy between perceived risk for self and actual risk Baseline: 36.9 (SD, 20.4) 1 month: 18.9 (SD, 28.6) 6 months: 17.1 (SD, 25.9)	After counseling, accuracy of perceived risk of breast cancer increased.	Susan G. Komen Foundation
Mikkelsen et al, 2007[161] Fair	**A vs. B vs. C** Perceived absolute lifetime risk of breast cancer (%) Mean within-group changes from baseline to 1-year followup: -6.6 (95% CI, -3.0 to -10.2) vs. 1.6 (95% CI, 3.6 to -0.5) vs. 1.1 (95% CI, 2.2 to 0.0) Mean between-group changes: -8.2 (95% CI, -12.2 to -4.1) counseling vs. group 1; -7.7 (95% CI, -11.4 to -4.0) counseling vs. group 2 Change in risk accuracy of perceived lifetime risk of breast cancer (%) Overestimate: -12 vs. 5 vs. 2 Accurate at 1-year followup: 16 vs. -5 vs. -2 (p=0.03 for A vs. B and	Genetic counseling helped to increase risk accuracy even 1 year after counseling.	Danish Cancer Society, Grant Number PP 02 010, the Center of Innovation and Development in Nursing Education in the County of Aarhus and Aarhus University Research Foundation

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Results	Conclusions	Funding source
	p=0.07 for A vs. C)		
Mikkelsen et al, 2009[162] Fair	**A vs. B vs. C** HADS-A score decreased from baseline to 1 year: 4.7% (95% CI, -3.5 to 12.8) vs. 2.5% (95% CI, -4.5 to 9.5) vs. 1.1% (95% CI, -2.3 to 4.7); decrease in anxiety in group 1 was in women in nonsystematic screening (7.0% [95% CI, -4.1 to 18.1]), with a slight increase in women in systematic screening (1.1% [95% CI, -7.5 to 9.8]) Baseline vs. 2-weeks followup vs. 6-months followup vs. 12-months followup Cancer-specific distress: 52% vs. 50% vs. 41% vs. 41% Comparing women referred for mammography vs. no genetic counseling (41% to 35%) or to a random sample from the general population (from 32% to 30%) with no counseling. More women with genetic counseling experienced decrease in cancer-specific distress; difference statistically significant when compared to general population (p=0.006) and subgroup of women with mammography screening (p=0.05).	An 11% (95% CI, 1.4 to 20.8) decrease in cancer-specific distress in genetic counseling group from baseline to 1-year followup exceeded decrease in groups 1 and 2, with significance in group 2 (p=0.006) and subgroup of group 1 in systematic screening (p=0.05).	Danish Cancer Society, Grant Number PP 02 010, the Center of Innovation and Development in Nursing Education in the County of Aarhus and Aarhus University Research Foundation, and the Danish Nurses' Organization
Pieterse et al, 2011[163] NA	Risk perception accuracy: N (%) Precounseling vs. immediately postcounseling vs. 6-months postcounseling Underestimation: 1 (3) vs. 5 (16) vs. 8 (24) Correct estimation: 0 (-) v. 10 (32) vs. 6 (18) Overestimation: 29 (97) vs. 16 (52) vs. 19 (57) Total number of counselees: 3 (unaffected group)	Counseling educates women on lifetime breast cancer risk; correct knowledge on breast cancer genetics decreased over time. Benefits gained immediately after counseling seem to remain over time.	Dutch Cancer Society supported original study (Grant number NIVEL 1999-2090); author supported by a post-doctoral fellowship from the Dutch Cancer Society.
Roshanai et al, 2009[164] Fair	The only significant difference between intervention and control was immediately after counseling and at 2 weeks, when controls showed more accurate estimation of risk; groups showed the same results at 8-months followup. No significant difference for anxiety or depression between control and intervention at any time point; both groups significantly decreased over time (p<0.01).	At 8-months followup, 74% of counselees in control and intervention groups had informed relatives; 96% of relatives of intervention counselees and 89% of relatives of controls reported being informed. The majority (75% of intervention relatives and 67% of controls) reported receiving sufficient information.	The Swedish Cancer Society
Prior report			
Bowen et al, 2002[57] Fair	Counseling on risk slightly changed levels of interest in genetic testing in women with a family history. Those who participated in counseling were less interested in genetic testing and less likely to view themselves as good candidates. Stigma and access beliefs about genetic testing were related to the effect of counseling on whether women thought they should participate in testing. As women gained more information, they were slightly less likely to want to participate in testing.	Individual counseling was more predictive of women's increased awareness than psychosocial group counseling.	The National Cancer Institute and the National Human Genome Institute (HG01190)

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Author, year Quality	Results	Conclusions	Funding source
Bowen et al, 2004[62] Fair	Women's perceived risk for breast cancer decreased by 50% for the 2 counseling groups relative to control ($p<0.01$). Cancer worry decreased in both counseling groups by 1 scale point ($p<0.05$). There were no differential effects of counseling type on perceived risk or cancer worry. Women in psychosocial counseling experienced more anxiety change than those in the other groups. Depression was not impacted by study group.	Some women reported high levels of attendance and satisfaction with counselors and counseling; women in the genetic counseling arm reported more frequently talking about concerns than did women in psychosocial groups. Perceived risk and worry can be reduced with both types of short-term interventions.	The National Human Genome Institute, the National Cancer Institute, and the National Office for Research on Women's Health (HG/CA01190)
Brain et al, 2002[166] Good	State anxiety: Significant main effect of time, with decreased anxiety from baseline to followup ($p=0.03$). Breast cancer worry: Significant overall reduction from baseline to followup. Significant interaction between risk information and time. Decline in women at low risk ($t(106)$, 5.92; $p<0.001$) and moderate risk ($t(443)$, 12.13; $p<0.001$), but not at high risk. Satisfaction: Significantly lower in high-risk group ($p<0.001$). Perception of risk: Marginally significant trend to increased perceived risk in high-risk women in the trial group. Interest in genetic testing: Effect of risk information not significant.	Specialists other than geneticists might provide assessment of breast cancer risk, reassuring those at reduced risk and targeting high-risk women for specialist genetic counseling and testing services. Low-risk women: Anxiety and cancer concerns were reduced with personal risk information. High levels of satisfaction, whether or not information based on detailed genetic analysis. High-risk women: Risk information, even unfavorable, does not appear to create significant anxiety. Concerns about breast cancer risk remained and they were less satisfied with consultation in either group. Implication: Breast cancer worry may impact quality of life for women who recognize they are at high risk.	The Medical Research Council, National Assembly for Wales, NHS R&D (Wales), and Imperial Cancer Research Fund. Dr. Gray is supported by Tenovus, a cancer charity
Burke et al, 2000[58] Fair	Significant differences between counseling and control groups in mean perceived risk of breast cancer ($F=27.9$; $p<0.009$). Significant differences over time in perceived risk for the counseling group ($F=65.9$; $p<0.001$). Interaction between group and time for perceived risk was significant ($F=50.6$; $p<0.001$). Low overestimators of breast cancer risk reduced risk estimates by an average of 19 percentage points after counseling, compared with high overestimators who reduced risk estimates by an average of 36 percentage points ($F=13.41$; $p<0.00001$). After counseling, those who perceived themselves as candidates for testing decreased from 82% to 60%; interest in testing was reduced from 91% to 60%. 82 (70%) liked the counseling very much. 65 (56%) found the counseling very useful and 26 (22%) found it moderately useful. After receiving risk estimates, 39 (33%) were a lot less worried and 37 (32%) were a little less worried.	Most participants saw a benefit to counseling and afterward had a more accurate understanding of their risk. Counseling reduced interested in genetic testing.	The National Institutes of Health (HGO1190)

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Author, year Quality	Results	Conclusions	Funding source
Cull et al, 1998[59] Good	Duration of Consultation: VB group spent less time with surgeon (mean, 11.8 min vs. 14.6; p< 0.05), but their time with geneticist was not significantly shorter. Risk Assessment: No significant difference between VB or VA in accuracy of estimate at baseline. VB retained accuracy from clinic to followup. VA were more likely to underestimate at followup (p<0.05). Understanding of Risk Information: Subjective: at baseline and at followup, no significant difference. Objective: VB had higher scores (p<0.01) and a higher proportion of correct responses to more items. Followup: no significant differences after adjusting for education level (t=0.34). Emotional Distress: No significant difference in groups in anxiety or distress levels. Use of Video and Family Discussion: VB: 94% watched video at least 1 time from start to finish. 76% reported it offered new information. VA: 41/42 who gave followup data watched the video at least once and 41% of them said it gave new information. In both VA and VB, most (66% and 65%, respectively) watched it alone and most discussed it with a partner.	Women who saw the video before their clinic visit were not deterred from attending. Compliance with the study and satisfaction with the clinic visit were higher among those who viewed the video beforehand.	The NHS R&D (Cancer) Programme and the Imperial Cancer Research Fund
Hopwood et al, 1998[167] Fair	GHQ scores: Compliance at baseline was 85% (n=34) and 94% at 3 months (n=148). Prevalence of psychological distress, with a cutoff score of >5, was 31% at baseline and 26% at 3 months. An examination of the 4 subscales of GHQ showed that 9.7% scored a ≥5 on the somatic scale, 14% on the anxiety subscale, and 3% each on the depression and suicidal ideation subscales at baseline. At 3 months, proportions were 12%, 15%, 6.8%, and 3.4%, respectively. When analysis was restricted to 105 women with evaluable assessments on all occasions, prevalence was 31% and 25%, respectively. Baseline scores compared with precounseling risk estimates showed no significant difference (p=0.087). Significant differences between psychological distress and perceived risk postcounseling (p=0.0053). Women with accurate risk knowledge postcounseling had significantly lower scores than those who underestimated (p=0.0034) or who overestimated (p=0.0447). Psychiatric Assessment Schedule: Psychiatric disorder was confirmed in 21 (13.3%) of the study participants at 3 months. Most women had multiple concerns, but none reported risk counseling as a precipitant for their distress. Estimation of risk: Prior to risk counseling, 10% accurately estimated risk of breast cancer, while 50% accurately estimated after (p=0.0000). More women continued to overestimate (17%) than underestimate (11%). In general, giving women an accurate estimate of their probability of breast cancer when they perceived it to be much lower did not appear to trigger	Prevalence rate for psychological distress when measured by a self-report questionnaire was double that ascertained by psychiatric interview, which is regarded as the gold standard. Interview data suggests that psychiatric morbidity was not apparently caused by the genetic counseling. This suggests that routine genetic risk consultations do not facilitate disclosure of distress or unresolved grief, and the use of a screening instrument together with a second-stage assessment interview should be explored further.	The Cancer Research Campaign

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Results	Conclusions	Funding source
	clinical anxiety or depression.		
Lerman et al, 1996[168] Fair	Breast cancer preoccupation: IES average score on measure of breast cancer preoccupation was 6.9 + 0.71 (mean + SE). No significant baseline difference in risk comprehension between groups; however, significant change in risk comprehension at 3-months followup due to movement in risk counseling group from overestimation to accurate or underestimation.	Among women with less formal education, counseling led to significant reductions in distress by the 3-months followup, suggesting a possible increased adherence to mammography.	Public Health Service grants ROICA57767 and K07CAOI604 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services
Lerman et al, 1999[60] Fair	Genetic testing intention: Family history and baseline genetic testing intentions both made significant independent contributions to 1-month genetic testing intentions. Women with stronger family history of cancer had greater increases in intentions. Only in African Americans, education plus counseling led to greater increases in intentions than education only (p=0.003). IES scores: All groups evidenced a reduction in distress from baseline to 1 month. However, this decrease, although not a significant difference, was smallest among African American women who received education plus counseling.	Overall: African American women were found to differ significantly from Caucasian women in the effects of the interventions on testing intentions and provision of a blood sample. Effects were independent of socioeconomic status and referral mechanism.	The National Institutes of Mental Health and National Human Genome Research Institute grant MH/HG54435
Lobb et al, 2004[169] Good	Anxiety: Women who had more aspects of genetic testing discussed had a decrease in anxiety after 4 weeks (p=0.03). Women receiving a letter summarizing their consultation had lower anxiety (p=0.012) and a trend toward less anxiety about breast cancer (p=0.089). Women who received ≥4 supportive communications were more anxious about breast cancer (p=0.000). Depression: Women whose consultants facilitated understanding more had a decrease in depression (p=0.052). Risk Accuracy: Women receiving a letter summarizing their consultation had increased risk accuracy (p=0.023).	Women who understood what was being presented to them had decreased depression. This can imply that women may feel overwhelmed with the amount of information they receive and may feel worse if they are not helped to understand it. Providing a written summary of the consultation helped with accurate risk perception.	The University of Sydney Cancer Research Fund
Watson et al, 1998[171] Good	CWS scores: For both groups, median score was 11 (range, 6-22) (95% CI, 10-12 for cases and 95% CI, 10-11 for controls); mean, 11.14 (SD, 3.23) for cases and mean, 11.39 (SD, 3.37) for controls. Scores fell in subjects given a tape of consultation from a median of 11 at baseline to 10 at 1 month, then 9 at 6 months. Relative risk scores: At 1-month followup, 41% accurately recalled their risk of developing cancer, 25% overestimated, 11% underestimated, 23% didn't know/didn't remember. Results suggest that risk figure, regardless of accuracy, doesn't reflect more general view about risk compared with average women. When rRisk figure was given as odds ratio compared with other formats (percentage or descriptive terms), 71% were accurate in recall compared with 25% when given in other formats. Risk questionnaire scores: Usefulness of information rated on a visual analog scale. Average ratings were high, ranging from 8.5 (population	Overall: GHQ-12 scores: For combined groups, median score was 1 (range, 0-11). 36 subjects had a score indicative of psychological morbidity (>3) at baseline and 31 at 1-month and 6-month followup.	NR

Appendix C6. Evidence Table of Genetic Counseling

Author, year Quality	Results	Conclusions	Funding source
	risk) to 9.1 (risk of gene in family). Risk of gene in family, lifetime risk, and risk before age 50 were rated significantly more useful than population risk, risk of no cancer by age 50, and risk of disease over next 5 years. Medical management uptake: No significant correlation between cancer worry change scores and either level of breast clinical exam (p=0.8) or mammography (p=0.8); no difference between cases and controls for rate of self-exam, doctor exam, or mammography at 6-month followup; no difference between groups for other health behaviors unaffected by whether consultation tape was received or not.		
Watson et al, 1999[170] Good	GHQ: One third had notable levels of distress. There was no statistically significant change in general mental health at each followup compared with precounseling level. Cancer Anxiety and Helplessness/IES: No statistically significant changes in levels of cancer-specific distress. Followup assessment revealed that 13% (35/268) had received some psychological intervention during the 12 months since attending the clinic. Of these, 7% (n=19) had received psychotropic medication, 4% (n=10) had engaged in psychological counseling, and 2% (n=6) had received both forms of intervention. Levels of state anxiety: Anxiety levels at precounseling were at similar levels to those reported in healthy women attending for breast cancer screening (mean, 38.7), with a significant downward shift immediately postcounseling (mean, 35.2; p<0.001). Perception of risk: Specific figures about risk, provided within genetic counseling, tend not to be remembered. Continual overestimators may be worrying unnecessarily and excessively about breast cancer risk and underestimators appear undisturbed by the information that their risk is greater than they thought. Underestimators were not significantly different from the rest of the sample in terms of their scores for intrusive and avoidant thoughts about breast cancer risk when assessed precounseling. However, at 12 months, their scores were significantly lower than the rest on each of the scales (avoidance, p=0.02; intrusion, p=0.006), indicating that in the long term they are less likely to report having intrusive thoughts about breast cancer risk. High levels of cancer-specific distress were found in pregenetic counseling, with 28% reporting that they worried about breast cancer "frequently or constantly" and 18% worry about breast cancer as a "severe or definite" problem. Following genetic counseling, levels of cancer-specific distress were unchanged. General mental health remained unchanged over time (33% psychiatric cases were detected pregenetic counseling, and 27% 12 months after genetic counseling).	High levels of cancer-related worry compare unfavorably to previously gathered data on general population risk samples. Genetic counseling does not alleviate cancer-specific distress in a substantial minority of women; this contradicts previous U.S. findings. A single counseling session may not shift worries in some women. General levels of psychological morbidity unaffected by genetic counseling. Substantial minority of women who do not benefit from counseling and continue to overestimate risk, and worry was unrelieved. Study highlights problems with genetic counseling (e.g., some women continue to overestimate risk despite being told otherwise). Anxiety is not alleviated by genetic counseling, and women who continue to overestimate their risk and worry about breast cancer are likely to go on seeking unnecessary screening.	The Cancer Research Campaign (CRC project CP1026)

Abbreviations: CASH = Cancer and Steroid Hormone Study; CI = confidence interval; CG = control group; FDR = first-degree relative; GHQ = General Health Questionnaire; FHC = family history clinic; GRACE = Genetic Risk Assessment in the Clinical Environment; HADS = Hospital Anxiety and Depression Scale; ICG = individual genetic counseling; IES = Impact of Event Scale; LCIS = lobular carcinoma in situ; NHS = National Health Service; NR = not reported; OR = odds ratio; PC = psychosocial counseling; PCP = primary care

provider; RCT = randomized, controlled trial; SD = standard deviation; SDR = second-degree relative; STAI = State-Trait Anxiety Inventory; VA = video after; VAS = Visual Analog Scale; VB = video before.

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria	Country
Prevalence high-risk					
Beristain et al, 2007[174]	NA	NR	Individuals with suspicious personal or family history.	Cases met 1 of the following criteria: 1) patients without family history of breast and/or ovarian cancer, but showing early onset breast cancer (age <40); 2) patients from families with 2 cases of female breast cancer, 1 diagnosed at age <50; 3) patients of families with ≥3 cases of female breast cancer; 4) patients from families with ≥1 case of breast cancer or ovarian cancer in association with ≥1 case of male breast cancer; 5) patients from families with ≥1 cases of ovarian cancer or breast and ovarian cancer in the same individual; 6) patients from families with ≥2 cases of ovarian cancer. Each index case was the youngest individual affected with breast and/or ovarian cancer alive in each family.	Basque Country, Spain
Konecny et al, 2011[183]	NA	High-risk clinics	Individuals referred for genetic analysis on the basis of family history.	Families were included if they met any of the following criteria: 1) the presence of ≥2 patients with diagnosed breast or ovarian cancer among the direct relatives and ≥1 case diagnosed at age <45; 2) the presence of bilateral breast or ovarian cancer among the direct relatives diagnosed at any age; 3) occurrence of duplex breast and ovarian cancer in ≥1 patient diagnosed at any age; 4) the presence of sporadic breast or ovarian cancer diagnosed at age <35 years; 5) the presence of ≥1 case of male breast cancer diagnosed at any age.	Slovakia
Nanda et al, 2005[193]	NA	Genetics clinic	Families presenting to high-risk clinic.	Families with ≥2 cases of breast cancer, ovarian cancer, or both among FDRs and SDRs. Families were excluded if any individual had previously been tested for a *BRCA1* or *BRCA2* mutation.	U.S.: University of Chicago, Mayo Clinic, Rush University, UCSF
Neuhausen et al, 2009[194]	Breast Cancer Family Registry	Population and clinic-based family registries	Probands and their families recruited through population and clinic-based registries.	Population-based families from the California Breast CFR recruited case probands <65 years at diagnosis; <70 years at diagnosis from the Ontario Breast CFR; and case probands stratified by age from the Australian Breast CFR. Clinic-based families from the Philadelphia and New York Breast CFRs recruited affected and unaffected probands with a family history of breast and/or ovarian cancer; families with ≥3 cases of breast or ovarian cancer, especially if ≥1 occurred before age 45, were recruited to the Utah Breast CFR; and affected and unaffected probands with ≥2 affected relatives were recruited to the Australian Breast CFR. Ashkenazi Jewish women with a personal or family history of breast cancer were recruited through the New York, Philadelphia, Ontario and Australian Breast CFRs.	U.S., Canada, Australia

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria	Country
Seymour et al, 2008[197]	Cancer Prevention Units in the Forli-Cesena and Ravenna provinces of north-central Italy	Genetics clinic	Women undergoing breast checkups who completed a questionnaire on family history.	Healthy or affected individuals from families meeting 1 of the following criteria: 1) ≥1 relative diagnosed with a) BC at age <36 years, b) BC and OC in the same patient at any age, c) bilateral BC at age <51 years, d) male BC at any age, e) OC of fallopian tube cancer at age <46 years; or 2) a) 2 relatives diagnosed with BC at age <51 years, b) 1 relative with BC at age <51 years and 1 relative with bilateral BC at any age, c) 1 relative with BC at age <51 years and 1 relative with OC or fallopian tube cancer at any age, d) 2 relatives diagnosed with OC of fallopian tube cancer at any age; or 3) ≥3 relatives diagnosed with BC at any age.	Italy
Tamboom et al, 2010[199]	Estonian Cancer Registry	North Estonia Medical Centre's Centre of Oncology and the Hematology and Oncology Clinic of Tartu University Hospital	Early onset, familial, and predictive cases.	Early onset cases were identified if diagnosed with breast cancer <45 years. Early onset cases with a familial history of breast or ovarian cancer were classified as familial cases. Familial cases were identified as individuals with breast or ovarian cancer, including early onset, with ≥1 relative with these cancers. Predictive testing cases included individuals with high-risk families (≥2 relatives diagnosed with breast or ovarian cancer) who did not have breast or ovarian cancer themselves.	Estonia
Tommasi et al, 2005[200]	Dipartimento Donna of the National Cancer Institute of Bari, Italy	Surgical department	Women with a first diagnosis of breast cancer undergoing surgery.	A preliminary investigation of cancer syndromes was performed by a surgeon and the patients eligible for genetic counseling were referred.	Italy
Vaziri et al, 2001[202]	Familial Cancer Registry of the Cleveland Clinic Foundation	Clinic	Breast and breast-ovarian cancer families recruited through the registry.	An affected proband with ≥2 family members with cancer; 2 of whom must have either breast cancer (<50 years) or ovarian cancer; and ≥1 with breast, ovarian, colon, prostate or pancreatic cancer. Cases must be present in ≥2 generations.	U.S.
Weitzel et al, 2005[319]	City of Hope's Cancer Screening & Prevention Program Network	High-risk clinics; Hereditary Cancer Registry	All patients presenting for genetic cancer risk assessment.	Probands of Hispanic origin who enrolled in the registry between October 1998 and October 2004 and underwent testing. Participants with Hispanic origin only on 1 parental side were eligible if that side was significant for a history of breast cancer.	Hispanic; U.S.
Unselected populations (Ashkenazi Jewish)					
Metcalfe et al, 2010[191]	NA	Article published in a national newspaper in May 2008	Ashkenazi or Sephardic Jews.	Women who self identified as (Ashkenazi or Sephardic) Jewish, who were between the ages of 25 and 80 years, and who resided in Ontario. Not selected on the basis of family or personal history of cancer.	Ontario, Canada

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Study design	Primary risk measure	Comparison group	Family history/risk level definition	N
Prevalence high-risk					
Beristain et al, 2007[174]	Post intervention series	Prevalence	NA	See inclusion/exclusion criteria	236 index cases
Konecny et al, 2011[183]	Post intervention series	Prevalence	NA	See inclusion/exclusion criteria	585 families
Nanda et al, 2005[192]	Post intervention series	Prevalence	NA	NR	155 families
Neuhausen et al, 2009[193]	Post intervention series	Prevalence	NA	See inclusion/exclusion criteria	*BRCA1:* 4531 probands *BRCA2:* 4084 probands 1385 Ashkenazi Jewish probands 1360 individuals
Seymour et al, 2008[191]	Post intervention series	Prevalence	NA	See inclusion/exclusion criteria	363 families 707 individuals
Tamboom et al, 2010[198]	Post intervention series	Prevalence	NA	See inclusion/exclusion criteria	64 early onset 47 familial 33 predictive
Tommasi et al, 2005[199]	Case series	Prevalence	NA	Patients were classified as having a family history of breast cancer if 1 of the following conditions was met: 1) ≥3 relatives (1st or 2nd degree) had breast or ovarian cancer; 2) 2 relatives <50 years had breast cancer; 3) 1 relative <36 years had breast cancer; 4) the patient had bilateral cancer and ≥1 relative with breast cancer (or a relative with bilateral cancer); 5) male breast cancer. The Myriad II program was used to compute the probability of finding a *BRCA1* mutation. Individuals were classified as having an increased risk if this probability was ≥10%, and a low risk when the probability was <10%.	100 patients
Vaziri et al, 2001[201]	Post intervention series	Prevalence	NA	See inclusion/exclusion criteria	104 families
Weitzel et al, 2005[320]	Post intervention series	Prevalence	NA	A calculated BRCA mutation probability of ≥5% by any model.	110 probands
Unselected populations (Ashkenazi Jewish)					
Metcalfe et al, 2010[190]	Post intervention series	NA	NA	NR	2080 women

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source
Prevalence high-risk					
Beristain et al, 2007[174]	NR	NR	*BRCA1* & *BRCA2*	The full coding sequences and intronic boundaries were amplified using PCR. CSGE method was used to screen. Genomic fragments with altered mobility patterns were sequenced.	Blood

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source
Konecny et al, 2011[183]	Gender: NR Mean age at diagnosis (*BRCA1* vs. *BRCA2*): 42.7 years (range: 22 to 75) vs. 46 years (range: 33 to 59) Race/ethnicity: Slovak	NR	*BRCA1* & *BRCA2*	A combination of PCR amplification, SSCP analysis, and direct sequencing was used. Allelic discrimination analysis was used to detect mutation p.Cys61Gly. The MLPA analysis was used.	Blood
Nanda et al, 2005[192]	Race/ethnicity: 50% Caucasian (nonHispanic, nonJewish) 28% African American 19% Ashkenazi Jewish 2% Hispanic 1% Asian	117/160 (73%)	*BRCA1* & *BRCA2*	80% were analyzed by Myriad using direct DNA sequencing; 20% were screened by SSCP or dHPLC, followed by sequencing of those with variant results. Individuals who self identified as Ashkenazi Jewish were initially screened for the 3 common founder mutations. Complete sequencing was performed only if the initial screening did not detect 1 of these founder mutations.	NR
Neuhausen et al, 2009[193]	Gender: 100% female Age (years) of mutation carriers at diagnosis *BRCA1* vs. *BRCA2* affected: <30: 43 vs. 21 30-39: 193 vs.107 40-49: 168 vs.100 50-59: 51 vs. 65 >60: 19 vs. 28 Unknown: 1 vs. 0 *BRCA2* affected: <30: 21 30-39: 107 40-49: 110 50-59: 65 >60: 28 Unknown: 0 Race/ethnicity 1385 Ashkenazi Jewish *BRCA1* vs. *BRCA2* probands excluding Ashkenazi Jewish: 63% vs. 61% nonHispanic white 12% vs. 13% Hispanic 9% vs. 10% African American 12% vs. 12% Asian/Pacific Islander 3% vs. 3% other/multiple race 1% vs. 1% unknown	NR	*BRCA1* & *BRCA2*	Initially, 2-D gel scanning, DHPLC, EMD and PTT. EGAN and CSGE have also been used in the California samples. More recently, majority of testing is performed by Myriad Genetic Laboratories using BRC-Analysis.	Blood and/or buccal samples and tumor tissue
Seymour et al, 2008[196]	100% female Median age at diagnosis: 46.6 years (range: 20 to 80) Race/ethnicity: Italian	NR	*BRCA1* & *BRCA2*	PCR amplification and direct sequencing. Variants were confirmed by resequencing the reverse DNA strand.	Blood

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source
Tamboom et al, 2010[198]	NR	NR	*BRCA1* & *BRCA2*	SSCP-HA followed by direct DNA sequencing and MDE. All mutations were confirmed using PCR.	Blood
Tommasi et al, 2005[199]	100% female Age: NR Race/ethnicity: Italian	NR	*BRCA1*	PCR amplification and pre-screening using dHPLC analysis, followed by DNA sequencing. If a mutation was identified, it was confirmed using a second sample from the patient.	Blood
Vaziri et al, 2001[201]	NR	NR	*BRCA1* & *BRCA2*	PCR amplification, CSGE, and PTT. Family-specific mutations were amplified and directly sequenced using tissue-derived genomic DNA.	Blood
Weitzel et al, 2005[320]	99% female Mean age at diagnosis: 37 years (for the 89 probands with a cancer diagnosis) Race/ethnicity: 100% Hispanic	98%	*BRCA1*	Full sequencing of exons and flanking intronic sequences by Myriad Genetic Laboratories. 5 specific *BRCA1* rearrangements for assays done after 2001.	NR
Unselected populations (Ashkenazi Jewish)					
Metcalfe et al, 2010[190]	100% female Mean age at enrollment: 49.3 years Race/ethnicity: 1886 (91%) reported 100% Ashkenazi Jewish ancestry 105 (5%) reported 75% Ashkenazi Jewish ancestry (3 grandparents) 56 (3%) reported 50% Ashkenazi Jewish ancestry (2 grandparents) 3 reported 25% Ashkenazi Jewish ancestry (1 grandparent) 17 reported Sephardic Jewish ancestry	NR	*BRCA1* & *BRCA2*	Tested for the 3 Jewish founder *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) mutations. All mutations were confirmed by direct sequencing.	Blood or saliva

Author, year	Parts of genes studied	Who was tested?	Results/conclusions	Quality considerations			
				Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Prevalence high-risk							
Beristain et al, 2007[174]	Exons and intronic boundaries	Proband	16/236 (6.8% of index cases) had mutations	NR	NR	NR	NA
Konecny et al, 2011[183]	Whole coding region	NR	*BRCA1*: 85/585 (15%) families *BRCA2*: 12/104 (12%) families	NR	NR	NR	NA

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Parts of genes studied	Who was tested?	Results/conclusions	Quality considerations			
				Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Nanda et al, 2005[192]	Full sequence	In each family, the individual with the highest probability of being a mutation carrier was tested.	*BRCA1*: 28% -Hispanic: 0% -Asian: 0% -African American: 16% -Caucasian: 31% -Ashkenazi Jewish: 41% *BRCA2*: 16% -Hispanic: 0% -Asian: 0% -African American: 12% -Caucasian: 15% -Ashkenazi Jewish: 28% African Americans were more likely to have sequence variants of unknown significance compared with Caucasian women (44% vs. 12%).	As previously described in Frank et al, 1998.	NR	NR	NA
Neuhausen et al, 2009[193]	Full sequence	Proband and affected family members; Ashkenazi Jewish women for the 3 founder mutations	*BRCA1* vs. *BRCA2* probands Excluding Ashkenazi Jewish: 233/4531 (5.1%) vs. 193/4084 (4.7%)	As defined by the BIC and Myriad Genetic Laboratories.	NR	Age and cancer status were reported.	NA
Seymour et al, 2008[196]	Coding regions and flanking introns	Proband and some relatives	*BRCA1* or *BRCA2*: 21/247 (8.5%) families	NR, although a distinction is made between deleterious and nondeleterious mutations.	Personal and family cancer status was reported by the proband and verified during genetic counseling sessions.	NR	NA
Tamboom et al, 2010[198]	Full sequence	Probands, families, and predictive cases	Early onset vs. familial vs. predictive *BRCA1* 4/64 (6%) vs. 6/47 (13%) vs. 1/33 (3%) *BRCA2* (16 familial cases only) Total: 2/16 (12.5%)	As defined by the BIC database or those which result in a stop codon.	Cancer status was reported by the proband and confirmed in the Estonian Cancer Registry.	Age and cancer status were reported.	NA
Tommasi et al, 2005[199]	Coding region	Proband	*BRCA1*: 7/100 (7%) patients	NR, although a distinction is made between deleterious and nondeleterious mutations.	Cancer status was reported by the proband and updated in genetic counseling.	NR	NA

Appendix C7. Evidence Table of Prevalence of *BRCA1* and *BRCA2* Mutations

Author, year	Parts of genes studied	Who was tested?	Results/conclusions	Quality considerations			
				Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Vaziri et al, 2001[201]	Coding region	Proband and affected family members	Patients vs. affected family members *BRCA1*: 18/104 (17.3%) vs. 18/25 (72%) *BRCA2*: 2/104 (1.9%) vs. 4/4 (100%)	NR	NR	NR	NA
Weitzel et al, 2005[320]	Exons and flanking intronic sequence	Proband	34 (31%) had deleterious mutations (25 in *BRCA1*, 9 in *BRCA2*)	NR	Cancer status was reported by the proband.	NR	NA
Unselected populations (Ashkenazi Jewish)							
Metcalfe et al, 2010[190]	Founder mutations	Individual	Prevalence of mutation: 22/2080 (1.1%) found to have 1 of 3 founder mutations *BRCA1*: 0.5% *BRCA2*: 0.6%	1 of 3 founder mutations.	Cancer status for the family was reported by the proband through questionnaire.	Age, cancer status, vital status, and prophylactic surgery were reported.	NA

Abbreviations: BC = breast cancer; BIC = Breast Cancer Information Core; CFR = Cancer Family Registry; CSGE = conformation sensitive gel electrophoresis; dHPLC = denaturing high performance liquid chromatography; EGAN = Exploratory Gene Association Networks; EMD = enzymatic mutation testing; FDR = first degree relative; IVS = intervening sequence; MDE = mutation detection enhancement; MLPA = mulitplex ligation dependent probe amplification; NA = not applicable; NR = not reported; OC = ovarian cancer; PCR = polymerase chain reaction; PTT = protein truncation test; SDR = second degree relative; SSCP-HA = single strand conformation polymorphism - hederoduplex analysis; UCSF = University of California, San Francisco.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria
BRCA uncertain or uninformative				
Kauff et al, 2005[182]	Memorial Sloan Kettering Cancer Center	Genetics clinic	BRCA mutation negative site-specific breast cancer kindreds with a living female proband. All probands, 1st-, and 2nd-degree relatives age >18 years at the time that BRCA test results were transmitted to the proband.	Probands were included if the kindred had ≥3 cases of breast cancer in the same lineage, 1 of the breast cancers in a kindred was diagnosed when the patient was age <50 years, no ovarian cancer was present anywhere in the lineage, and BRCA mutation screening did not detect a deleterious or unclassified missense mutation in the proband's *BRCA1* or *BRCA2* gene. If the proband reported her heritage to be exclusively Ashkenazi, testing negative for the 3 Ashkenazi founder mutations was sufficient for inclusion. The proband was defined as the youngest living individual with breast cancer in the kindred who had personally undergone BRCA mutation testing. If the family had no member who had both been diagnosed with breast cancer and had undergone genetic testing, the proband was defined as the first unaffected individual in the kindred who underwent testing.
Metcalfe et al, 2009[189]	NA	Genetics clinic	All female FDRs of the breast cancer cases age >18 years at the time the pedigree was drawn.	Inclusion: In database of families who have received testing for *BRCA1/2* at 1 of 2 Canadian centers between 1993 and 2003, ≥1 woman affected with breast cancer had been tested and was found not to carry a *BRCA1* or *BRCA2* mutation.
BRCA true negative				
Bernholtz et al, 2012[175] **[True negative group only]**	Israeli Cancer Registry	Oncogenetics unit, Sheba medical center	Jewish, female mutation carriers and their family members referred for oncogenetic counseling.	High-risk status was assigned based on: 1) FDR with breast and ovarian cancer, 2) FDR with bilateral breast cancer and ≥1 breast cancer diagnosed at age <50 years, 3) 1st- or 2nd-degree male relative who developed breast cancer, 4) FDRs or SDRs with ovarian cancer, 5) 3 FDRs or SDRs diagnosed with breast cancer at any age, or 6) 1 FDR and 1 SDR with breast cancer diagnosed at age <50 years. Excluded if nonJewish origin and/or unwillingness to participate.
Domchek et al, 2010[177]	Memorial Sloan Kettering Cancer Center and University of Pennsylvania	Genetics clinic	Women who do not carry a known family mutation in *BRCA1* or *BRCA2*.	Women who had genetic testing at the University of Pennsylvania or Memorial Sloan Kettering Cancer Center who agreed to participate in research were considered for inclusion. Women were eligible if they were a close relative of an individual with a known deleterious *BRCA1* or *BRCA2* mutation, had undergone genetic testing for the known family mutation in *BRCA1* or *BRCA2*, had ≥1 followup since having genetic testing, had no prior cancer diagnosis at the time of their genetic testing (apart from in situ cervical cancer or nonmelanoma skin cancer), and had not undergone bilateral mastectomy prior to genetic testing or subsequent to genetic testing.
Gronwald et al, 2007[180]	NA	18 hospitals in Poland	Women who do not carry a known family mutation in *BRCA1* or *BRCA2.*	The probands were unselected breast cancer patients diagnosed before age 50 years who were found to carry a *BRCA1* or *BRCA2* mutation. Living sisters of probands were included in this study if they received genetic testing for the family mutation.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria
Harvey et al, 2011[181]	Australian Cancer Incidence and Mortality data	1 of 16 family cancer clinics in Australia and New Zealand; Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab)	Women who were blood relatives of mutation carriers who tested negative for the known mutation in their family.	Women were eligible if they were 1) blood relatives (not married to) of mutation carriers with a known pathogenic, large deletion, or splice site mutation in *BRCA1* or *BRCA2*; 2) had tested negative for the known mutation in their family; 3) had no personal history of cancer at enrollment (other than in situ cervical carcinoma or nonmelanoma skin cancer); and 4) had not had risk-reducing surgery before enrollment in kConFab.
Korde et al, 2011[184]	NCI cohort	NR	Mutation negative women in families with known deleterious *BRCA1/2* mutations.	All bloodline individuals within 3 degrees of relatedness to a known mutation carrier. Excluded because of missing date of birth or because researchers had not had contact with the individual or a family member within ≥3 degrees of relatedness.
Kramer et al, 2005[185] **[Mutation Negative Group Only]**	NCI	Families participating in research studies	Self or physician-referred families.	Analysis was restricted to 23 families with a known *BRCA1* mutation out of a larger cohort of 60 HBOC families.
Kurian et al, 2011[186]	BCFR	Population-based cancer registries	Women with incident breast cancer and their female 1st-degree family members, including mothers and full sisters.	Inclusion: Northern California site: Diagnosed with breast cancer at age <65 years through the Greater Bay Area Cancer Registry. Ontario site: Diagnosed at age <70 years through the Ontario Cancer Registry. These 2 sites recruited all patients diagnosed between ages 18 and 34 years or having a family history of cancer suggestive of increased genetic susceptibility, and a random sample of patients without these features. Australian site: All women diagnosed from age 18 to 39 years and random samples of women diagnosed from age 40 to 59 years through the Victorian and New South Wales Cancer Registries. Most probands were enrolled between 1996 and 2000; from 2001 and 2009, contributing sites recruited families with specific criteria of interest, including oversampling of racial and ethnic minorities.
Rowan et al, 2007[196]	NA	Familial breast cancer center	Women who do not carry a known family mutation in *BRCA1* or *BRCA2*.	Inclusion: Resident in Ontario, Canada ages 30 to 70 years. A FDR or SDR with a documented *BRCA1* or *BRCA2* mutation, the participant being negative for this mutation, and no history of breast, ovarian, or other cancer at the date of disclosure of the participant's genetic test result.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria
Smith et al, 2007[198]	M6-ICE Study	Genetics clinic	Women who do not carry a known family mutation in *BRCA1* or *BRCA2*	Families were identified from those being tested for *BRCA1/2* mutations in specialist genetic clinics, and detailed 3-generation family history was elicited. Families were only included if a *BRCA1/2* mutation was identified. Patients were only included if they have breast or ovarian cancer and tested negative for the family mutation.
van der Kolk et al, 2010[201] **[Testing Negative Group Only]**	University Medical Center Groningen	Genetics clinic	Women who do not carry a known family mutation in *BRCA1* or *BRCA2*	Screening is carried out if the family history meets 1 of the following: 1) 1 breast cancer case at age <35 years, 2) 2 breast cancer cases in 1st-degree relatives with 1 case at age <50 years, 3) ≥3 FDRs with breast cancer in 2 successive generations, 4) the occurrence of breast and ovarian cancer in FDRs, and 5) the occurrence of male breast cancer.
BRCA positive-single				
Chen et al, 2006[122]	Cancer Genetics Network	282 Ashkenazi Jewish families were population-based, the remainder were from genetics clinics	Families presenting to high-risk clinic.	Families were recruited from 8 centers including: Georgetown University, University of Pennsylvania, Duke University, Johns Hopkins University, Baylor College of Medicine, MD Anderson Cancer Center, University of Texas Southwestern Medical School, and Huntsman Cancer Institute. Criteria for inclusion varied across centers, but most families had a positive family history of breast or ovarian cancer. On average, there were >3 diagnoses of breast or ovarian cancer per family. There were 282 Ashkenazi Jewish families recruited at Baylor that were population-based.
Finkelman et al, 2012[179] **[Prospective participants only]**	Prevention and Observation of Surgical End Points (PROSE) Consortium	22 international centers in the PROSE consortium	Jewish and nonJewish women with a confirmed disease-associated *BRCA1/2* mutation.	Participants were excluded if they did not have a confirmed disease-associated *BRCA1/2* mutation or if they had a mutation in both *BRCA1* and *BRCA2.* For BC analysis, participants were excluded if they had BC or were censored before ascertainment, or if they were missing necessary data to determine followup. For OC analyses, participants were excluded if they had OC or were censored before ascertainment, or if they were missing necessary data to determine followup.
Lubinski et al, 2012[187]	26 centers in Canda, United States, and Poland	Clinical centers	Unaffected women with a *BRCA1* mutation.	A woman was eligible if she was a carrier of a deleterious mutation in *BRCA1*, was between age 25 and 65 years at baseline, and if she did not have a prior mastectomy or known diagnosis of breast or ovarian cancer.
Marroni et al, 2004[188]	NA	Clinical centers	Families receiving BRCA testing.	Eligibility criteria for genetic testing varied across centers and within centers over time; families with multiple cases of breast or ovarian cancer or early-onset cancer cases were preferentially selected.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria
Metcalfe et al, 2010[190]	Hereditary Breast Cancer Clinical Study Group	33 centers in 6 countries	Women who were known to be carriers of a deleterious mutation in *BRCA1* or *BRCA2*.	A woman was eligible if molecular analysis established that she was a carrier of a deleterious mutation in *BRCA1* or *BRCA2*. For estimation of breast cancer risk: Ages 25 to 65 years at the time of completion of the baseline questionnaire, did not have breast cancer or a prophylactic mastectomy at or before baseline, and had been followed for ≥2 years after baseline. Followed until development of breast cancer, prophylactic mastectomy, or death, whichever occurred first. For ovarian cancer risk estimation: Ages 25 to 65 years at baseline, no ovarian cancer diagnosis or prophylactic oophorectomy at baseline, ≥2 years of followup. Followed until the development of ovarian or fallopian tube cancer, prophylactic oophorectomy, death, or date of last followup, whichever occurred first.
Risch et al, 2006[1895]	Ontario Cancer Registry	Registry for ovarian cancer	All patients diagnosed with invasive and borderline ovarian cancer.	All patients diagnosed from January 1, 1995 to December 31, 1999 with invasive ovarian cancer and from January 1, 1995 to December 31, 1997 with borderline ovarian tumors. Ages 20 to 79 years and resident in Ontario at the time of diagnosis of a new primary tumor.
BRCA positive-multi				
Al-Mulla et al, 2009[172]	NA	NR	Patients and their family members in moderate- or high-risk families.	Moderate- or high-risk families.
Antoniou et al, 2006[173]	INHERIT BRCAs	Network of referring physicians	Families with family history suggestive of a genetic component.	Family meets ≥1 of the following criteria: 1) ≥4 individuals with breast and/or ovarian cancer diagnosed at any age in FDRs or SDRs, 2) 3 FDRs affected with breast and/or ovarian cancer at any age, or 3) deleterious mutation already identified in the *BRCA1/2* genes. 8 additional families that did not meet those criteria were recruited when the analysis of pedigrees was suggestive of a genetic component (e.g., monozygotic twins affected with breast cancer at an early age; 4 related individuals with early-onset breast cancer; 1 case of male breast cancer plus a women affected with early breast cancer). Age >18 years and mentally competent.
Evans et al, 2008[178]	NA	Genetics clinic	Families presenting to high-risk clinic.	Families were identified from those being tested for *BRCA1/2* mutations in specialist genetic clinics, and detailed 3-generation family history was elicited. Families were only included if a *BRCA1* or *BRCA2* mutation was identified.
Kramer et al, 2005[185] **[Mutation Carrier Group Only]**	NCI	Families participating in research studies	Self- or physician-referred families.	Analysis was restricted to 23 families with a known *BRCA1* mutation out of a larger cohort of 60 HBOC families.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Data source/ parent study	Setting	Population	Inclusion/exclusion criteria
Milne et al, 2008[192]	NA	Genetics clinic	Families testing positive for deleterious mutations in *BRCA1* or *BRCA2*.	Families were selected for mutation testing if they contained ≥3 cases of breast or ovarian cancer in the same family line, ≥2 FDRs diagnosed with breast cancer before age 50 years, ≥1 case of breast cancer and 1 case of ovarian or bilateral breast cancer in the same family line, ≥1 woman with both breast and ovarian cancer, and/or ≥1 case of male breast cancer. Once a mutation was identified in the family, the family was eligible only if ≥1 other member was tested for the family mutation.
van der Kolk et al, 2010[201] **[Mutation Carriers Group Only]**	University Medical Center Groningen	Genetics clinic	Families presenting to high-risk clinic.	Screening is carried out if the family history meets 1 of the following inclusion criteria: 1) 1 breast cancer case at age <35 years, 2) 2 breast cancer cases in FDRs with 1 case at age <50 years, 3) ≥3 FDRs with breast cancer in 2 successive generations, 4) the occurrence of breast and ovarian cancer in FDRs, and 5) the occurrence of male breast cancer.

Author, year	Country	Study design	Primary risk measure	Comparison group	Family history/ risk level definition	N
BRCA uncertain or uninformative						
Kauff et al, 2005[182]	U.S.	Retrospective cohort study	SIR	Age-specific cancer incidence rates from the SEER program.	See inclusion/exclusion criteria. Family history was collected via questionnaire sent to the proband.	165 probands 583 FDRS or SDR
Metcalfe et al, 2009[189]	Ontario, British Columbia	Retrospective cohort study	Cumulative incidence SIR	Expected rates for Ontario and British Columbia were obtained from the registry data recorded in "Cancer Incidence in Five Continents (Volume VII)."	Each family contained breast cancer diagnosed before age 50 years, or 3 cases of breast cancer diagnosed at any age. Family history of cancer diagnosis was based on report from the proband or another family member.	365 families 874 breast cancers at baseline 1492 FDRs who did not have breast cancer at baseline
BRCA true negative						
Bernholtz et al, 2012[235] **[True negative group only]**	Israel	Post intervention series	SIR	Israeli Cancer Registry	See inclusion/exclusion criteria.	884 families 1318 female individuals 307 were noncarriers true negatives
Domchek et al, 2010[177]	U.S.	Cohort Families: penetrance	SIR	Expected number of cases were based on SEER 2013 incidence rates for invasive breast and ovarian cancer and for in situ breast cancer from 1992 to 2005 in women age ≥18 years (all races).	See inclusion/exclusion criteria.	249 families 405 true negatives were identified 378 had followup information

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Country	Study design	Primary risk measure	Comparison group	Family history/ risk level definition	N
Gronwald et al, 2007[180]	Poland	Cohort Families: penetrance	OR	Expected number of breast cancer cases was determined for Poland from the "Cancer Incidence in Five Continents (Volume VIII)," using age-specific estimates.	NR	188 families 261 sisters (140 received genetic testing)
Harvey et al, 2011[181]	Australia	Prospective	SIR	Australian Cancer Incidence and Mortality data	See inclusion/exclusion criteria. Women were considered at risk from enrollment until 1 of the following events: bilateral mastectomy, bilateral oophorectomy, invasive cancer diagnosis (other than nonmelanoma skin cancer), death, or last followup.	722 mutation-negative women
Korde et al, 2011[184]	U.S.	Cohort Families: penetrance	Observed to expected risk ratio	Age-, race-, and calendar time-specific expected number of breast cancer cases were derived from the SEER 2009 Cancer Registry.	Degree of relatedness to closest relative with known BRCA mutation (1st, 2nd, or 3rd-degree). Adjustment for intact ovaries vs. oophorectomy age category.	395 women 28 families
Kramer et al, 2005[185] **[Mutation Negative Group Only]**	U.S.	Post intervention series	Cumulative risk	NA	NR	23 families 673 females total 353 were *BRCA1* mutation negative for the known family mutation
Kurian et al, 2011[186]	Melbourne and Sydney, Australia, Ontario, Canada, and Northern California, U.S.	Cohort Families: penetrance	Risk ratio, HR	Baseline incidence rates were estimated by combining carrier prevalence estimates with population-based breast cancer incidence rates, specific for each proband's country of residence, and for probands from the Northern California BCFR (which oversampled racial and ethnic minorities) for race/ethnicity, by using categories of African American, Asian American, Hispanic, and nonHispanic white.	NR	Probands: Australia (n=799) Canada (n=1034) U.S. (n=1214) FDRs: approximately 9,000

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Country	Study design	Primary risk measure	Comparison group	Family history/ risk level definition	N
Rowan et al, 2007[195]	Ontario, Canada	Cohort Families: penetrance	SIR	The expected number was estimated from the age-specific breast cancer rates for the Ontario population from 1993 to 1997 ("Cancer Incidences in Five Continents")	NR	104 subjects 64 families
Smith et al, 2007[197]	Manchester and Birmingham, England	Cohort Families: penetrance	SIR	Expected numbers were calculated using incidence rates for the period 1975 to 2004 from the North Western Cancer Registry, using age-, sex-, and calendar period-specific estimates.	NR	277 families 258 individuals tested negative for the family mutation (28 with breast cancer, 4 with ovarian cancer)
van der Kolk et al, 2010[201] **[Testing Negative Group Only]**	Netherlands	Cohort Families: penetrance	SIR	Dutch cancer registries	NR	185 families 111 segregating *BRCA1* 74 segregating *BRCA2* 1188 women total 128 noncarriers for *BRCA1* 74 noncarriers for *BRCA2*
BRCA positive-single						
Chen et al, 2006[122]	U.S.	Post intervention series	Age-specific cumulative risk; RR	To estimate the hazard of breast or ovarian cancer in noncarriers, age-conditional probabilities from SEER were used.	NR	676 Ashkenazi Jewish families 1272 families of other ethnicities 1948 counselees had genetic testing performed (1 from each pedigree)
Finkelman et al, 2012[179] **[Prospective participants only]**	U.S.	Prospective	HR	NA	NR	2362 BC analyses (1874 nonJewish vs. 488 Jewish) 3787 OC analyses (3034 nonJewish vs. 753 Jewish)

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Country	Study design	Primary risk measure	Comparison group	Family history/ risk level definition	N
Lubinski et al, 2012[187]	Canada, U.S., and Poland	Prospective	Cumulative incidence, HR, age-specific cancer and incidence rates	North American cohort	NR	1477 women 614 North America 863 Poland
Marroni et al, 2004[188]	Italy	Post intervention series	Cumulative incidence	Cancer registry data	NR	568 families 80 segregating *BRCA1* 52 segregating *BRCA2* 435 not segregating a BRCA mutation
Metcalfe et al, 2010[220]	Canada, U.S., Poland, Austria, Italy, France	Post intervention series	Penetrance	NA	A) ≥1 FDR or SDR with breast or ovarian cancer, b) no FDR or SDR with these cancers.	3011 women
Risch et al, 2006[1895]	Ontario, Canada	Case series	Cumulative incidence	NA	NR	1171 women 977 with invasive ovarian cancer (75 were *BRCA1* mutation carriers and 54 were *BRCA2* mutation carriers) 194 with borderline tumors None of the patients with borderline tumors were BRCA mutation carriers
BRCA positive-multi						
Al-Mulla et al, 2009[172]	Yorkshire and Humberside, U.K.	Post intervention series	Cumulative incidence, HR	NA	High-risk: Members of families with 4 confirmed cases of breast and/or ovarian cancer, with breast cancer occurring before age 60 years or ovarian cancer at any age. Moderate risk: Families with 3 cases of cancer.	241 patients and their family members 131 families 219 subjects with available clinical and mutation data
Antoniou et al, 2006[173]	French Canadian	Post intervention series	Cumulative risk	NA	NR	191 families 25 families segregating *BRCA1* 27 families segregating *BRCA2*

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Country	Study design	Primary risk measure	Comparison group	Family history/ risk level definition	N
Evans et al, 2008[178]	Manchester and Birmingham, England	Post intervention series	Age-specific cumulative risk	NA	NR	385 families 2466 individuals 223 families segregating *BRCA1* 162 families segregating *BRCA2*
Kramer et al, 2005[185] **[Mutation Carrier Group Only]**	U.S.	Post intervention series	Cumulative risk	NA	NR	23 families 673 females
Milne et al, 2008[191]	Spain	Post intervention series	HR, cumulative risk	NA	NR	319 families 155 families segregating *BRCA1* 164 families segregating *BRCA2*
van der Kolk et al, 2010[201] **[Mutation Carriers Group Only]**	Netherlands	Post intervention series	Cumulative incidence	NA	NR	185 families 1188 women total 111 segregating *BRCA1* 74 segregating *BRCA2*

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source	Parts of genes studied
BRCA uncertain or uninformative						
Kauff et al, 2005[182]	Mean age: 51.6 years 100% female 67% Ashkenazi Jewish ancestry Followup: Mean, 40.6 months (range, 15.3 to 82.4 months)	165/207 (80%)	*BRCA1* & *BRCA2*	NR	NR	NR
Metcalfe et al, 2009[189]	Baseline: Mean age: 48.2 years (range, 17 to 99) 100% women Race/ethnicity: NR Followup: Mean age: 54.3 years (range, 24 to 101) Mean followup period: 6.1 years (range, 1 to 10 years)	NR	*BRCA1* & *BRCA2*	Methods changed over time and between centers but used a combination of PTT, DGGE, dHPLC, and direct sequencing	NR	All coding regions

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source	Parts of genes studied
BRCA true negative						
Bernholtz et al, 2012[235] **[True negative group only]**	100% female Mean age at testing: 43.0 years (SD, 13.0; range, 19.7 to 92.8) Mean age at diagnosis: 54.1 years (SD, 12.9; range, 48.1 to 60.1) Mean age at diagnosis, *BRCA1*: 55.5 (SD, 12.5) Mean age at diagnosis, *BRCA2*: 54.7 (SD, 15.35) Race/ethnicity: Ashkenazi Jewish Median followup time: 7.2 years	NR	*BRCA1 & BRCA2*	PCR and restriction enzyme digests. An assay as previously described in Shiri et al, 2000. Full sequence analysis performed by Myriad Genetics and other private labs.	NR	Mutant alleles of founder mutations and full sequence for all others
Domchek et al, 2010[177]	100% female Median age at genetic testing: 44 years (range, 18 to 91) Race/ethnicity: 91% Caucasian 5.1% African American 0.8% Hispanic/Latino 3.2% unknown	378/405 (93%)	*BRCA1* & *BRCA2*	Direct sequencing. Individuals of Ashkenazi Jewish descent were also tested for the 3 founder mutations in *BRCA1* (185delAG, 5382insC) and *BRCA2* (6174delT).	NR	Family mutation
Gronwald et al, 2007[180]	100% female Mean age: NR Race/ethnicity: Polish	188/198 (95%) families	*BRCA1*	See Lubinski et al 2006 reference.	NR	Family mutation
Harvey et al, 2011[181]	100% female Median age at enrollment: 43.0 years (range, 18 to 88) Race/ethnicity: NR Median followup time: 6.1 years (range, 0.1 to 12.4)	NR	*BRCA1 & BRCA2*	NR	Blood	NR
Korde et al, 2011[184]	100% female Mean age at cohort entry: 31.3 years Race/ethnicity: NR	395/415 (95%)	*BRCA1* & *BRCA2*	Mutation status was based on either direct testing for the family mutation or direct inference (participants were inferred to be mutation-negative if they were descendents of an individual who tested negative).	NR	Family mutation

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source	Parts of genes studied
Kramer et al, 2005[185] **[Mutation Negative Group Only]**	100% female Age: NR Race/ethnicity: NR	NR	*BRCA1*	Various methods were used to screen for mutations in the families, with results confirmed by direct sequencing. Ultimately, affected individuals from all families negative by screening methods were fully sequenced by Myriad Genetics. In addition, all families with no mutation detected by sequencing were studied (by Myriad) for the presence of large germline deletions in *BRCA1*. After a mutation was found in a family, other members were offered clinical mutation testing for the known mutation.	NR	Full sequence
Kurian et al, 2011[186]	100% female Race/ethnicity: 61% Caucasian 11% African American 11% Hispanic 14% Asian 2% other Average age at diagnosis (breast vs. ovarian) (years): *BRCA1* families: 42 vs. 54 *BRCA2* families: 44 vs. 51 Neither: 51 vs. 50	NR	*BRCA1* & *BRCA2*	U.S.: Exon grouping analysis (EGAN) or capillary exon grouping analysis (cEGAN). Ontario and USA: RNA/DNA-based protein truncation test with complementary 5' sequencing or complete gene sequencing by Myriad. Australia: Exon and flanking intron sequencing, protein truncation, 2-dimensional gel scanning, site-specific testing for founder mutations, multiplex ligand dependent probe amplification, and BRACAnalysis by Myriad (full sequencing of *BRCA1* and *BRCA2* with testing for 5 large rearrangements in *BRCA1*). For all sites, all mutations were confirmed by sequencing.	NR	U.S.: Coding regions and splice sites. Ontario and U.S.: Complete gene. Australia: Exon and flanking introns, or founder mutations, or full gene.
Rowan et al, 2007[195]	100% female Age: 30 to 70 years Race/ethnicity: NR Median followup time: 8 years (range, 1 to 10 years)	NR	*BRCA1* & *BRCA2*	Mutation status was based on direct testing.	NR	NR

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source	Parts of genes studied
Smith et al, 2007[197]	100% female Median age: 50 years (range, 23 to 87) Race/ethnicity: NR	NR	*BRCA1* & *BRCA2*	Patients with breast or ovarian cancer who tested negative for the family mutations had a 2nd blood sample taken, and ≥2 techniques (sequencing, single-strand conformational polymorphism, protein truncation test) were used to establish the negative status. In addition, the mutation was confirmed by testing ≥2 samples from the index case or from another family member. Confirmation of mutation status for women who tested negative for the family mutation but who did not have breast or ovarian cancer was not reported.	Blood	Family mutation
van der Kolk et al, 2010[201] **[Testing Negative Group Only]**	NR	NR	*BRCA1* & *BRCA2*	Denaturing gradient gel electrophoresis, the protein truncation test, direct sequencing, and multiplex ligation-dependent probe amplification.	NR	NR
BRCA positive-single						
Chen et al, 2006[122]	2.7% male Mean age: 52.8 years Race/ethnicity: 35% Ashkenazi Jewish	NR	*BRCA1* & *BRCA2*	An array of techniques were used for *BRCA1*, including SSCP (n=209), sequencing (n=499), targeted mutation screening (n=8), sequencing for mutations 185delAG and 5382insC (n=10), CSGE (n=378), SSCP plus ASO (n=18), targeted mutation screening plus sequencing (n=60), targeted mutation screening plus CSGE (n=21), or other (n=28). For *BRCA2*, the techniques were SSCP (n=178), sequencing (n=509), CSGE (n=260), ASO (n=9), ASO plus CSGE (n=18), ASO plus sequencing (n=60), or other (n=63).	NR	NR
Finkelman et al, 2012[179] **[Prospective participants only]**	NonJewish vs. Jewish 100% female Mean age at ascertainment, BC: 39.1 (range, 2.0 to 89.3) vs. 42.7 (range, 10.2 to 90.4) Mean age at ascertainment, OC: 41.5 (range, 2.0 to 89.3) vs. 45.1 (range, 10.2 to 90.4) Race/ethnicity: NR Mean followup time, BC: 5.2 (range, 0.0 to 33.3) vs. 4.7 (range, 0.0 to 33.1) Mean followup time, OC: 5.6 (range, 0.0 to 33.3) vs. 5.0 (range, 0.0 vs. 33.1)	NR	*BRCA1* & *BRCA2*	NR	NR	NR

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source	Parts of genes studied
Lubinski et al, 2012[187]	North America vs. Poland 100% female Mean age: 43.6 years (range, 25 to 74) vs. 40.1 years (range, 25 to 74) Race/ethnicity: NR Mean followup time: 4.8 (range, 0 to 14.9) vs. 4.0 (range, 0 to 10	NR	*BRCA1*	NR	NR	NR
Marroni et al, 2004[188]	100% female Age: NR Race/ethnicity: NA (Italian)	NR	*BRCA1* & *BRCA2*	3 centers used both direct automatic sequencing and PTT-SSCP, 1 center used both PTT-SSCP and FAMA, and the last center used PTT-SSCP only.	NR	NR
Metcalfe et al, 2010[220]	NR	NR	*BRCA1* & *BRCA2*	NR	NR	NR
Risch et al, 2006[1895]	100% female Mean age: NR Race/ethnicity: 44% British Isles 28% Mixed European 11% French Canadian 17% Other	1171/2338 (50%) eligible subjects	*BRCA1* & *BRCA2*	All samples were screened for 11 common mutations (3 in Ashkenazi Jewish and 6 in French Canadian). If no mutations were found, exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* were then screened with the protein truncation test. If no mutations were found, remaining coding exons and exon-intron boundaries were screened using fluorescent multiplex DGGE for *BRCA1* and dHPLC for *BRCA2*. All variants were confirmed by direct DNA sequencing.	Blood	Coding exons and exon-intron boundaries.
BRCA positive-multi						
Al-Mulla et al, 2009[172]	40 (18%) males 179 (82%) females Mean age: 47.7 years Race/ethnicity: NR	NR	*BRCA1* & *BRCA2*	Level 1: Amplification refractory mutation system PCR for *BRCA1* exons 2 and 20 and *BRCA2* exon 11, multiplex ligation-dependent probe amplification of exon 13 Level 2: Direct sequencing of exon 11 Level 3: SSCP analysis and sequencing of all *BRCA1* coding exons	Blood	Mutations at exon 2 (185delAG) and exon 20 (5382insC) of *BRCA1*, exon 11 (6147delT) of BRCA2; duplication of exon 13 (Exon13dup6kb) and exon 11; all *BRCA1* coding exons.
Antoniou et al, 2006[173]	NR	NR	*BRCA1* & *BRCA2*	Level 1: Panel of 18 truncating mutations Level 2: Full length *BRCA1/2* sequencing by Myriad using comprehensive BRCAnalysis Level 3: Multiplex ligation probe amplification to detect deleterious rearrangements	Blood	Full sequence

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Demographics	Participation rate	Genes included	Laboratory methods	Tissue source	Parts of genes studied
Evans et al, 2008[178]	100% female Age: NR Race/ethnicity: NR	NR	*BRCA1* & *BRCA2*	A whole gene test, including a test for large deletions.	NR	NR
Kramer et al, 2005[185] **[Mutation Carrier Group Only]**	100% female Age: NR Race/ethnicity: NR	NR	*BRCA1*	Various methods were used to screen for mutations in the families, with results confirmed by direct sequencing. Ultimately, affected individuals from all families negative by screening methods were fully sequenced by Myriad Genetics. In addition, all families with no mutation detected by sequencing were studied (by Myriad) for the presence of large germline deletions in *BRCA1*. After a mutation was found in a family, other members were offered clinical mutation testing for the known mutation.	NR	Full sequence
Milne et al, 2008[191]	NR	NR	*BRCA1* & *BRCA2*	A range of methods.	NR	NR
van der Kolk et al, 2010[201] **[Mutation Carriers Group Only]**	NR	NR	*BRCA1* & *BRCA2*	Denaturing gradient gel electrophoresis, the protein truncation test, direct sequencing, and multiplex ligation-dependent probe amplification.	NR	NR

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
BRCA uncertain or uninformative						
Kauff et al, 2005[182]	Proband	Observed vs. expected BC: 19 vs. 6.07; SIR, 3.13 (95% CI, 1.88 to 4.89); p<0.001 OC: 1 vs. 0.66; SIR, 1.52 (95% CI, 0.02 to 8.46); p=0.48	NR	Cancer status was reported by the proband by questionnaire.	Collected data included age and cancer status. Not reported whether prophylactic surgery or vital status were collected.	NA
Metcalfe et al, 2009[189]	≥1 woman affected with breast cancer was tested in each family	BC: SIR, 3.9 (95% CI, 3.1 to 5.0); p<0.0001 OC: SIR, 0.85 (95% CI, 0.23 to 3.12); p=0.82	NR	Cancer status was reported by the proband and other family members by telephone interview.	Collected data included age, cancer status, prophylactic surgery, and vital status.	NA

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
BRCA true negative						
Bernholtz et al, 2012[235] **[True negative group only]**	All mutation carrying families and 1318 female individuals genotyped for mutation carriers from within the 884 families.	Observed in study vs. expected in Israeli population BC: 20 vs. 23.8; SIR, 0.84 (95% CI, 0.51 to 1.30) BC <50 years: 9 vs. 6.4; SIR, 1.41 (95% CI, 0.64 to 2.67) BC >50 years: 11 vs. 17.42; SIR, 0.63 (95% CI, 0.31 to 1.13) No significant difference in age at diagnosis in true negatives between *BRCA1* and *BRCA2* (p=0.347). Mean age of diagnosis in *BRCA1* carriers was significantly younger than diagnosis among true negatives within *BRCA1* families (p=0.001) but not among families with a *BRCA2* mutation (p=0.061).	NR	NR	Information was collected on age and cancer status. It is not clear if information was available on prophylactic surgery and vital status.	NA
Domchek et al, 2010[177]	All subjects were tested for the known mutation in the family.	Observed vs. expected Invasive BC: 2 vs. 3.8; age-adjusted SIR, 0.52 (95% CI, 0.13 to 2.09) In situ BC: 2 vs. 0.9; age-adjusted SIR, 2.3 (95% CI, 0.57 to 9.19) OC: 0 vs. 0.4	NR	Cancer status was obtained by personal report or from a family member.	Information was collected on age, cancer status, vital status, and prophylactic surgery. DCIS and invasive cancer were reported separately.	NA
Gronwald et al, 2007[180]	140/261 (54%) of sisters received direct testing. Genotypes are assigned probabilistically for untested women, adjusted for cancer status and vital status.	Observed vs. expected in study vs. expected in Polish population BC: 1/72 (1.4%) vs. 2.5 vs. 1.2; OR, 21/17 (5.8%) affected sisters was a phenocopy	NR	It is not reported how cancer status was determined.	Not reported if information was collected on prophylactic surgery. Did not distinguish between DCIS and invasive breast cancer.	NA

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Harvey et al, 2011[181]	Unaffected mutation negative women coming from families with known mutations.	SIR of BC in the observed cohort compared with the most recent BC incidence rates from the Australian Cancer Incidence and Mortality data 1st-, 2nd- or 3rd-degree relatives: 1.14 (95% CI, 0.51 to 2.53) 1st- or 2nd-degree relatives: 1.29 (95% CI, 0.58 to 2.88) No family history: 0.48 (95% CI, 0.12 to 1.93)	NR	Cancer status was verified by pathology reports.	Information was collected on age, cancer status, prophylactic surgery, and vital status.	NA
Korde et al, 2011[184]	All subjects tested, or genotype was available by direct inference.	Observed vs. expected BC: 10 vs. 12; O/E, 0.82 (95% CI, 0.39 to 1.51); O/E of invasive disease only, 0.95 (95% CI, 0.45 to 1.74)	NR	Cancer status was obtained from the subject or a family member by questionnaire. All cancer diagnoses were confirmed by review of the pathology reports.	Information was collected on age, cancer status, vital status, and prophylactic surgery. Did not distinguish between DCIS and invasive breast cancer.	NA
Kramer et al, 2005[185] **[Mutation Negative Group Only]**	All women in the family who agree to testing. Women were inferred positive based on having a child who was found to carry the mutation. Women were inferred negative based on having a parent that tested negative for the family mutation. A total of 451/673 (67%) had a known or inferred genotype.	Observed BC: 5/353 mutation-negative women Cumulative risk of BC at age 50 years: 0.017 (SE, 0.012) Cumulative risk of BC at age 70 years: 0.068 (SE, 0.033)	NR	Cancer status was initially reported by family members by questionnaire. Reported cancers were confirmed through death certificates, medical records, pathology reports, and central review of pathology slides.	Information was collected on prophylactic surgery, age, cancer status, and vital status.	NA

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Kurian et al, 2011[186]	All probands tested. If a proband tested positive for a mutation, her FDRs who had provided DNA samples were tested for the same mutation. Untested FDRs were assigned probabilities of mutation carriage conditional on the known genotypes in the family.	BC risk True negative vs. FDRs from families without *BRCA1/2* mutations: RR, 0.39 (95% CI, 0.04 to 3.81) Carriers vs. noncarriers of the risk allele for an unobserved gene that represents all unobserved genetic and nongenetic factors: HR, 13.4 (95% CI, 8.7 to 22.5)	Mutations were classified as deleterious if they were protein-truncating, missense, or slice-site mutations as defined by the Breast Cancer Information Core.	It is not clear how family cancer status information was collected or verified.	It is not clear whether information was collected on prophylactic surgery or vital status. Did not distinguish between DCIS and invasive breast cancer.	NA
Rowan et al, 2007[195]	All subjects tested.	Observed vs. expected BC: 3 vs. 1.0; SIR, 2.9 (95% CI, 1.0 to 8.6) OC: 0 vs. NR	NR	Personal cancer history was collected by survey.	It is not clear whether information was collected on prophylactic surgery. Did not distinguish between DCIS and invasive breast cancer.	NA
Smith et al, 2007[197]	Multiple members of each family were tested. Untested individuals had genotypes assigned probabilistically based on age and cancer status.	SIR of BC All relatives: 5.3 (95% CI, 3.5 to 7.7) All FDRs: 5.0 (95% CI, 2.9 to 7.8) FDRs whose cases of BC and OC are explained by the identified mutation: 3.2 (95% CI, 2.0 to 4.9) All FDRs testing negative for the family mutations who were unaffected at the time of testing: 2.1 (95% CI, 0.4 to 6.2) SIR of OC: 4.6 (95% CI, 1.2 to 11.7) Phenocopies (i.e., women who test negativ e for the family *BRCA1*/*2* mutation but who develop breast or ovarian cancer) constitute up to 24% of tested women with breast cancer after the identification of the mutation in the proband.	NR	Cancer status was reported by a family member and confirmed by means of hospital or pathology records, regional cancer registries, or death certification.	Information was collected on age, cancer status, vital status, and prophylactic surgery. Did not distinguish between DCIS and invasive breast cancer.	NA

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
van der Kolk et al, 2010[201] **[Testing Negative Group Only]**	Probands and some family members. Noncarriers were defined as women who tested negative for a known familial mutation in either *BRCA1* or *BRCA2*.	Observed vs. expected BC in *BRCA1* group: 5 vs. 2.5; age- and period-adjusted SIR, 2.0 (95% CI, 0.7 to 4.7) OC in *BRCA1* group: 0 vs. 0.3; age- and period-adjusted SIR, 0 (95% CI, 0 to 12) BC in *BRCA2* group: 4 vs. 1.6; age- and period-adjusted SIR, 2.5 (95% CI, 0.7 to 6.3) OC in *BRCA2* group: 0 vs. 0.2; age- and period-adjusted SIR, 0 (95% CI, 0 to 20.4)	NR	Cancer status was reported by the family. Cancer cases were confirmed by hospital or pathology records or else through a first degree family member.	DCIS was included as breast cancer. Information was collected on age, cancer status, vital status, and prophylactic surgery.	NA
BRCA positive-single						
Chen et al, 2006[122]	Proband	*BRCA1* carriers vs. *BRCA2* carriers Cumulative BC risk at age 70: 0.46 (95% CI, 0.39 to 0.54) vs. 0.43 (95% CI, 0.36 to 0.51) Cumulative OC risk at age 70: 0.39 (95% CI, 0.30 to 0.50) vs. 0.22 (95% CI, 0.14 to 0.32)	NR	NR	It is not clear if information was collected on prophylactic surgery or vital status.	The retrospective likelihood approach was used.
Finkelman et al, 2012[179] **[Prospective participants only]**	Proband	BC vs. OC *BRCA1*, 185delAG (ref nonCJM): HR, 1.23 (95% CI, 0.87 to 1.73) vs. 0.97 (95% CI, 0.58 to 1.63) *BRCA1*, 5382insC (ref nonCJM): HR, 1.53 (95% CI, 0.96 to 2.45) vs. 0.61 (95% CI, 0.27 vs. 1.38) *BRCA2*, 6174delT (ref nonCJM): HR, 0.35 (95% CI, 0.18 to 0.69) vs. 1.34 (95% CI, 0.48 to 3.73) Jewish (ref nonJewish): HR, 0.76 (95% CI, 0.56 to 1.01) vs. 0.93 (95% CI, 0.59 to 1.46) RRSO (ref no): HR, 0.62 (95% CI, 0.47 to 0.83) vs. 0.08 (95% CI, 0.04 to 0.16) No significant difference in BC hazard reduction from RRSO was observed in specific CJM carriers (joint Wald test; p=0.61).	NR	NR	Information was collected on prophylactic surgery, age, cancer status, and vital status.	Cumulative incidence of cancer based on method adapted from Antoniou et al, 2003.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Lubinski et al, 2012[187]	Proband	North America vs. Poland Cumulative incidence: 15.9% (95% CI, 12.0 to 19.8) vs. 12.1% (95% CI, 8.0 to 16.2) Average annual risk of BC: 2.4% (95% CI, 1.8 to 2.9) vs. 1.7% (95% CI, 1.2 to 2.1) Penetrance to age 70: 71.7% vs. 48.6% Penetrance to age 70 after adjusting for oophorectomy: 76.3% vs. 57.5% Residence in Poland vs. North America: adjusted HR, 0.54 (95% CI, 0.34 to 0.86); p=0.01 Adjusted for oophorectomy, age at study entry, age of menarche, parity (0, 1, 2, 3, 4+), oral contraceptive use (ever/never), tamoxifen use (ever/never), hormone replacement therapy (ever/never), smoking (ever/never), regular alcohol use (ever/never), and family history (number of FDRs and SDRs with BC).	NR	Cancer status was reported by the proband and 70% were confirmed with pathology reports.	Information was collected on prophylactic surgery, age, cancer status, and vital status.	Theoretical penetrance curves up to age 70, for age-specific cancer rates calculated based on 5-year intervals.
Marroni et al, 2004[188]	Probands. Not reported if other family members tested.	Penetrance (*BRCA1* vs. *BRCA2*) BC by age 50: 27% (95% CI, 20 to 34) vs. 26% (95% CI, 18 to 34) BC by age 70: 39% (95% CI, 27 to 52) vs. 44% (95% CI, 29 to 58) OC by age 50: 14% (95% CI, 7 to 22) vs. 3% (95% CI, 0 to 7) OC by age 70: 43% (95% CI, 21 to 66) vs. 15% (95% CI, 4 to 26)	NR	Cancer status was reported by family members for FDRs and SDRs of the proband.	It is not clear if information was collected on prophylactic surgery	Parameter estimates are based on the retrospective likelihood, the likelihood of the genetic data (the observed test results) conditional on the phenotype. Obtained penetrance estimates via a Metropolis-Hastings Markov Chain Monte Carlo

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
						(MCMC) method implemented in BRCAPRO.
Metcalfe et al, 2010[220]	Proband	0 FDRs vs. 1 FDR vs. ≥2 FDRs diagnosed with BC at age ≤50 years *BRCA1* penetrance for BC by age 70: 56% vs. 57% vs. 72% *BRCA2* penetrance for BC by age 70: 38% vs. 46% vs. 85% 0 FDRs vs. 1 FDR vs. ≥2 FDRs diagnosed with OC *BRCA1* penetrance for OC by age 70: 39% vs. 55% vs. 68%	NR	Cancer status was reported by the proband.	Information was collected on age, cancer status, prophylactic surgery, and vital status.	Age and mutation specific cancer rates were calculated for the 2 sites of cancer for 5-year intervals. Based on these rates, penetrance curves were constructed by applying the observed cancer rates annually to a theoretical cohort of healthy women from age 25 to 70 years.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Risch et al, 2006[1895]	Proband	*BRCA1* vs. *BRCA2* Cumulative incidence for BC by age 80: 90% (95% CI, 77 to 97) vs. 41% (95% CI, 26 to 60) Cumulative incidence for OC by age 80: 24% (95% CI, 15 to 38) vs. 8.4% (95% CI, 3.9 to 17)	Founder mutations; shortened, non-functional proteins; substitutions producing premature termination codons; mutations reported previously as documented in the BIC database or elsewhere.	Investigators reviewed pathology reports to determine eligibility for the proband. Family history information was reported by the proband through telephone interview.	It is not clear if information was collected on prophylactic surgery	Cumulative incidence of cancer to age 80 years for all cancer sites was based on Ontario general population age-specific incidence and mortality data. The DevCan computer program was used to calculate cancer site specific incidence according to mutation status. The sum of the incidence to age 80 years for the 3 groups (non carriers, *BRCA1* carriers, and *BRCA2* carriers) totaled the population incidence.
BRCA positive-multi						
Al-Mulla et al, 2009[172]	Probands and their family members.	Median age at onset for BC (years) 185delAG mutation in exon 2: 55 4184delTCAA mutation in exon 11: 47 Exon 13 duplication: 41	NR	Not clear.	Information was collected on age, cancer status, and vital status.	Cox proportional hazards regression adjusting for clustering within families using robust standard errors by the method of Lin and Wei.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
Antoniou et al, 2006[173]	Families were included that had ≥1 mutation carrier identified and ≥1 further family member had DNA testing after the mutation carrier was identified.	Cumulative risk (*BRCA1* vs. *BRCA2*) BC by age 50: 20% (95% CI, 0 to 45) vs. 21% (95% CI, 0 to 55) BC by age 70: 72% (95% CI, 0 to 93) vs. 75% (95% CI, 0 to 97) OC by age 50: 1% (95% CI, 0 to 10) vs. 0.4% (95% CI, 0 to 2) OC by age 70: 38% (95% CI, 0 to 78) vs. 49% (95% CI, 0 to 81)	NR	Cancer status of family members was reported by the proband. In most instances, the diagnoses of breast and/or ovarian cancer were confirmed by examining a pathology report.	It was not reported whether prophylactic surgery was collected.	Penetrance parameters were estimated by maximum likelihood using a modified segregation analysis implemented in MENDEL.
Evans et al, 2008[178]	Index case and some family members. Testing is offered to all blood relatives. Where possible, all affected women with breast/ovarian cancer are tested.	*BRCA1* vs. *BRCA2* Penetrance of BC to age 70: 68% (95% CI, 65 to 71) vs. 75% (95% CI, 72 to 78) Risk of OC to age 70: 60% (95% CI, 65 to 71) vs. 30% (95% CI, 26 to 35) There was evidence of a strong cohort effect with women born after 1940 having a cumulative risk of 22% for breast cancer by age 40 years compared to 8% in women born before 1930 (p=0.0005).	NR	Cancer status of family members was reported by the proband for 1st, 2nd, and 3rd degree relatives. All cases of breast or abdominal cancers are confirmed by means of hospital/pathology records, cancer registries, or death certification.	Information was collected on age, cancer status, prophylactic surgery, and vital status. DCIS was included as breast cancer.	Penetrance analysis was performed by including all mutation positive individuals and appropriate numbers of untested FDRs on a proportional basis.
Kramer et al, 2005[185] **[Mutation Carrier Group Only]**	All women in the family who agree to testing. Women were inferred positive based on having a child who was found to carry the mutation. Women were inferred negative based on having a parent that tested negative for the family mutation. A total of 451/673 (67%) had a known or inferred genotype.	Risk of BC (SE) *BRCA1* carriers at age 50: 0.44 (0.07) *BRCA1* carriers at age 70: 0.76 (0.08) 10-year BC risk (SE) in mutation carriers BC free with intact ovaries vs. same who have undergone oophorectomy At age 40 years: 0.32 (0.13) vs. 0.11 (0.10) At age 50 years: 0.28 (0.14) vs. 0.19 (0.12) At age 60 years: 0.25 (0.18) vs. 0.14 (0.13)	NR	Cancer status was initially reported by family members by questionnaire. Reported cancers were confirmed through death certificates, medical records, pathology reports, and central review of pathology slides.	Information was collected on prophylactic surgery, age, cancer status, and vital status.	Cumulative, age specific probabilities of developing breast cancer were estimated using the Kaplan-Meier product-limit method, with age as the time variable, modified to account for late entry. Analysis was repeated with oophorectomy as a censoring

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
						event. A Cox proportional hazards model incorporated oophorectomy as a time-dependent covariate to estimate the effect oophorectomy on the incidence of breast cancer. To provide estimate of the absolute risk of breast cancer by age in mutation carriers, oophorectomy was treated as time-fixed covariate as defined at the beginning of a given age interval. Followup time was divided into 10 year intervals. A competing risks model (with death as the competing risk) was then used to estimate the 10 year cumulative incidence.
Milne et al,	Probands and ≥1	*BRCA1*	Deleterious if	Cancer status was	Information was	Penetrance

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
2008[191]	family member were tested.	Penetrance to age 70: 52% (95% CI, 26 to 69) for BC and 22% (95% CI, 0 to 40) for OC *BRCA2* Cumulative risk to age 70: 47% (95% CI, 29 to 60) for BC and 18% (95% CI, 0 to 35) for OC	they a) were classified as "clinically important" by the BCIC; b) produced a premature stop codon at or before codon 1853 in BRCA; c) were protein truncating mutations occurring before exon 27 in *BRCA2*; d) were single base changes occurring at highly conserved bases of the splice donor of acceptor site and predicted to adversely affect splicing or shown to have other functional consequen-ces; and/or e) produced an amino acid change with strong evidence of	reported by the proband, and confirmed by other family members, when possible. Attempts were made to confirm the details of all reported cancers, including requesting pathology reports where possible.	collected on age, cancer status, prophylactic surgery, and vital status.	parameters were estimated by maximum likelihood using a modified segregation analysis implemented in MENDEL.

Appendix C8. Evidence Table of Penetrance of BRCA-Related Cancer

Author, year	Who was tested?	Results/conclusions	Quality considerations			
			Definition of clinically significant	How was cancer status ascertained?	Confounders	Method
			pathogenicity			
van der Kolk et al, 2010[201] **[Mutation Carriers Group Only]**	Probands and some family members. Obligate carriers were defined if a child as well as a parent or sibling carried a BRCA mutation.	Cumulative incidence (*BRCA1* vs. *BRCA2*) BC by age 70 excluding index cases: 60% (95% CI, 55 to 66) vs. 78% (95% CI, 69 to 88) OC by age 70 excluding index cases: 52% (95% CI, 45 to 59) vs. 13% (95% CI, 7.4 to 19)	NR	Cancer status was reported by the family. Cancer cases were confirmed by hospital or pathology records or else through a first degree family member.	DCIS was included as breast cancer. Information was collected on age, cancer status, vital status, and prophylactic surgery.	Cumulative incidence was estimated using Kaplan-Meier survival analysis.

Abbreviations: ASO = allele specific oligohybridization; BC = breast cancer; BCFR = Breast Cancer Family Registry; BCIC = Breast Cancer Information Core; CI = confidence interval; CJM = common Jewish mutations; CSGE = conformation sensitive gel electrophoresis; DCIS = ductal carcinoma in situ; DGGE = denaturing gradient gel electrophoresis; dHPLC = denaturing high performance liquid chromatography; DNA = deoxyribonucleic acid; FAMA = fluorescence assisted mutation analysis; FDR = first-degree relative; HBOC = hereditary breast and ovarian cancer; HR = hazard ratio; INHERIT = INterdisciplinary HEalth Research International Team on BReast CAncer Susceptibility; MCMC = Metropolis-Hastings Markov Chain Monte Carlo; NA = not applicable; NCI = National Cancer Institute; NR = not reported; O/E = observed to expected ratio; OC = ovarian cancer; OR = odds ratio; PCR = polymerase chain reaction; PROSE = Prevention and Observation of Surgical End Points Consortium; PTT = protein truncation test; RNA = ribonucleic acid; RR = relative risk; SDR = second-degree relative; SE = standard error; SEER = Surveillance, Epidemiology, and End Results; SIR = standardized incidence ratio; SSCP = single strand conformation polymorphism.

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/Setting
Current report						
Arver et al, 2004[235] NA	Psychological	To prospectively evaluate the psychological consequences during the 1st year following pre-symptomatic testing with respect to anxiety, depression, and QOL in self-referred individuals tested for breast/ovarian or colon cancer genes known in their families.	Before and after	Eligible: NR Enrolled: 66 Analyzed: 63 at week 1 and 2 months, 61 at 6 months, 59 at 12 months	Sweden	Clinical Genetic Unit, Karolinska University Hospital, Stockholm
Dagan and Shochat, 2009[236] Fair Same population as Shochat and Dagan, 2010[247]	Psychological Cancer worry	To investigate the association between *BRCA1/2* status and HR-QOL in Ashkenazi asymptomatic women.	Case-control	Eligible: 152 (39 carriers, 77 noncarriers, 36 controls) Enrolled: 73 (17 carriers, 20 noncarriers, 36 controls) Analyzed: 73 (17 carriers, 20 noncarriers, 36 controls)	Israel	Rambam Health Care Campus oncogenetic clinic
Ertmanski et al, 2009[237] NA	Psychological	To predict which women might suffer from abnormally high levels of anxiety and depression after receiving a positive genetic test result.	Before and after	Eligible: NR Analyzed: 56	Poland	Women seeking genetic testing at cancer genetics center in Poland. Women who tested positive for BRCA were included in analysis.
Foster et al, 2007[238] Fair	Cancer worry	To assess long-term impact of genetic testing for breast/ovarian cancer predisposition in a clinical cohort.	Prospective cohort	Eligible: NR Analyzed: 154	U.K.	Recruited from 9 U.K. centers between 1997 and 2000
Geirdal et al, 2005[240] Good Same population as Geirdal and Dahl, 2008[239]	Psychological	To explore psychological distress in women at risk of FBOC and HNPCC cancer and without access to genetic testing, and to compare them with mutation carriers and with healthy women from the general population.	Prospective cohort	Eligible: 10,321 (253 FBOC, 10,000 normal controls, 68 *BRCA1* mutation carriers) Enrolled: 10,244 (176 FBOC, 10,000 normal controls, 68 *BRCA1* mutation carriers) Analyzed: 10,244 (176 FBOC, 10,000 normal controls, 68 *BRCA1* mutation carriers)	Norway	Section for Genetic Counseling, Department of Cancer Genetics, The Norwegian Radium Hospital
Geirdal and Dahl, 2008[239] Good Same population as Geirdal et al, 2005[240]	Psychological	To examine how coping strategies used by women with FBOC were associated with caseness of anxiety disorder and to explore if a similar pattern of associations were observed in the carrier group.	Prospective cohort	Eligible: 333 (253 FBOC, 80 *BRCA1* mutation carriers) Enrolled: 242 (174 FBOC, 68 *BRCA1* mutation carriers) Analyzed: 242 (174 FBOC, 68 *BRCA1* mutation carriers)	Norway	Section for Genetic Counseling, Department of Cancer Genetics, The Norwegian Radium Hospital

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/Setting
Graves et al, 2012[241] NA	Psychological	To examine long-term psychosocial outcomes in a large U.S. sample.	Case-series	Eligible: 655 Enrolled: 464 Analyzed: 107 (unaffected)	U.S.	Women at the Lombardi Comprehensive Cancer Center Familial Cancer Registry
Julian-Reynier et al, 2011[242] Good	Risk perception	To describe the sequences of preventive decisions made by women up to 5 years after disclosure of their test results and the surveillance/surgical options chosen by various age groups.	Prospective cohort	Eligible: 331 Analyzed: 246	France	French Cancer Genetic Network
Kinney et al, 2005[243] Poor	Psychological	To evaluate the effect of receiving genetic test results on general and cancer-specific psychological distress in African Americans at high risk for carrying a deleterious *BRCA1* mutation.	Prospective cohort	Eligible: NR Analyzed: 52	U.S.	Members of a high-risk African American kindred that was identified previously with the *BRCA1* mutation
Low et al, 2008[244] Fair	Psychological	To examine the relationship between mutation carrier status, personal cancer history, and the potential positive impact of genetic testing.	Prospective cohort	Eligible: NR Analyzed: 47	U.S.	UCLA Familial Cancer Registry and Genetic Evaluation Program
Metcalfe et al, 2012[249] NA	Psychological	To report on cancer-related distress levels, uptake of cancer risk reduction options, and the resulting breast and ovarian cancer risk in Jewish women 2 years after receiving a postive BRCA mutation result.	Before and after	Eligible: 22 Enrolled: 19 Analyzed: 17	Canada	Jewish women responding to a newspaper ad
Reichelt et al, 2004[245] Good	Psychological	To examine the short-term psychological impact of receiving definite results concerning *BRCA1* mutation status in a clinical setting.	Prospective cohort	Eligible: 301 Enrolled: 244 Analyzed: 209	Norway	Unit of Medical Genetics, The Norwegian Radium Hospital
Reichelt et al, 2008[246] NA	Psychological	To examine the levels of psychological and cancer-specific distress at 18 months after getting genetic test results in women with demonstrated *BRCA1* mutations and to explore associations with baseline characteristics.	Before and after	Eligible: NR Analyzed: 181	Norway	Section for Hereditary Cancer, Department of Medical Genetics, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/Setting
Shochat and Dagan, 2010[247] Fair Same population as Dagan and Schochat,2009[236]	Insomnia	To investigate the association between positive genetic diagnosis for *BRCA1/2* founder mutations and symptoms of insomnia in Ashkenazi asymptomatic women.	Case-control	Eligible: 152 (39 carriers, 77 noncarriers, 36 controls) Enrolled: 73 (17 carriers, 20 noncarriers, 36 controls) Analyzed: 73 (17 carriers, 20 noncarriers, 36 controls)	Israel	Rambam Health Care Campus oncogenetic clinic between 1996 and 2006
van Dijk et al, 2006[248] Good	Cancer worry	To assess whether the pedigree-based familial risk estimation and the personal cancer history can explain cancer worry and distress among women who receive an uninformative DNA test result.	Prospective cohort	Eligible: NR Enrolled: 133 Analyzed: 132	The Netherlands	Department of Clinical Genetics in Leiden or Rotterdam between 1995 and 2002, in families where a BRCA mutation was already detected
Prior report						
Meiser et al, 2002[250] Good	Psychological	To study the psychological adjustment of women who have undergone testing for *BRCA1/2* breast and ovarian cancer susceptibility.	Prospective cohort	Eligible: NR Enrolled: 143 (30 carriers, 60 noncarriers, and 53 controls) Analyzed: 140 (30 carriers, 59 noncarriers, and 51 controls)	Australia	Women in outreach clinics who had *BRCA1/2* testing, were healthy with a family history of breast or ovarian cancer, and approached 1 of 14 familial cancer clinics (FCC) and 6 associated clinics

Author, year Quality	Demographics	Inclusion/Exclusion criteria	Risk level definition	Mutation status	Measures
Current report					
Arver et al, 2004[235] NA	Mean age of 40.5 years (SD 11.1)	Inclusion: Healthy females belonging to a family with a known mutation in 1 of the genes *(BRCA1, BRCA2, MLH1*, *MSH2*), wishing for genetic testing, age ≥18 years, Swedish speaking Exclusion: Individuals with cancer and men	Women with a 50% or 25% risk of being gene carriers	BRCA carriers and noncarriers	Hospital Anxiety and Depression Scale (HADS, each subscale 0 to 21) Swedish SF-36 Health Survey (SF-36, scale NR)
Dagan and Shochat, 2009[236] Fair Same population as Shochat and Dagan, 2010[247]	Mean age of 51.5 years (SD 8.9) Carriers: 51.4 years (SD 9.1) Non-carriers: 54.5 years (SD 9.4) Controls: 50.0 years (SD 8.3)	Inclusion: Asymptomatic *BRCA1/2* carriers and noncarriers who had genetic testing at Rambam Health Care Campus Control: Age-matched low-risk community control, with no family history of breast/ ovarian cancer and not tested for *BRCA1/2* mutations Exclusion: Major chronic illnesses, pregnancy, age ≤1 year	FDR and/or SDR with breast or ovarian cancer and/or relative with other cancer	BRCA carriers and noncarriers	Health-Related Quality of Life (HR-QOL, scale NR) Cancer Related Worry (CRW, scale NR) The Brief Symptom Inventory (BSI, scale NR)

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Demographics	Inclusion/Exclusion criteria	Risk level definition	Mutation status	Measures
Ertmanski et al, 2009[237] NA	NR for women without breast cancer	Inclusion: Women who tested positive for BRCA mutation and completed both baseline and followup measures Exclusion: NR	Positive family history of early onset breast or ovarian cancer	BRCA positive	State-Trait Anxiety Inventory (STAI, scale 1 to 10) Impact of Events Scale (IES, scale 0 to 75)
Foster et al, 2007[238] Fair	Median age 42 years (range: 23-72)	Inclusion: Unaffected by cancer and from families with a *BRCA1/2* mutation identified in an affected blood relative Exclusion: NR	50% risk of inheriting a *BRCA1/2* mutation, this was lower if intervening relative had died	BRCA carriers and noncarriers	General Health Questionnaire (GHQ-28, scale 0 to 28) Cancer worry scale-revised (CWS-R, scale 6 to 24)
Geirdal et al, 2005[240] Good Same population as Geirdal and Dahl, 2008[239]	Mean age (years): FBOC: 40.5 (SD 9.7) *BRCA1* carriers: 42.0 (SD 10.6) Controls: 42.5 (SD 10.9)	Inclusion: Self-referred or referred from doctors to Section for Genetic Counseling, at risk for FBOC or BRCA positive Controls: random sample of age-matched women completing same questionnaires Exclusion: NR	Family history of ≥2 FDRs (or SDR though males) with early onset (<50 years) breast cancer and/or multiple cases of breast cancers in the same lineage compatible with dominant inheritance in the family and/or a combination of early onset breast cancer and ovarian cancer in the family	BRCA positive FBOC, mutation status unknown	Hospital Anxiety and Depression Scale (HADS, each subscale 0 to 21) General Health Questionnaire (GHQ-28, scale 0 to 84) Beck Hopelessness Scale (BHS, scale 0 to 20) Impact of Event Scale (IES, IES-I subscale 0 to 35 and IES-A subscale 0 to 40)
Geirdal and Dahl, 2008[239] Good Same population as Geirdal et al, 2005[240]	Mean age (years): FBOC: 40.5 (SD 9.7) *BRCA1* carriers: 42.0 (SD 10.6)	Inclusion: FBOC: Women age ≥18 years, had been to genetic counseling at Section for Genetic Counseling *BRCA1* positive: Women age ≥18 years, had been to genetic counseling and testing at Section for Genetic Counseling, carried a demonstrable mutation Exclusion: FBOC: Any identifiable mutation in family, diagnosed with breast or ovarian cancer *BRCA1* positive: Diagnosed with breast or ovarian cancer	Family history of ≥2 FDRs (or SDRs though males) with early onset (<50 years) breast cancer and/or multiple cases of breast cancers in the same lineage compatible with dominant inheritance in the family and/or a combination of early onset breast cancer and ovarian cancer in the family	BRCA positive FBOC, mutation status unknown	Hospital Anxiety and Depression Scale (HADS, anxiety subscale 0 to 21) Coping Orientation to Problems Experienced Scale (COPE, scale varied for each coping strategy)
Graves et al, 2012[241] NA	NR for women without breast cancer	Inclusion: Women ages 25 to 75 years, received *BRCA1/2* test results, and were at least 3 years postdisclosure at the time of the study Exclusion: Not reported	NR	47/107(43.9%) BRCA positive 60/107 (56.1%) BRCA true negative	Impact of Events Scale (IES, scale 0 to 75) State-Trait Anxiety Inventory (STAI, scale 20 to 80)
Julian-Reynier et al, 2011[242] Good	Mean age (years) Carriers: 37.2 Noncarriers: 41.7	Inclusion: *BRCA1/2* mutation carriers and noncarriers in the same families Exclusion: NR	*BRCA1/2* mutation carriers or members of families where a mutation was identified	101/246 (41%) *BRCA1/2*	Perception of personal risk of cancer (6-point Likert scale) Preventive health behaviors

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Demographics	Inclusion/Exclusion criteria	Risk level definition	Mutation status	Measures
Kinney et al, 2005[243] Poor	NR for women without breast cancer	Inclusion: Women age ≥18 years and members of the family identified in the genetic linkage study as having *BRCA1* mutation Exclusion: NR	All women from *BRCA1* mutation positive family	*BRCA1* carriers and noncarriers	State-Trait Anxiety Inventory (STAI, scale 1 to 10) Impact of Events Scale (IES, scale 0 to 75) Center for Epidemiologic Studies-Depression (CES-D, scale NR)
Low et al, 2008[244] Fair	NR for women without breast cancer	Inclusion: Age ≥18 years with family history of breast, ovarian, or other cancer consistent with *BRCA1/2* heredity and/or 10% prior probability of carrying a *BRCA1/2* mutation based on published risk assessment data Exclusion: Did not complete followup data	Personal and/or family history consistent with *BRCA1/2* heredity and/or 10% prior probability of carrying a *BRCA1/2* mutation	BRCA positive and negative Variant of uncertain significance was grouped with negative results	Impact of Events Scale-Revised (IES-R, scale NR) Brief COPE (scale NR) Emotional Approach Coping Scale (scale NR) Post-Traumatic Growth Inventory (PTGI, scale 0 to 105)
Metcalfe et al, 2012[249] NA	Mean age of 46 years (range: 28-67)	Inclusion: Women self-identified as Jewish, ages 25 to 70 years, residing in Ontario, and positive for a BRCA mutation Exclusion: Not reported	All were positive for BRCA mutation	8/19 (42%) *BRCA1* 11/19 (58%) *BRCA2*	Impact of Events Scale (IES, scale 0 to 75, IES-I subscale 0 to 35, IES-A subscale 0 to 40)
Reichelt et al, 2004[245] Good	Mean age (years): Tested: 43.9 (SD 11.7) Not tested: 33.0 (SD 11.7)	Inclusion: Age ≥18 years and risk based on clinical criteria Exclusion: None	50% risk for FDRs to carriers 25% risk for SDRs through males to carriers	BRCA carriers and noncarriers Unknown status, for those who refused testing	Hospital Anxiety and Depression Scale (HADS, each subscale 0 to 21) General Health Questionnaire (GHQ-28, scale 0 to 84) Beck Hopelessness Scale (BHS, scale 0 to 20) Impact of Event Scale (IES, IES-I subscale 0 to 35 and IES-A subscale 0 to 40)
Reichelt et al, 2008[246] NA	NR for women without breast cancer	Inclusion: Women age >18 years, with a known *BRCA1* mutation in a close relative Exclusion: None	Known *BRCA1* mutation in close relative	BRCA positive and negative	Hospital Anxiety and Depression Scale (HADS, scale 0 to 42) Impact of Events Scale-Intrusive subscale (IES-I, scale 0 to 35)

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Demographics	Inclusion/Exclusion criteria	Risk level definition	Mutation status	Measures
Shochat and Dagan, 2010[247] Fair Same population as Dagan and Schochat,2009[236]	Mean age of 51.5 years (SD 8.9) Carriers: 51.4 years (SD 9.1) Noncarriers: 54.5 years (SD 9.4) Controls: 50.0 years (SD 8.3)	Inclusion: Asymptomatic *BRCA1/2* carriers and noncarriers who had undergone genetic testing at Rambam Health Care Campus Control: Age-matched low-risk community control, with no family history of breast/ovarian cancer and not tested for *BRCA1/2* mutations Exclusion: Major chronic illnesses, pregnancy, age ≤1 year	FDR and/or SDR with breast or ovarian cancer and/or relative with other cancer	BRCA carriers and noncarriers	Wrist activity monitors Daily sleep log Pittsburgh Sleep Quality Index (PSQI, each subscale 4-point Likert) Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF, scale 0 to 120) The Brief Symptom Inventory (BSI, scale NR) Cancer Related Worry (CRW, scale NR)
van Dijk et al, 2006[248] Good	NR for women without breast cancer	Inclusion: Women from a family with a previously detected BRCA mutation, age ≥18 years, and had not previously received genetic counseling elsewhere Exclusion: NR	BRCA mutation previously detected in family and individuals with a probability of mutation detection of ≥10%; women with an uninformative result were separated into 2 risk groups, 1) <30% personal risk estimate for low risk and 2) ≥30% personal risk estimate for high-risk	BRCA positive, true negative, and uninformative results	Impact of Events Scale (IES, scale 0 to 75) Breast cancer worry question of "During the last 2 weeks, how often did you worry about developing breast cancer?" (Likert scale ranging from 1=almost never to 4=almost all the time)
Prior report					
Meiser et al, 2002[250] Good	Mean age of 40 years (SD 11.1)	Inclusion: Eligible for genetic testing and at risk for developing hereditary breast cancer with an affected living relative to provide blood sample Exclusion: History of breast or ovarian cancer, limited English literacy, and being tested for founder mutations only	25% mutation (*BRCA1/2*) carrier risk: Subjects from high-risk family with closest affected relative or relative with a *BRCA* mutation is 2nd degree 50% risk: Subjects from high-risk family who has either a 1st degree affected relative or unaffected relative with a known pathogenic *BRCA1/2* mutation	BRCA carriers and noncarriers	Miller Behavioural Style Scale (scale NR) Impact of Events Scale (IES, scale 0 to 75) State-Trait Anxiety Inventory (STAI, scale 20 to 80) Beck Depression Inventory (BDI, scale 0 to 63)

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
Current report				
Arver et al, 2004[235] NA	1995-1999 At 1 week, 2, 6, and 12 months	**Pretest vs. 1 week posttest vs. 2 months posttest vs. 6 months posttest vs. 1 year posttest** Mean on psychological scale HADS-A (estimated from graph): 5.6 vs. 4.6 vs. 4.0 vs. 4.0 vs. 4.2; p<0.001 over time, only pretest is above normal value HAD-D (estimated from graph): 2.4 vs. 2.4 vs. 2.4 vs. 2.4 vs. 2.6; p=NS SF-36 general health (SD): 78.7 (19.2) vs. 78.8 (18.1) vs. 79.6 (20.2) vs. 81.0 (20.1) vs. 81.0 (20.3); p=NS SF-36 vitality: 67.0 (21.9) vs. 66.4 (19.8) vs. 71.9 (21.8) vs. 68.2 (25.4) vs. 69.3 (23.4); p=NS SF-36 social function: 87.3 (15.6) vs. 86.5 (20.0) vs. 91.1 (17.5) vs. 89.1 (19.4) vs. 89.0 (18.2); p=NS SF-36 role emotional: 83.8 (30.5) vs. 82.5 (34.8) vs. 79.2 (38.6) vs. 88.0 (29.2) vs. 86.2 (33.1) SF-36 mental health: 77.4 (18.7) vs. 74.9 (20.0) vs. 80.1 (19.5) vs. 78.6 (17.9) vs. 78.3 (19.6); p=NS	Anxiety went down over time, however depression and QOL were not affected. The results were not separated out by carriers and noncarriers though.	King Gustav V's Jubilee Fund and the Swedish Cancer Society
Dagan and Shochat, 2009[236] Fair Same population as Shochat and Dagan, 2010[247]	January 2006-November 2007 Mean followup of 8.0 years (SD 1.9)	**Carriers (n=17) vs. noncarriers (n=20) vs. controls (n=36)** Mean on psychological scale (SD) CRW: 0.75 (0.5) vs. 0.67 (0.5) vs. 0.45 (0.4); p=NS BSI total: 0.66 (0.7) vs. 0.35 (0.4) vs. 0.50 (0.4); p=NS HR-QOL total: 74.4 (19.2) vs. 80.3 (13.7) vs. 83.0 (10.2); p=NS HR-QOL role limitation due to emotional problems subscale: 74.5 (36.4) vs. 91.7 (21.3) vs. 97.2 (9.3); p<0.01 HR-QOL role limitation due to physical problems subscale: 79.4 (30.9) vs. 85.0 (28.6) vs. 95.1 (13.1); p=0.05	Carriers had higher QOL distress regarding role limitation due to emotional problems and physical problems compared to noncarriers and controls.	NR
Ertmanski et al, 2009[237] NA	January 2005-December 2007 At 1 month and 1 year	**Pretest vs. 1 month posttest vs. 1 year posttest** Mean STAI-Anxiety: 6.6 vs. 6.5 vs. 6.5 At 1 month posttest, IES mean score was 23.8, which is considered a low level of negative psychological reaction	For women not affected by breast cancer themselves, testing positive for the BRCA mutation did not increase anxiety and did not have a negative psychological impact.	Polish Ministry of Science and Higher Education grant number 2 PO5 D 129 29
Foster et al, 2007[238] Fair	1997-2000 3 years	**Carriers (n=53) vs. noncarriers (n=101)** Mean on psychological scales (SD) GHQ at baseline: 2.7 (4.6) vs. 2.6 (3.8); p=NS GHQ at 3 year posttest: 4.5 (6.3) vs. 3.7 (5.3); p=0.03 for carriers baseline vs. posttest; p=NS for between-groups differences CWS-R at baseline: 11.7 (3.1) vs. 11.5 (3.4); p=NS CWS-R at 3 year posttest: 10.4 (3.6) vs. 9.3 (2.1); p=0.03 for carriers baseline vs. posttest; p=NS for between-groups differences	Overtime cancer worry decreased for both carriers and noncarriers, while general distress increased for both groups, with 18% of carriers and 17% of noncarriers identified as cases using the GHQ-28 at 3 year followup.	Award C1226/A137 from Cancer Research U.K.

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
Geirdal et al, 2005[240] Good Same population as Geirdal and Dahl, 2008[239]	January 2000-December 2001	**FBOC (n=176) vs. carriers (n=68) vs. controls (n=10,000)** Mean differences on psychological scales (SD) HADS-D: 2.4 (2.9) vs. 1.7 (2.4) vs. 3.2 (2.9); p<0.05 FBOC vs. carriers HADS-A: 5.2 (3.8) vs. 4.2 (3.6) vs. 4.5 (3.5); p<0.05 FBOC vs. carriers GHQ-28: 3.3 (5.4) vs. 2.3 (4.0) vs. NR; p<0.05 FBOC vs. carriers IES-I: 10.2 (8.7) vs. 9.8 (7.6) vs. NR; p=NS IES-A: 8.3 (7.9) vs. 8.4 (7.6) vs. NR; p=NS BHS: 3.7 (2.5) vs. 3.8 (2.6) vs. NR; p=NS	Women in FBOC group, but who had not undergone genetic testing were more anxious, more depressed, and higher general distress than women who were known to be BRCA mutation carriers.	Norwegian Foundation for Health and Rehabilitation, National Council for Mental Health, Norway, and a donation from Edith Kongshe, Oslo
Geirdal and Dahl, 2008[239] Good Same population as Geirdal et al, 2005[240]	January 2000-December 2001	**FBOC (n=174) vs. carriers (n=68)** Mean HADS-A: 5.3 (SD, 3.9) vs. 4.2 (SD, 3.6); p=0.04 Prevalence of HADS-defined anxiety: 24% vs. 24%; p=NS Mean (SD) on subscales of COPE with significant differences, higher scores=strategy used more often Active coping: 10.2 (3.2) vs. 8.7 (3.2); p=0.002 Planning: 9.1 (3.5) vs. 7.9 (3.7); p=0.01 Suppression of competing activities: 6.7 (2.7) vs. 5.2 (2.3); p<0.001 Focus on and venting of emotions: 8.1 (3.6) vs. 6.2 (2.7); p<0.001 Seeking instrumental support: 10.2 (3.6) vs. 7.4 (3.1); p<0.001 Seeking emotional support: 9.4 (3.3) vs. 7.9 (2.7); p=0.003 Acceptance: 12.4 (3.1) vs. 13.3 (2.9); p=0.01 Mental disengagement: 6.7 (2.8) vs. 6.0 (2.2); p=0.03 NS COPE subscales: positive reinterpretation and growth, restraint coping, denial, behavioral disengagement, turning to religion, and use of humor	Women in FBOC group, but who had not undergone genetic testing were more anxious than *BRCA1* mutation carriers. FBOC groups used many more coping strategies compared with *BRCA1* mutations carriers, however mutation carriers were more accepting of their breast cancer risk than those in the FBOC group and therefore may not have used other coping strategies.	Norwegian Foundation for Health and Rehabilitation, National Council for Mental Health, Norway, and a donation from Edith Kongshe, Oslo
Graves et al, 2012[241] NA	Years NR Median of 5 years posttest	**Logistic regression bivariate analysis (statistically significant associations)** Positive genetic test with genetic testing distress: p=0.03 Negative genetic test with positive experiences: p=0.008 **Multiple regression analysis (statistically significant associations)** Genetic testing distress Model 1 adjusting for marital status, pretest cancer distress, and receipt of RRM accounted for 13% of variance in genetic testing distress; p=0.003 Model 2 adjusting for model 1 and genetic test result (positive or true negative) accounted for an additional 12% of variance in genetic testing distress; p=0.00001 Positive experiences Model 1 adjusting for income and pretest cancer distress accounted for 8% of variance in positive; p=0.04 Model 2 adjusting for model 1 and genetic test result (positive or	Among unaffected women, *BRCA1/2* carriers reported higher genetic testing distress and lower positive experiences compared with *BRCA1/2* true negatives.	Department of Defense grant DAMD BC021733, Jess and Mildred Fisher Center for Familial Cancer Research, and Lombardi Comprehensive Cancer Center's Familial Cancer Registry and Clinical and Molecular Epidemiology Shared Resources

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
		true negative) accounted for an additional 6% of variance in positive experiences; p=0.008		
Julian-Reynier et al, 2011[242] Good	2000-2006 5 years	**Carriers (n=101) vs. noncarriers (n=145)** Change from before test result to after test result of those who perceived personal risk as high Breast cancer risk: +18% vs. -47%; p=0.016 for carriers change and p<0.001 for noncarriers change Ovarian cancer risk: +20% vs. -27%; p=0.007 for carriers change and p<0.001 for noncarriers change	Carriers perception of risk increased after receiving genetic test results, while noncarriers perception of risk decreased.	Institute National du Cancer
Kinney et al, 2005[243] Poor	Year NR 4 month	Noncarriers unaffected with breast cancer decreased anxiety from baseline to 1 month followup; p=0.001, data not shown	Noncarriers anxiety went down after receiving genetic test results.	National Human Genome Research Institute, National Institute of Nursing Research and the National Cancer Institute
Low et al, 2008[244] Fair	September 1998-Fall 2003 Average of 20.9 months	**Carriers (n=7) vs. noncarriers (n=40)** Mean on psychological scale (SE) PTGI total score (estimated from graph): 14 vs. 22; p=NR IES-R at 1-month posttest: 5.83 (2.47) vs. 1.37 (0.10); p<0.05 Approach coping score: 2.32 (0.18) vs. 2.37 (0.14); p=NS	Women with BRCA positive mutations reported greater distress after testing than noncarriers, but did not report differences in positive life changes.	STOP CANCER Research Career Development Award
Metcalfe et al, 2012[249] NA	Years NR 2 years	**Pretest vs. 1 year posttest vs. 2 years posttest** Mean IES-I (SD): 1.1 (1.9) vs. 10.9 (8.6) vs. 6.9 (6.2); p=0.02 Mean IES-A (SD): 4.1 (8.7) vs. 12.9 (8.2) vs. 10.4 (9.4); NS Mean IES-total (SD): 5.2 (10.5) vs. 23.8 (14.5) vs. 17.2 (14.5); p=0.05 **2 years posttest clinical distress levels** 2/19 (11%) severe distress (score ≥44) 4/19 (21%) moderate distress (score 26-43) 7/19 (37%) mild distress (score 9-25) 6/19 (32%) subclinical distress (score <9)	Intrusive behaviors increased 1 year posttest but decreased by 2 years, with most women (69%) scoring in the mild or subclinical distress level at 2 years	NR
Reichelt et al, 2004[245] Good	September 1997-October 1999 6 weeks	**Carriers (n=141) vs. noncarriers (n=68)** Mean on psychological scales (SD) at followup; all p=NS IES-I: 9.8 (7.6) vs. 9.3 (8.0) IES-A: 8.4 (7.6) vs. 7.6 (7.4) HADS-A: 4.2 (3.6) vs. 4.1 (3.9) HADS-D: 1.7 (2.4) vs. 2.3 (2.7) GHQ-28: 2.3 (4.0) vs. 2.4 (4.5) BHS: 3.8 (2.6) vs. 4.0 (2.8) **Tested (n=244) vs. not tested (n=57)** Mean on psychological scales (SD) at baseline IES-I (subscale 0 to 35): 8.8 (7.5) vs. 8.9 (7.3); p=NS IES-A (subscale 0 to 40): 8.0 (7.1) vs. 7.7 (7.3); p=NS	Women who chose to get tested had higher baseline depression than those who decided not to get tested. There were no differences at followup between women who were tested and found to be mutation carriers and those who were not mutation carriers.	A grant from the Norwegian Research Council

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
		HADS-A (subscale 0 to 21): 4.4 (3.8) vs. 4.1 (3.2); p=NS HADS-D (subscale 0 to 21): 2.0 (2.6) vs. 1.3 (1.8); p<0.05 GHQ (scale 0 to 84): 2.5 (4.2) vs. 2.0 (3.2); p=NS BHS (scale 0 to 20): 4.0 (2.7) vs. 3.7 (2.1); p=NS		
Reichelt et al, 2008[246] NA	September 1997-October 1999 At 6 weeks and 8 months	**Pretest vs. 6 weeks posttest vs. 18 months posttest** Mean psychological scales (SD) HADS: 6.6 (6.1) vs. 6.2 (6.1) vs. 6.9 (6.9); p=NS IES-I: 9.3 (7.8) vs. 9.0 (7.8) vs. 8.7 (7.9); p=NS	This study did not separate out women without cancer by carrier status. Results show no differences in distress before testing or up to 18 months after testing.	Norwegian Research Council grant number 115586/320
Shochat and Dagan, 2010[247] Fair Same population as Dagan and Schochat, 2009[236]	January 2006-November 2007 Mean followup of 8.0 years (SD 1.9)	**Carriers (n=17) vs. noncarriers (n=20) vs. controls (n=36)** Reported sleep problems (PSQI >5): 53% vs. 20% vs. 28%; p=0.03 for carriers vs. other groups <u>Mean on sleep measures (SD)</u> PSQI total: 7.29 (4.34) vs. 3.94 (2.49) vs. 4.21 (2.80); p=0.013 for carriers vs. noncarriers Sleep latency (minutes, recorded by wrist monitor): 12.23 (14.36) vs. 5.41 (5.93) vs. 9.44 (8.05); p=NS Sleep duration (minutes, recorded by wrist monitor): 435.96 (47.68) vs. 407.46 (55.56) vs. 434.40 (52.19); p=NS Sleep efficiency (%, recorded by wrist monitor): 94.46 (10.65) vs. 96.80 (2.43) vs. 97.26 (2.85); p=NS Wake after sleep onset (minutes, recorded by wrist monitor): 18.08 (23.90) vs. 12.82 (10.64) vs. 11.51 (10.03); p=NS <u>Correlations between PSQI total score and other measures</u> CRW: 0.417 vs. 0.125 vs. 0.029; p=NS BSI: 0.437 vs. 0.546 vs. 0.057; p=0.013 for noncarriers MFSI-SF: 0.418 vs. 0.315 vs. 0.430; p=0.009 for controls <u>Linear regression model predictors of PSQI total score (poor sleep quality)</u> Menopausal symptoms and lower level of education combined accounted for 12.6% of the variance; p=0.019 Menopausal symptoms, lower level of education, and fatigue combined accounted for 23.0% of the variance; p=0.001 Menopausal symptoms, lower level of education, fatigue, and carrier status combined accounted for 28% of the variance; p<0.001	Carriers reported more sleep problems compared to noncarriers and healthy controls. However, actual sleep duration, latency and wakefulness after sleep onset were not significantly different between groups.	NR

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
van Dijk et al, 2006[248] Good	1998-2002 At 1 and 7 months	**Positive (n=22) vs. true negative (n=41) vs. uninformative low risk (n=35) vs. uninformative high risk (n=34)** Mean on psychological scales (SD) IES at pretest: 21.55 (14.70) vs. 14.85 (11.99) vs. 13.54 (11.97) vs. 22.53 (14.22); p<0.05 for uninformative low risk group vs. positive and true negative groups IES at 1 month following test result: 24.14 (13.21) vs. 10.85 (13.62) vs. 7.40 (8.57) vs. 14.38 (12.41); p<0.05 for positive group vs. other groups IES at 7 months following test result: 24.09 (15.57) vs. 8.32 (13.30) vs. 6.31 (8.44) vs. 14.00 (14.51); p<0.05 for positive group vs. other groups and p<0.05 for uninformative high risk group vs. uninformative low risk group Breast cancer worry at pretest: 2.41 (0.73) vs. 1.88 (0.87) vs. 1.94 (0.73) vs. 2.21 (0.81); p<0.05 positive group vs. true negative and uninformative low risk groups Breast cancer worry at 1 month following test result: 2.64 (1.00) vs. 1.29 (0.75) vs. 1.51 (0.66) vs. 1.68 (0.81); p<0.05 for positive group vs. other groups Breast cancer worry at 7 months following test result: 2.18 (0.96) vs. 1.24 (0.70) vs. 1.37 (0.55) vs. 1.59 (0.66); p<0.05 for positive group vs. other groups	Women unaffected with breast cancer but with a positive mutation had higher levels of distress and cancer worry. However, at times they were similar in their level of distress and cancer worry as those who received an uninformative test result but were at high risk.	The Dutch Cancer Society Grant number UL 98-1740

Appendix C9. Evidence Table of Distress After Genetic Testing

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
Prior report				
Meiser et al, 2002[250] Good	November 1996-October 2000 12 months	**Carriers (n=30) vs. noncarriers (n=59) vs. controls (n=51)** Baseline mean scores (SD); p=NS for all Breast cancer worry: 13.1 (13.1) vs. 13.4 (14.6) vs. 16.0 (14.8) STAI: 36.1 (11.2) vs. 33.6 (12.1) vs. 33.6 (10.7) BDI: 5.5 (5.7) vs. 6.3 (6.7) vs. 5.9 (5.6) 7-10 day followup mean scores (SD) Breast cancer worry: 21.2 (14.4) vs. 13.9 (16.1) vs. 14.9 (12.3); p=0.005 carriers vs. controls; p=NR carriers vs. noncarriers STAI: 38.5 (13.8) vs. 31.6 (11.1) vs. 36.8 (12.1); p=0.024 noncarriers vs. others BDI: 5.3 (6.2) vs. 5.7 (7.0) vs. 7.2 (6.8); p=NS 4 month followup mean scores (SD) Breast cancer worry: 17.7 (18.6) vs. 8.1 (13.5) vs. 13.1 (13.5); p=NS carriers vs. controls; p=NR carriers vs. noncarriers STAI: 36.8 (15.3) vs. 32.2 (10.8) vs. 36.3 (14.2); p=NS BDI: 6.2 (8.7) vs. 3.6 (5.4) vs. 6.4 (6.3); p=0.024 noncarriers vs. others 12 month followup mean scores (SD) Breast cancer worry: 16.1 (14.9) vs. 8.2 (14.2) vs. 12.3 (14.8); p=0.045 carriers vs. controls, p=NR carriers vs. noncarriers STAI: 31.7 (10.5) vs. 36.2 (12.9) vs. 39.0 (12.2); p=0.007 noncarriers vs. control BDI: 4.0 (5.1) vs. 5.4 (6.4) vs. 6.9 (7.00); p=NS	Those without deleterious BRCA mutations derive psychological benefits from genetic testing. Those who test positive for deleterious BRCA mutations may anticipate a sustained increase in breast cancer distress following disclosure, although no other adverse effects were found in this group	Project Grants Nos. 970929 and 113877 from National Health and Medical Research Council of Australia

Abbreviations: BDI = Beck Depression Inventory; BHS = Beck Hopelessness Scale; BSI = Brief Symptom Inventory; CES-D = Center for Epidemiologic Studies-Depression Scale; COPE = Emotional Approach Coping Scale; CRW = Cancer-Related Worry; CWS-R = Cancer Worry Scale-Revised; FBOC = familial breast ovarian cancer; FCC = family cancer clinic; FDR = first-degree relative; GHQ = General Health Questionnaire; HADS = Hospital Anxiety and Depression Scale; HNPCC = hereditary nonpolyposis colorectal cancer; HR-QOL = Health Related-Quality of Life; IES = Impact of Events Scale; MSFI-SF = Multidimensional Fatigue Symptom Inventory-Short Form; NR = not reported; NS = not significant; PSQI = Pittsburgh Sleep Quality Index; PTGI = Post-Traumatic Growth Inventory; SD = standard deviation; SDR = second-degree relative; SE = standard error; SF-36 = Swedish SF-36 Health Survey; STAI = State-Trait Anxiety Inventory; UCLA = University of California, Los Angeles.

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Design	Purpose	Population/ setting	Inclusion/exclusion criteria	Risk level definitions
Breast cancer					
Cortesi et al, 2006[273] NA Modena Study Group for Familial Breast and Ovarian Cancer participants	Prospective cohort (Expected incidence ratio derived from registry data)	To describe the results of an intensive surveillance program and document effectiveness of the program in selecting individuals at risk of breast cancer	Italy Women with increased risk of breast cancer	Inclusion Women age >18 years with *BRCA1/2* mutations discovered through genetic testing or increased risk for breast cancer relative to the general population based on Gail model, Claus tables, and modified BRCAPro model (adapted to the Italian population) and study defined criteria: ≥3 relatives diagnosed with breast or ovarian cancer in 2 different generations; ≥1 of these 3 relatives must be FDR of 1 of the other 2, in case of male interposition, a relationship of different degree is allowed; ≥1 breast cancer diagnosed at age <35 years regardless of family history; ≥1 breast cancer and 1 ovarian cancer in the same woman, regardless of family history; ≥1 male breast cancer, regardless of family history; 1 sporadic breast cancer or ovarian cancer Exclusion Women with symptoms suggestive of breast cancer; women with a personal history of breast cancer	Risk level was defined by Gail model, Claus tables, modified BCAPRO model, and study defined criteria (see Inclusion). Carrier (Gail model lifetime risk of 50%-85%): presence of mutant BRCA genes. High-risk (Gail model lifetime risk of 30%-50%): ≥3 relatives with breast cancer (or ovarian cancer) in 2 different generations; 1 breast/ovarian cancer case is a FDR of the other 2; ≥1 case has been diagnosed at age <40 years or with bilateral breast cancer; breast cancer diagnosed at age <35 years, regardless of family history; breast and ovarian cancer in same woman, regardless of family history. Intermediate risk (Gail model lifetime risk of 18%-29%): male breast cancer, regardless of family history. Slightly increased risk (Gail model lifetime risk of 6%-18%): breast/ovarian cancer without any of the described criteria.
Leach, 2005[274] NAMARIBS study	Prospective cohort, one-arm	To compare contrast enhanced MRI with mammography for breast cancer screening in women genetically predisposed to breast cancer	U.K. Women attending 1 of 22 participating centers in the U.K. with increased breast cancer risk	Inclusion Asymptomatic women ages 35 to 49 years fulfilling 1 of the following: known carrier of a deleterious *BRCA1/2* or TP53 mutation; FDR of someone with 1 of these deleterious mutations; strong family history of breast or ovarian cancer or both; or family history consistent with classic Li-Fraumeni syndrome. Aim was to include women whose affected FDRs had ≥60% chance of being a *BRCA1/2* mutation carrier or women with an annual risk of at least 0.9%. Exclusion Women with previous breast cancer, those with any cancer such that prognosis was <5 years, participants who had predictive genetic testing during study and whose results were negative, women who developed cancer during study period.	Known carrier of a deleterious *BRCA1/2* or TP53 mutation; FDR of someone with 1 of these deleterious mutations; strong family history of breast or ovarian cancer or both; or family history consistent with classic Li-Fraumeni syndrome.

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Design	Purpose	Population/ setting	Inclusion/exclusion criteria	Risk level definitions
Le-Petross et al, 2011[276] NA	Retrospective analysis of prospective cohort, one-arm	To investigate the efficacy of alternating screening mammography and breast MRI every 6 months in women with a genetically high risk of developing breast cancer for breast cancer detection	United States Women at increased genetic risk of breast cancer at single institution	Inclusion Women age ≥18 years, having undergone alternating screening mammography and breast MRI every 6 months at study institution, either confirmed *BRCA1/2* carriers or FDR of confirmed *BRCA1/2* carrier. Exclusion Women with history of breast cancer, who had calculated lifetime risk of breast cancer >20%, or who did not undergo a screening MRI, women who used chemoprevention or underwent bilateral prophylactic mastectomy, those with metastatic disease, undergoing treatment, or high BMI preventing MRI, women lost to followup, or died during original trial.	Based on BRCA status.
Rijnsburger et al, 2010[278] See also Kriege et al, 2004[277] NA Dutch MRISC study	Prospective cohort (Registry data/data from another prospective study used for cancer characteristics comparison)	To evaluate the long-term results of the Dutch MRI Screening (MRISC) study, including separate analyses of *BRCA1/2* mutation carriers and survival results	The Netherlands Women with increased familial or genetic predisposition for breast cancer attending academic and/or cancer centers at 6 sites	Inclusion Women ages 25 to 75 years with cumulative lifetime risk of breast cancer ≥15% due to genetic or familial predisposition (women could be tested before age 25 years if family member diagnosed before age 30). Exclusion Women with symptoms suggestive of breast cancer or who had a personal history of breast cancer; women proven not to have a mutation in a family with a proven mutation.	Based on cumulative lifetime risk determined using modified Claus tables: *BRCA1/2* carriers, or other mutations: 50%-85% risk. High-risk: 30%-50% risk. Moderate risk (no documented gene mutation): 15%-30% risk.
Ovarian Cancer					
Hermsen et al, 2007[281] NA	Prospective cohort, one-arm (staging compared to 2 external comparison groups; unscreened family members with cancer, combined data from multiple studies)	To assess efficacy of annual gynecological screening, accounting for compliance to protocol	The Netherlands Women with BRCA mutation screened at 6 University Family Cancer Clinics	Inclusion Women with *BRCA1/2* mutation screened at 1 of participating centers. Exclusion Women with symptoms at first visit, who had only 1 visit, or who were found to have cancer at first screening visit.	Based on BRCA status.

Author, year Quality	N	Baseline Demographics	Screening method and interval	Scoring criteria

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	N	Baseline Demographics	Screening method and interval	Scoring criteria
Breast cancer				
Cortesi et al, 2006[273] NA Modena Study Group for Familial Breast and Ovarian Cancer participants	1325 enrolled 48 mutation carriers (37 *BRCA1* and 11 *BRCA2*) 674 high risk 257 intermediate risk 346 slightly increased risk	Mean age at surveillance (range), years Carrier: 42 (20-75) High-risk: 42 (15-75) Intermediate risk: 43 (19-67) Slightly increased risk: 40 (18-75)	From 1994 to September 2000 all women underwent: A) Mammography B) Ultrasonography C) CBE D) Transvaginal ultrasound and serum CA 125 levels Testing interval varied by assessed risk (see below). From October 2000 mutation carrier surveillance modified to include: E) CE MRI BRCA risk: Started at age 25 with annual mammography and MRI, biannual CBE and ultrasound plus transvaginal ultrasound and serum CA 125 levels. High risk: Started at age 30 with mammography every 2 years until age 36 and then annually, biannual CBE and ultrasound plus annual transvaginal ultrasound and serum CA 125 levels. Intermediate risk: Started at age 30 with mammography every 2 years until age 40 and then annually, biannual CBE and ultrasound plus annual transvaginal ultrasound and serum CA 125 levels. Slightly increased risk: Started at age 30 with 1 mammogram < 40 years, then every 18 to 24 months, and annual CBE and ultrasound. Note: if possible, all exams performed on the same day during the 2nd week of the menstrual cycle in premenopausal women; additional investigation using fine needle aspiration or core biopsy performed as required.	
Leach, 2005[274] NAMARIBS study	649 analyzed 82 (13%) with known *BRCA1* mutation 38 (6%) with known *BRCA2* mutation	Median age at entry, years: 40 (range, 31-55; only 1 woman age >50 years)	All women underwent: A) Annual mammography from age 35 years (or younger if FDR developed cancer at age <35 years) B) Annual CE MRI Note: if possible, exams done on same day, between days 6 and 16 of menstrual cycle. Note: In women with equivocal results, high specificity MRI exam or repeat screening MRI done 2 to 6 weeks later, followed by ultrasound, fine needle aspiration, localization, and tissue sampling by conventional methods as appropriate. Note: 93% of mammographic examinations were 2-view, 7% 1-view.	Scoring system based on morphological and dynamic contrast uptake characteristics validated against histology (area under receiver operator curve=0.88 [95% CI, 0.83-0.94]) and diagnostic accuracy tested using subset of present study and 100 symptomatic cases (sensitivity, 91% [95% CI, 83-96]; specificity, 81% [95% CI, 79-83]). Note: All scoring was double reported; in statistical analysis, scoring system was paired to BIRADS as follows: for MRI; score of B, suspicious = BIRADS 0, 3, or 4 and score of A, malignant = BIRADS 5; for mammography; score M3, indeterminate = BIRADS 0-3, M4, suspicious = BIRADS 4, and M5, malignant = 5.

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	N	Baseline Demographics	Screening method and interval	Scoring criteria
Le-Petross et al, 2011[276] NA	321 screened 73 analyzed (37 [51%] *BRCA1*, 36 [49%] *BRCA2*)	Median age at entry, years: 44 (range, 23-75) Mean age at diagnosis, years: 51 (range, 43-64)	All women underwent CBE every 6 months plus: A) Mammography every 6 months alternating with B) MRI every 6 months Note: Ultrasound used to evaluate abnormal screen findings, biopsy as required.	BIRADS
Rijnsburger et al, 2010[278] See also Kriege et al, 2004[277] NA Dutch MRISC study	2275 enrolled 2157 analyzed (422 *BRCA1*, 172 *BRCA2*, 5 other mutation, 1069 high risk, 489 moderate risk)	Mean age at entry, years: Cohort: 40.1 (range, 19-75) *BRCA1*: 38.7 *BRCA2*: 40.0 High risk: 40.8 Moderate risk: 40.0	All women underwent: A) Biannual CBE B) Annual mammography C) Annual contrast enhanced MRI Note: Both imaging investigations performed on same day or time period when possible, between day 5 and 15 of menstrual cycle. Note: When 1 of the examinations reported "probably benign finding" or "need additional imaging evaluation" (BI-RADS 3 or 0), further investigation undertaken by ultrasonography. Malignancy diagnosis based on histological findings.	BIRADS
Ovarian Cancer				
Hermsen et al, 2007[281] NA	883 (683 *BRCA1*, 200 *BRCA2*) 459 for analysis of screening/ compliance (data available for all screen visits)	Median age, years: *BRCA1*: 40 (range, 21-76) *BRCA2*: 44 (range, 25-77)	All women underwent: A) Annual serum CA-125 measurement B) Annual TVUS Starting at age 35 years or 5 years earlier than youngest diagnosed ovarian cancer in the family Note: Biannual screens were done in some centers during the study period, but this was not systematically adopted.	CA-125: >35kU1-1 abnormal if resulted in extra screen visit or diagnostic operation. TVUS: Abnormal or normal.

Author, year Quality	Duration/ Followup	Outcome: Test characteristics	Cancer incidence
Breast Cancer			
Cortesi et al, 2006[273] NA Modena Study Group for Familial Breast and Ovarian Cancer participants	1992-2005 Median, 55 months (range, 1-151 months)	44 breast cancers detected; 64% (n=28) invasive, 36% (n=16) DCIS 36 screen-detected Carriers: n=5 cancers (4 invasive, 1 DCIS) High risk: n=23 (14 invasive, 9 DCIS) Intermediate risk: n=11 (8 invasive, 3 DCIS) Slightly increased risk: n=5 (2 invasive, 3 DCIS) <u>Sensitivity, A vs. B vs. A+B vs. E</u> All: 78% (28/36) vs. 50% (18/36) vs. 97% (35/36) vs. 100% (4/4) Carriers: 50% (2/4) vs. 75% (3/4) vs. 75% (3/4) vs. 100% (4/4) High risk: 90% (19/21) vs. 52% (11/21) vs. 100% (21/21) Intermediate risk: 50% (4/8) vs. 450% (4/8) vs. 100% (8/8) Slightly increased risk: 100% (3/3) vs. 0% (0/3) vs. 100% (3/3)	Breast cancer incidence in study population vs. expected incidence All: SIR, 4.9 (95% CI, 1.6-7.6), p<0.001 Carriers: SIR, 20.3 (95% CI, 3.1-83.9), p<0.001 High-risk: SIR, 4.5 (95% CI, 1.5-8.3), p<0.001 Intermediate risk: SIR, 7.0 (95% CI, 2.0-17.1), p=0.0018 Slightly increased risk: SIR not significantly increased Note: SIR=ratio of observed to expected number of cancers; expected number of cancers based on Modena Cancer Registry rates from 1998 to 2002 in 5-year age groups from 25 to >85 years; observed women-years at risk were multiplied by expected cancer incidence to estimate total number of cancers expected.

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Duration/ Followup	Outcome: Test characteristics	Cancer incidence
Leach, 2005[274] NAMARIBS study	Study recruitment 1997-2003 Variable screening episodes per individual but screening continued until each women had at least 2 annual scans (in 2004)	**All cancers (n=35)** Sensitivity (95% CI), A vs. B 40% (24-58) vs. 77% (60-90), p=0.01 A plus B: 94% (81-99) Specificity (95% CI), A vs. B 93% (92-95) vs. 81% (80-83), p<0.0001 A plus B: 77% (75-79) PPV (95% CI), A vs. B 10% (5.8-17) vs. 7.3% (4.9-10) NPV (95% CI), A vs. B 99% (98-99) vs. 99% (99-100) Area under receiver operator curve, A vs. B 0.70 (0.68-0.72) vs. 0.85 (0.84-0.87), p=0.035 **Excluding DCIS (n=6)** Sensitivity (95% CI), A vs. B 31% (15-51) vs. 86% (68-96), p=0.0009 A plus B: 97% (82-100) ***BRCA1* carriers or relative with *BRCA1* mutation (n=139)** Sensitivity (95% CI), A vs. B 23% (5-54) vs. 92% (64-100), p=0.004 A plus B: 92% (64-100) Excluding 1 DCIS case: 25% (5.5-57) vs. 100% (74-100) Specificity (95% CI), A vs. B 92% (88-94) vs. 79% (75-83), p<0.0001 A plus B: 74% (69-78) PPV (95% CI), A vs. B 9.1% (1.9-24) vs. 14% (7.2-23) ***BRCA2* carriers or relative with *BRCA2* mutation (n=86)** Sensitivity (95% CI), A vs. B 50% (21-79) vs. 58% (28-84), p=1.0 A plus B: 92% (62-100) Excluding 3 DCIS cases: 33% (7.5-70) vs. 67% (30-93), p=0.45 Specificity (95% CI), A vs. B 94% (91-97) vs. 82% (77-87), p=0.0001 A plus B: 78% (72-83) PPV (95% CI), A vs. B 9.1% (1.9-24) vs. 14% (7.2-23) Note: Anonymous testing was restricted to women with breast cancer so that women with BRCA-positive relatives but no breast cancer themselves were not tested; sensitivities refer only to tested mutation carriers, specificities are only preliminary estimates. **Incident screens (n=15 cancers, n=1217 noncancers); observed incidence rate was 1.9% per year** Sensitivity (95% CI), A vs. B Any cancer: 40% (16-68) vs. 80% (52-96), p=0.11	15 incident cancers, observed incidence rate was 1.9% per year

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Duration/ Followup	Outcome: Test characteristics	Cancer incidence
		Excluding 6 DCIS cases: 31% (15-51) vs. 86% (68-96), p=0.0009 A plus B: 97% (82-100) Any cancer, excluding *BRCA1* carriers/relatives: 50% (28-72) vs. 68% (45-86), p=0.45 Any cancer, excluding *BRCA2* carriers/relatives: 35% (16-57) vs. 87% (66-97) A plus B: 96% (78-100) Specificity (95% CI), A vs. B All cancers: 94% (92-95) vs. 81% (79-83), p<0.0001	
Le-Petross et al, 2011[276] NA	Records from 1997-2009 Median followup, 2 years (range, 1-6 years) Median number of screening cycles, 2 (range, 1-6 cycles); 29% completed 1 cycle, 31% completed 2 cycles, 25% completed 3 cycles, 15% completed 4, 5, or 6 cycles	Sensitivity, (95% CI), A vs. B Not able to report vs. 92% (0.76-1.00) Specificity, (95% CI), A vs. B 82% (0.72-0.92) vs. 87% (0.79-0.95) 12/13 cancers identified on MRI (1/13 on prophylactic mastectomy), but not mammography 6 months prior; no cancer detected by mammography alone; no cancer palpable by CBE 5/13 cancers detected on targeted ultrasound post MRI detection	13 cancers detected (10 invasive, 3 DCIS) in 11 patients 5/13 cancers detected on first screening cycle (likely prevalent), 8/13 incident cancers Number of cancers detected by cycle in 11 patients: Post cycle 1: 5 cancers Post cycle 2: 2 cancers Post cycle 3: 3 cancers Post cycle 4: 1 cancer
Rijnsburger et al, 2010[278] See also Kriege et al, 2004[277] NA Dutch MRISC study	1999-2006 Median, 4.9 years; mean, 4.0 years (range, 0.1 to 6.3 years); followup post diagnosis for mortality Relapse: Median, 5.0 years (range, 1.7-8.4 years)	Number of screen-detected breast cancers; total, invasive, DCIS *BRCA1*: 21/35, 19/31, 2/4 *BRCA2*: 15/18, 12/13, 3/5 Other mutation: 1/5, 0/0, 1/1 High risk: 26/27, 22/23, 4/4 Moderate risk: 15/16, 11/11, 4/5 Total: 78/97, 64/78, 14/19 Screening method comparisons based on 75 breast cancers with data that included results for both imaging methods Sensitivity (95% CI), A vs. B vs. C Any breast cancer: 21% (12-32) vs. 41% (30-53) vs. 71% (59-81), p=0.0016 for B vs. C Invasive: 22% (11.8-32) vs. 36% (24-49) vs. 77% (65-87), p<0.00005 for B vs. C DCIS: 15% (1.9-45) vs. 69% (39-91) vs. 39% (14-68), p=0.388 for B vs. C	Incidence of cancer per population group; total, invasive, DCIS *BRCA1*: 35, 31, 4 *BRCA2*: 18, 13, 5 Other mutation: 5, 0, 1 High risk: 27, 23, 4 Moderate risk: 16, 11, 5 Total: 97, 78, 19

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Duration/ Followup	Outcome: Test characteristics	Cancer incidence
		Mutation (any breast cancer) *BRCA1*: 13% (2.8-34) vs. 25% (9.8-47) vs. 67% (45-84), p=0.0129 for B vs. C *BRCA2*: 7.7% (0.2-36) vs. 62% (33-86) vs. 69% (39-91), p=1.0 for B vs. C Risk group (any breast cancer) High: 32% (13-56) vs. 46% (24-68) vs. 77% (55-92) Moderate: 33% (9.9-65) vs. 47% (21-73) vs. 67% (38-88) *BRCA1* vs. *BRCA2* sensitivity of methods compared: Mammography, p =0.04; all other comparisons between groups and screening methods were nonsignificant. Specificity of methods did not differ between groups. Specificity (95% CI), A vs. B vs. C Any breast cancer: 98% (97.5-98.2) vs. 95 (94.0-95.1) vs. 90 (88.9-90.4) Mutation (any breast cancer) *BRCA1*: 97% (95.7-97.9) vs. 95% (93.0-95.9) vs. 91% (89.1-92.6) *BRCA2*: 98% (96.4-99.4) vs. 94% (90.9-96.0) vs. 92% (88.7-94.5) Risk group (any breast cancer) High: 98% (97.7-98.7) vs. 95% (93.8-95.3) vs. 89% (87.9-90.1) Moderate: 98% (96.9-98.6) vs. 95% (93.5-95.9) vs. 90% (87.8-91.0) PPV (95% CI), A vs. B vs. C Any breast cancer: 10% (5.7-17) vs. 8.5% (5.8-12) vs. 7.7% (5.8-9.9) Mutation (any breast cancer) *BRCA1*: 8.8% (1.8-24) vs. 9.5% (3.6-20) vs. 14% (8.5-22) *BRCA2*: 14% (0.4-58) vs. 26% (12-45) vs. 23% (11-39) Risk group (any breast cancer) High: 9.8% (3.7-20) vs. 5.3% (2.6-9.5) vs. 4.5% (2.6-7.1) Moderate: 12% (3.4-28) vs. 8.5% (3.5-17) vs. 6.2% (3.0-11)	
Ovarian Cancer			
Hermsen et al, 2007[281] NA	1993-2005 1473 person-years	15 cancers diagnosed in cohort Based on 459 women with data on each visit: 7 cancers diagnosed (2 prevalent, 2 interval, 3 incident) Sensitivity (95% CI), A vs. B vs. A+B All cancers: 42% (14-70) vs. 25% (1-50) vs. 42% (14-70) Excluding occult cancers: 71% (38-100) vs. 43% (6-80) vs. 71% (38-100) Specificity (95% CI), A vs. B vs. A+B All cancers: 99% for all (CI range, 98-100) Excluding occult cancers: 99% for all (CI range, 98-100) PPV (95% CI), A vs. B vs. A+B All cancers: 33% (9-57) vs. 20% (0-40) vs. 23% (5-40) Excluding occult cancers: 33% (9-57) vs. 20% (0-40) vs. 23% (5-40) NPV (95% CI), A vs. B vs. A+B All cancers: 99% (99-100) for all	10 cancers diagnosed during followup 5 screen-detected 6.5 cases expected Based on 459 women with data on each visit: 7 cancers diagnosed (2 prevalent, 2 interval, 3 incident) SIR (95% CI) Overall: 1.5 (0.7-2.8) *BRCA1*: 1.7 (0.8-3.1) *BRCA2*: unable to estimate, no event observed Optimally screened women-years (interval between screen visits <13 months): 1.6 (0.5-3.6) Note: Expected number of cases based on data from population-based studies of breast cancer cases, families of *BRCA1/2* carriers; SIR=expected/observed cases based on reference curves derived from refitting BOADICEA

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Duration/ Followup	Outcome: Test characteristics	Cancer incidence
		Excluding occult cancers: 100% for all (CI range, 99-100)	model of genetic susceptibility to breast cancer and including data from population-based studies of breast cancer families and cases.

Author, year Quality	Outcome: Cancer characteristics Interval cancers	Outcome: Disease-free survival Mortality	Conclusions	Funding source
Breast Cancer				
Cortesi et al, 2006[273] NA Modena Study Group for Familial Breast and Ovarian Cancer participants	Staging: 61% (n=17) stage I; 25% (n=7) stage II; 7% (n=2) stage III; 7% (n=2) stage IV Size: 29% (n=8) <10 mm in diameter; 36% (n=10) were 10-15 mm in diameter; 32% (n=9) >15 mm in diameter; 1 was inflammatory breast cancer Nodal status: 36% (n=10) node positive Interval cancers: n=8, all identified with CBE; interval cancer rate, 1.3 per 1000; diagnosed with CBE only (n=4); CBE plus ultrasound (n=3); CBE plus ultrasound plus mammography (n=1); time interval from last negative screen to diagnosis ranged from 1 to 14 months DCIS: Screening sensitivity for DCIS increased with age; low rate (65%) in women <50 years; high rate (93%) in oldest age group	Post treatment, 4 recurrences and 3 deaths (2 for disease progression, 1 from heart failure). Actuarial 5 year survival rate was 93%.	Rate of cancer detected in women at high risk for breast cancer was significantly higher than expected in an age-matched general population. Results support increased screening surveillance program to identify and monitor high-risk individuals.	Italian consortium for Hereditary Breast and Ovarian Cancer; COFIN-MURST 2003-2005; Faondazione Cassa di Risparmio di Modena; Associazione Angela Serra per la ricerca sul Cancro
Leach, 2005[274] NAMARIBS study	Grade: 10% (3/29) grade 1; 24% (7/29) grade 2; (66%) 19/29 grade 3 Size: 38% (11/29) were <10 mm in greatest dimension; 14% (4/29) were 10-14 mm in greatest dimension; 17% (5/29) were 15-19 mm; 31% (9/29) were ≥20 mm in greatest dimension; average tumor size = 15 mm Nodal status: 81% (21/26) cancers node-negative Interval cancers: n=2 (1 considered benign on MRI and 1 considered benign on mammography; method of detection NR)	NR	Contrast-enhanced MRI is more sensitive than mammography for breast cancer detection in women with familial risk for breast cancer. Specificity was acceptable for both. Detected tumors were small and mostly node negative, suggesting that annual screening with mammography and contrast-enhanced MRI would detect most tumors in this risk group.	Grant from U.K. Medical Research Council; MRI cost paid from subvention funding for research from U.K. National Health Service
Le-Petross et al, 2011[276] NA	Size on MRI: Mean, 14 mm (range, 1-30 mm) Nodal status: 9% (1/11) women node-positive Interval cancers: n=0	NR	Screening women at increased genetic risk of breast cancer by alternating mammography with MRI every 6 months has a higher cancer yield than	NR

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Outcome: Cancer characteristics Interval cancers	Outcome: Disease-free survival Mortality	Conclusions	Funding source
			studies that screened using both modalities at the same time point.	
Rijnsburger et al, 2010[278] See also Kriege et al, 2004[277] NA Dutch MRISC study	<u>Characteristics of detected breast cancer (includes 78 screen-detected cancers and 11 interval cancers)</u> Tumor size: 40% (30/76) <1 cm, 39% (29/76) 1-2 cm, 20% (15/76) >2 cm; p1=0.003, p2=0.0045 Nodal status negative: 69% (50/72); p1=0.42, p2=1 Histology: 29% (21/72) grade 1, 32% (23/72) grade 2, 39% (28/72) grade 3; p1<0.001, p2=0.15 p1=overall comparison between subgroups p2=comparison between *BRCA1* and *BRCA2* Note: Age at diagnosis, number of interval cancers, and estrogen and progesterone receptor status significantly different between subgroups <u>Number of interval cancers (total, invasive, DCIS)</u> *BRCA1*: 10/35, 10/31, 0/4 *BRCA2*: 1/18, 1/18, 0/5 Other mutation: 0/0, 0/0, 0/0 High risk: 1/27, 1/23, 0/4 Moderate risk: 1/16, 0/11, 1/5 Total: 13/97, 12/78, 1/19 Note: denominator includes 6 breast cancers detected at prophylactic mastectomy <u>Kriege 2004 breast cancer characteristics, study group vs. control 1 vs. control 2 (based on 50 screen-detected cancers in study group, 1500 in control group 1, and 45 in control group 2)</u> Number of DCIS: 6 vs. 120 vs. 0 Invasive tumor size ≤1 cm: 19/44 vs. 193/1380 vs. 5/45; p<0.001 vs. control 1, p<0.04 vs. control 2 Nodal status negative: 28/44 vs. 657/1380 vs. 17/45; p<0.001 vs. control 1, p=0.001 vs. control 2 Histological grade 1: 19/44 vs. 99/1380 vs. 4/45; p<0.001 vs. control 1, p=0.01 vs. control 2 Note: Control 1 = National Cancer Registry data of women with breast cancer diagnosed in 1998; Control 2 = participants diagnosed with breast cancer between 1996 and 2002 participating in a prospective study of gene mutation.	<u>Disease-free and overall survival in 89 patients</u> 11/93 patients with breast cancer had relapse, 7/11 were mutation carriers 5 patients had distant metastasis, all were mutation carriers 4 patients died, 3/31 (9.7%) *BRCA1* and 1/16 (6.3%) *BRCA2* Cumulative metastasis-free and overall survival at 6 years in 43 mutation carriers with invasive cancer were 84% and 93%; other groups had 100% cumulative survival	Sensitivity of MRI superior to mammography for detection of breast cancer in women at increased risk. *BRCA1*-associated cancers have younger age at diagnosis, lower mammographic sensitivity, high number of interval cancers, low number of DCIS, and unfavorable tumor size at diagnosis.	Dutch government; Cancer Genomics Center

Appendix C10. Evidence Table of Intensive Screening Interventions

Author, year Quality	Outcome: Cancer characteristics Interval cancers	Outcome: Disease-free survival Mortality	Conclusions	Funding source
Ovarian Cancer				
Hermsen et al, 2007[281] NA	Stage: 80% (8/10) stage III/IV (4/5 incident, 4/5 interval cancers) vs. 77% (20/26) in unscreened family members with cancer Interval cancers: n=5	After mean followup of 28 months from diagnosis: 3/15 cases died of ovarian cancer	Annual screening with TVUS and serum CA-125 is an ineffective method for detecting ovarian cancer in women at increased risk due to family history.	Biocare Foundation

*Incident plus interval cancer.

Abbreviations: BIRADS = Breast Imaging- Reporting and Data System; BMI = body mass index; CA-125 = cancer antigen-125; CBE = clinical breast examination; CE = contrast enhanced; CI = confidence interval; DCIS = ductal carcinoma in situ; FDR = first-degree relative; MRI = magnetic resonance imaging; MRISC = Magnetic Resonance Imaging Screening Study; NA = not applicable; NPV = negative predictive value; NR = not reported; PPV = positive predictive value; SIR = standardized incidence ratio; TP53 = tumor protein 53; TVUS = transvaginal ultrasound; US = ultrasound.

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Design	Purpose	Intervention	Country/population/ setting	Inclusion/exclusion criteria
Tamoxifen vs. placebo					
IBIS-I[287] Fair See also Cuzick 2002[328]	RCT	To report the updated analysis of IBIS-I, focusing on the period after active treatment was completed	Oral tamoxifen 20 mg/day or placebo Groups directly compared, no expected incidence rates but baseline risk assessed using complex model for 10-year risk of ≥5%	United Kingdom (60% of participants), Europe, Australia, and New Zealand (37% of participants) Women at increased risk of breast cancer Recruited from family history clinics, relatives of women with breast cancer, breast screening centers, general practitioners, and the media	Inclusion Women had to have risk factors for breast cancer indicating ≥2-fold RR if they were ages 45 to 70 years, 4-fold RR if ages 40 to 44 years, or 10-fold RR if ages 35 to 39 years. Specifically, women were eligible from age 45 years if they had 1) mother or sister diagnosed with breast cancer before age 50 years, 2) 2 FDRs or SDRs with breast cancer at any age, or 3) FDR with breast cancer at any age, and were nulliparous or had previous hyperplastic benign lesion. Women were eligible from age 40 years if they had 1) atypical ductal or lobular hyperplasia, 2) FDR with bilateral breast cancer at any age, 3) 2 FDRs or SDRs with breast cancer, 1 of whom was diagnosed before age 50 years. Women were eligible from age 35 years if they had either 1) lobular carcinoma in situ or 2) 2 FDRs with breast cancer, both diagnosed before the age of 50 years. Any woman with estimated 10-year risk of ≥5% based on complex model was eligible after approval by study chairman. Exclusion Women with any previous invasive breast cancer, aside from nonmelanoma skin cancer, previous deep vein thrombosis or pulmonary embolism, current users of anticoagulants, or those wishing to become pregnant.
NSABP P-1[284] Fair See also Fisher et al, 1998[71]	RCT	To update the findings from the NSABP P-1 Trial after 7 years of followup	Oral tamoxifen 20 mg/day vs. placebo Note: 2 groups compared directly, no expected incidence rates	United States and Canada Women at increased risk for breast cancer Recruited through 133 clinical centers	Inclusion Women at increased risk for breast cancer due to 1) age ≥60 years, 2) ages 35 to 59 years with 5-year predicted risk of ≥1.66% by Gail model, 3) history of lobular carcinoma in situ, as well as 10 years of life expectancy, no clinical or mammographic evidence of breast cancer, not pregnant and not planning on becoming pregnant during study, normal white blood cell and platelet counts, normal hepatic and renal function, available for followup, have undergone endometrial sampling. Exclusion Women who had taken hormone replacement therapy, oral contraception, or androgens within 3 months of randomization; history of DVT or pulmonary embolism.

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Design	Purpose	Intervention	Country/population/ setting	Inclusion/exclusion criteria
Royal Marsden[285] Fair See also Powles et al, 1998[70]	RCT	To identify any long-term prevention of breast cancer associated with tamoxifen treatment after 20 years of followup of the Royal Marsden trial	Oral tamoxifen 20 mg/day or placebo Note: 2 groups compared directly, no expected incidence rates	United Kingdom Women at increased risk of breast cancer Recruited from breast clinics at Royal Marsden Hospital	Inclusion Healthy women ages 30 to 70 years, with no clinical or screening evidence of breast cancer; at increased risk of breast cancer because of family history; with 1) ≥1 FDR age <50 years at breast cancer diagnosis, 2) 1 FDR with bilateral breast cancer, or 3) 1 FDR with breast cancer diagnosed at any age plus ≥1 other affected FDR or SDR with breast cancer; personal history of benign breast biopsy and FDR with breast cancer and those using hormone replacement therapy also eligible. Exclusion Women with history of any cancer, DVT, or pulmonary embolism; risk of pregnancy; or using oral contraceptives.
Italian Randomized Tamoxifen Prevention[286] Fair See also Veronesi et al, 1998[72]	RCT	To update the results of the Italian Randomized Tamoxifen Prevention Trial after 11 years of followup, focusing on the occurrence of breast cancer	Oral tamoxifen 20 mg/day vs. placebo Note: 2 groups compared directly, no expected incidence rates	Italy (97% of patients), South America, Greece Healthy women at average risk for breast cancer Recruited via national advertising and through gynecologists	Inclusion Healthy women ages 35 to 70 years who had a hysterectomy for reasons other than neoplasm. Exclusion Women with severe concurrent illness or history of cardiac disease, endometriosis, and suspected or certain previous DVT.
Raloxifene vs. placebo					
RUTH[73] Good See also Barrett-Connor et al, 2006[299]	RCT	To provide further details about breast cancer incidence by tumor characteristics, duration of treatment, and subgroup in the RUTH trial	Oral raloxifene 60 mg/day vs. placebo Note: 2 groups compared directly, no expected incidence rates (5-year risk of invasive breast cancer at baseline based on Gail model)	Multinational Postmenopausal women at increased risk of coronary events Recruited through 177 sites in 26 countries, including the U.S.	Inclusion Women age ≥55 years; ≥1 year from final menstrual period; with documented coronary heart disease or increased risk for coronary heart disease based on risk factors (older age, diabetes, hypertension, smoking, hyperlipidemia). Exclusion Women suspected of having breast cancer or those with a history of breast cancer; recent myocardial infarction, coronary artery bypass grafting or percutaneous coronary angioplasty, or severe heart failure; history of venous thromboembolism; recent unexplained uterine bleeding; life expectancy <5 years; chronic liver or renal disease; recent use of oral or transdermal estrogens or current use of sex hormones or SERMs.

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Design	Purpose	Intervention	Country/population/ setting	Inclusion/exclusion criteria
MORE and CORE[288] Good See also Cummings et al, 1999[74]	RCT	To assess the effect of raloxifene, indicated for osteoporosis treatment and prevention, on invasive breast cancer in subgroups of postmenopausal women by defined risk factors for breast cancer	MORE: Oral raloxifene 60 or 120 mg/day vs. placebo CORE: Oral raloxifene 60mg/day vs. placebo Note: 5-year predicted risk based on the modified Gail model score at baseline of CORE trial per each woman's risk factors	Multinational Postmenopausal women with osteoporosis Recruited from 180 clinical centers in 25 countries, including the U.S.	Inclusion Women age ≤80 years; ≥2 years postmenopausal; with documented osteoporosis. Exclusion Women with a history of breast cancer, invasive endometrial cancer, stroke, or venous thromboembolism in the preceding 10 years. Note: only eligibility requirement for CORE was to have been enrolled in MORE trial.
Tamoxifen vs. raloxifene					
STAR[289] Good See also Vogel et al, 2006[329]	RCT	To update the findings from the STAR trial	Oral tamoxifen 20 mg/day vs. oral raloxifene 60 mg/day Note: expected breast cancer incidence rates based on Gail model of risk per woman's risk factors	United States and Canada Women with increased risk for breast cancer Recruited from nearly 200 clinical centers	Inclusion Women age ≥35 years; postmenopausal; 5-year predicted breast cancer risk ≥1.7% (per Gail model); not taking tamoxifen, raloxifene, hormone therapy, oral contraceptives, or androgens for ≥3 months before randomization; not currently on warfarin or cholestyramine; no history of stroke, transient ischemic attack, pulmonary embolism, or DVT; no atrial fibrillation; no uncontrolled diabetes or uncontrolled hypertension; no psychiatric condition that would interfere with adherence; performance status that would not restrict normal activity; no history of previous malignancy except basal cell or squamous cell carcinoma of the skin, carcinoma in situ of the cervix, or LCIS of the breast.

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Assignment/attrition	Demographics	Surveillance	Duration/follow up
Tamoxifen vs. placebo				
IBIS-I[287] Fair See also Cuzick 2002[328]	Tamoxifen vs. placebo 7154 randomized: 3579 vs. 3575 4861 (68%) completed 5 years of treatment: 2287 (64%) vs. 2574 (72%) Approximately 85% of women returned ≥1 questionnaire during posttreatment followup	Tamoxifen vs. placebo Mean (SD) age, years: 50.7 (7.0) vs. 50.8 (6.7) 3913 (55%) ages 45 to 54 years Family history: 6939 (97%) of women reported some family history of breast cancer Cuzick 2002 FDR with breast cancer at age ≤50 years: 1689/3573 (47%) vs. 1744/3566 (49%) FDR with bilateral breast cancer: 579/3573 (16%) vs. 601/3566 (17%) ≥2 FDRs or SDRs with breast cancer: 2204/3573 (62%) vs. 2206/3566 (62%)	During treatment, women followed every 6 months by clinic visit or phone call. Compliance measured by pill counts at each 6 month visit. Posttreatment, followed by annual mailed questionnaire for women in U.K. and Europe or annual clinic visits for women in Australia and New Zealand.	5 years of treatment Median followup was 95.6 months
NSABP P-1[284] Fair See also Fisher et al, 1998[71]	Tamoxifen vs. placebo 57,641 approached 14,453 agreed to be medically evaluated for eligibility 13,954 met eligibility requirements 13,388 randomized; 6681 vs. 6707 13,207 had followup and were included in analysis; 6597 vs. 6610 Note: withdrawal rate between year 6 and 7 of followup was higher in the placebo vs. tamoxifen group, resulting in different amounts of information for groups during this period	Age distribution at randomization: 39% ages 35-49 years 31% ages 50-59 years 30% age ≥60 years FDRs with breast cancer, n (tamoxifen vs. placebo): None: 1548 (26%) vs. 1597 (24%) 1: 3763 (57%) vs. 3738 (57%) 2: 1072 (16%) vs. 1094 (17%) ≥3: 214 (3.2%) vs. 181 (2.7%)	NR	5 years of treatment Mean followup was 6.2 years
Royal Marsden[285] Fair See also Powles et al, 1998[70]	2508 consented 14 withdrew consent prior to randomization 1250 randomized to tamoxifen, 12 excluded from analysis (all previous DCIS) 1238 analyzed 1244 randomized to placebo, 11 excluded from analysis (10 previous DCIS, 1 invasive cancer) 1233 analyzed Note: self-reported compliance was 8% less in the tamoxifen arm vs. placebo (p=0.002); difference seen at 1 year and remained constant over treatment period	Tamoxifen vs. placebo Median age, years (range): 47 (31-70) vs. 47 (30-70) Age <50 years: n=774 vs. 749 FDRs or SDRs with breast cancer, n: 0/not known: 8 vs. 10 1: 373 vs. 372 2: 476 vs. 496 3: 257 vs. 228 4: 81 vs. 82 ≥5: 43 vs. 45 Note: no significant differences between groups	Followup visits every 6 months with clinical breast exam and assessment for acute toxicity. Data forms completed at each visit. Medical problems and changes to family history were recorded at each visit. Mammography done annually.	8 years of treatment Median followup was 158 months

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Assignment/attrition	Demographics	Surveillance	Duration/follow up
Italian Randomized Tamoxifen Prevention[286] Fair See also Veronesi et al, 1998[72]	Tamoxifen vs. placebo 13,419 recruited 4,989 refused 1499 ineligible 527 not contactable 996 missing 5408 randomized: 2700 vs. 2708 2119 withdrew: 1085 vs. 1034 (56 for ineligibility, 99 due to major changes in protocol, 394 for major adverse events, 1407 voluntarily, 154 lost to followup, 9 deaths) 3289 completed 5 years of treatment: 1615 vs. 1674	Median age at entry: 51 years FDRs with breast cancer, n: None: 2359 (87%) vs. 2407 (89%) ≥1: 341 (13%) vs. 301 (11%) Note: no differences between groups on any baseline characteristics	During treatment, women had a physical and blood tests every 6 months and mammography annually. After trial completion, or in case of dropout, women were followed annually. Information about major endpoints (death, adverse events, cancer diagnosis) continuously collected.	Mean duration of treatment, 4.2 years Mean followup, 9.1 years (cancers other than breast endpoint) 11.2 years (breast cancer endpoint)
Raloxifene vs. placebo				
RUTH[73] Good See also Barrett-Connor et al, 2006[299]	Raloxifene vs. placebo 10,101 enrolled and randomized: 5044 vs. 5057 Completed 5 years of followup: 4060 (80%) vs. 3979 (79%) Note: 71% of placebo and 70% of raloxifene group took 70% of study medication based on pill counts	Raloxifene vs. placebo Mean age at baseline, years (SD): 67.5 (6.6) vs. 67.5 (6.7) Family history of breast cancer, n: 452 (9.8%) vs. 445 (9.7%) Note: no differences between groups on any baseline characteristics Note: Unable to determine number without family history per group because of missing values not accounted for	Breast cancer risk assessment at baseline. Clinical breast exam at baseline and every 2 years after. Mammogram within 1 year of randomization and every 2 years after. Participants attended study visits or contacted by telephone semiannually to assess adherence, adverse events, and outcomes of interest.	Median exposure to drug was 5.05 years Median followup, 5.6 years (analysis of data collected before February 2006)
MORE and CORE[288] Good See also Cummings et al, 1999[74]	7705 randomized in MORE: 2557 to raloxifene 60 mg/day, 2572 to raloxifene 120 mg/day, 2567 to placebo 4011 enrolled in CORE: 2725 to raloxifene 60 mg/day, 1286 to placebo	Characteristics at beginning of MORE Age ≥65 years, n (%): 4621/7705; 2563 (60%) of combined raloxifene groups; 1550 (60%) of placebo group Age <65 years, n (%): 3084 total; 2058 (40%) of combined raloxifene groups; 1026 (40%) of placebo group Family history of breast cancer, n (%): 949/7705; 636 (13%) of combined raloxifene groups; 313 (12%) of placebo group Note: no significant differences between groups at baseline in MORE or CORE	MORE: Mammograms at baseline, 2, 3, 4 years and optional at year 1. Biannual study visits for clinical breast exam and questions about breast cancer diagnosis, biopsy, surgery since last visit. CORE: Mammograms within 1 year of study entry and at 2 and 4 years. Annual study visits for clinical breast exam and questions about breast cancer diagnosis, biopsy, surgery since last visit.	4 years of treatment in MORE, 4 years in CORE (median time from end of MORE to enrollment in CORE, 10.6 months (range, 2.6-62 months); mean time from randomization in MORE to end of CORE, 7.8 years (includes period between trials)

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Assignment/attrition	Demographics	Surveillance	Duration/follow up
Tamoxifen vs. raloxifene				
STAR[289] Good See also Vogel et al, 2006[329]	184,460 screened; 88,092 excluded for breast cancer risk <1.7% 96,368 had breast cancer risk ≥1.7%; 20,616 consented to screening for medical eligibility 20,168 met eligibility criteria; 421 did not want to participate 19,747 randomized; 9872 assigned to tamoxifen; 9875 assigned to raloxifene 19,471 original analysis (274 lost to followup; 146 in tamoxifen group, 128 in raloxifene group; 2 excluded for bilateral mastectomy prior to randomization) 19,490 update analysis; 9736 in tamoxifen group; 9754 in raloxifene group (followup collected on 20 women missing from original; 1 excluded due to breast cancer diagnosis before randomization) Note: adherence to 5 years of therapy was within study limits; since unblinding (April 2006), women who had not completed 5-year course of tamoxifen were offered option to switch to raloxifene for remaining portion of treatment course, which 879 women did	Characteristics at entry of women included in the STAR update analysis Mean age, years: 58.5 (SD 7.4) Age distribution: 9% <50 years; 50% ages 50-59 years; 32% ages 60-69 years; 8.8% age ≥70 years FDRs with breast cancer, n (%); tamoxifen vs. raloxifene: None: 2838 (29) vs. 2791 (27) 1: 5046 (52) vs. 5135 (53) 2: 1532 (16) vs. 1561 (16) ≥3: 320 (3.3) vs. 267 (2.7)	Followup occurred at 6 months after treatment initiation and every 6 months thereafter for 5 years. After 5 years, followup occurred annually. Biannual clinical breast exam and annual mammography. Annual gynecological examinations, complete blood count, routine serum chemistry tests. Outcomes assessed at each visit and verified with medical reports when applicable.	Mean duration of treatment was 43.5 months (SD, 20.7) for tamoxifen group and 46.8 months (SD, 20.0) for raloxifene group Median followup, 81 months (analysis cutoff March 2009)

Trial Quality	Results	Conclusions	Funding source
Tamoxifen vs. placebo			
IBIS-I[287] Fair See also Cuzick 2002[328]	Number of events and rate of breast cancers; tamoxifen vs. placebo: Total breast cancers: 142 vs. 195; rate,* 4.97 vs. 6.82; RR, 0.73 (95% CI, 0.58-0.91) Invasive breast cancers: 124 vs. 168; rate,* 4.34 vs. 5.88; RR, 0.74 (95% CI, 0.58-0.94) DCIS: 17 vs. 27; rate,* 0.60 vs. 0.94; RR, 0.63 (95% CI, 0.32-1.20)	Risk reducing effect of tamoxifen persists after ≥10 years of followup in a cohort of women, in which 97% reported some family history of breast cancer.	Cancer Research United Kingdom; National Health and Medical Research Council Australia

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Results	Conclusions	Funding source
NSABP P-1[284] Fair See also Fisher et al, 1998[71]	Tamoxifen vs. placebo Number and rates* of invasive breast cancer by number of FDRs with breast cancer: None: 33 vs. 62; rate,* 3.48 vs. 6.47; difference, 2.99; RR, 0.54 (95% CI, 0.34-0.83) 1: 73 vs. 124; rate,* 3.16 vs. 5.52; difference, 2.36; RR, 0.57 (95% CI, 0.42-0.77) 2: 32 vs. 52; rate,* 4.91 vs. 7.84; difference, 2.93; RR, 0.63 (95% CI, 0.39-0.99) ≥3: 7 vs. 12; rate,* 5.48 vs. 11.24; difference, 5.76; RR, 0.49 (95% CI, 0.16-1.34)	In women with FDRs with breast cancer, tamoxifen reduced the incidence of invasive breast cancer versus placebo; statistically significant reduction of risk for those with 1 or 2 FDRs with breast cancer, nonsignificant with ≥3 relatives	National Cancer Institute; U.S. Department of Health and Human Services
Royal Marsden[285] Fair See also Powles et al, 1998[70]	Tamoxifen vs. placebo Invasive ER-positive breast cancer events and rate* by family history, number of relatives: 0-2: 14 vs. 28; rate, 2.7 vs. 5.3; HR, 0.51 (95% CI, 0.27-0.96); p=0.04 ≥3: 9 vs. 19; rate, 3.9 vs. 9.1; HR, 0.43 (95% CI, 0.19-0.95); p=0.04 P for interaction (between tamoxifen and placebo, after adjusting for menopausal status at randomization, HT use during treatment) = 0.004	In women with a family history of breast cancer (FDRs or SDRs with breast cancer), less invasive ER-positive breast cancer observed in tamoxifen arm than in placebo arm; statistically significant	National Health Service; Cancer Research U.K.
Italian Randomized Tamoxifen Prevention[286] Fair See also Veronesi et al, 1998[72]	Tamoxifen vs. placebo Number and rates of breast cancer by number of FDRs with breast cancer: None: 46 vs. 64; rate,* 1.75 vs. 2.41; difference, 0.66; RR, 0.73 (95% CI, 0.50-1.06) ≥1: 16 vs. 10; rate,* 4.29 vs. 3.00; difference, -1.29; RR, 1.43 (95% CI, 0.65-3.15)	In women with ≥1 FDR with breast cancer, more breast cancer observed in tamoxifen arm than in placebo arm; not statistically significant difference	Italian National Research Council; Italian Foundation for Cancer Research; American-Italian Cancer Foundation; Italian League Against Cancer
Raloxifene vs. placebo			
RUTH[73] Good See also Barrett-Connor et al, 2006[299]	Raloxifene vs. placebo Number of cases (annualized rate†, %) of invasive breast cancer by family history: No: 29 (0.13) vs. 53 (0.25); HR, 0.53 (95% CI, 0.34-0.84) Yes: 8 (0.34) vs. 9 (0.39); HR, 0.89 (95% CI, 0.34-2.31) P for interaction=0.34	In women with a family history of breast cancer (FDR with breast cancer), raloxifene reduced the incidence of invasive breast cancer versus placebo; nonsignificant reduction	Eli Lilly and Company

Appendix C11. Evidence Table of Risk-Reducing Medications

Trial Quality	Results	Conclusions	Funding source
MORE and CORE[288] Good See also Cummings et al, 1999[74]	Raloxifene vs. placebo Number of cases, incidence rates, and risk reduction of invasive breast cancer by family history: No: 36 (0.8%) vs. 42 (1.9%); rate†, 15 vs. 35; absolute risk reduction, 20 Yes: 3 (0.5%) vs. 13 (4.2%); rate†, 9 vs. 81; absolute risk reduction, 72 Risk for invasive breast cancer in women receiving raloxifene vs. placebo by family history: No: HR, 0.42 (95% CI, 0.27-0.66) Yes: HR, 0.11 (95% CI, 0.03-0.38) p=0.04 for interaction between family history of breast cancer and treatment Adjusted risk for invasive breast cancer in women receiving raloxifene by family history: No: HR, 0.55 (95% CI, 0.36-0.84); p=0.005 Yes: HR, 0.16 (95% CI, 0.06-0.42); p<0.001	Raloxifene was associated with significantly lower incidence of invasive breast cancer over 8 years of followup in women at higher risk of breast cancer Statistically significant interaction between treatment and risk reduction by family history status in women with family history of breast cancer (FDR with breast cancer); raloxifene associated with 89% reduction in risk of invasive breast cancer vs. placebo; risk reduction present after adjustment Family history of breast cancer was a risk factor for breast cancer in the placebo group, but not the raloxifene group	Costs of publication of this article defrayed in part by payment of page charges; funding source NR
Tamoxifen vs. raloxifene			
STAR[289] Good See also Vogel et al, 2006[329]	Number of events and annual rates of invasive breast cancer by number of FDRs with breast cancer; tamoxifen vs. raloxifene: None: 82 vs. 105; rate,* 4.77 vs. 6.17; difference, -1.40; RR, 1.29 (95% CI, 0.96-1.75) 1: 112 vs. 135; rate,* 3.51 vs. 4.10; difference, -0.59; RR, 1.17 (95% CI, 0.90-1.51) ≥2: 53 vs. 70; rate,* 4.44 vs. 5.96; difference, -1.52; RR, 1.34 (95% CI, 0.93-1.96)	In women with a FDR with breast cancer, tamoxifen reduced the incidence of invasive breast cancer more than raloxifene, though difference not statistically significant	National Cancer Institute; U.S. Department of Health and Human Services

* Per 1000 women-years.
†Per 10,000 women-years.

Abbreviations: CI = confidence interval; DCIS = ductal carcinoma in situ; DVT = deep vein thrombosis; ER = estrogen receptor; FDR = first-degree relative; HR = hazard ratio; HT = hormone therapy; IBIS-I = International Breast Cancer Intervention Study; LCIS = lobular carcinoma in situ; MORE = Multiple Outcomes of Raloxifene Evaluation Trial; NR = not reported; CORE = Continuing Outcomes Relevant to Evista Trial; NSABP = National Surgical Adjuvant Breast and Bowel Project; RCT = randomized, controlled trial; RR = relative risk; RUTH = Raloxifene Use for the Heart Trial; SD = standard deviation; SDR = second-degree relative; SERM = selective estrogen receptor modulator; STAR = Study of Tamoxifen and Raloxifen Trial.

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Design	Purpose	Sample size	Population/setting	Demographics
Salpingo-oophorectomy or oophorectomy vs. no oophorectomy					
Domchek et al, 2010[292] Fair	Prospective cohort	To assess the relationship of RRM or RRSO with cancer outcomes.	Eligible: 2482 Analyzed: 1458 with no prior breast cancer (935 *BRCA1*, 523 *BRCA2*)	1974-2008 U.K., Europe, and North America Women from 22 centers in the PROSE consortium	NR
Kramer et al, 2005[185] Fair Note: only oophorectomy performed	Prospective cohort	To assess whether population differences in oophorectomy prevalence might significantly influence breast cancer penetrance estimates in *BRCA1* mutation families.	Eligible: 673 (98 *BRCA1* positive, 23 from *BRCA1* families)	Year: NR U.S. Women from self-referred and physician-referred families affected by hereditary breast/ovarian cancer with a *BRCA1* mutation and participating in ongoing studies at the National Cancer Institute	NR Mean, 2.7 cases of breast cancer and 3.0 cases of ovarian cancer per family diagnosed before ascertainment
Olson et al, 2004[296] NA Note: only oophorectomy performed	Retrospective cohort	To estimate the potential risk reduction of breast cancer for women who underwent oophorectomy and had a family history of breast cancer but unknown BRCA status.	Eligible: 851 Analyzed: 634	1970-1994 U.S./review of Mayo Clinic Surgical Index Followup survey completed by patient or surrogates (if patient deceased)	Surrogate respondent vs. self-respondent Age at surgery, years (n): 21-30: 1 (4%) vs. 16 (3%) 31-40: 1(4%) vs. 88 (14%) 41-50: 11 (41%) vs. 319 (53%) 51-60: 14 (52%) vs. 184 (30%) Age at questionnaire response (followup) of self-respondents, years (n): 31-40: 9 (1%) 41-50: 48 (8%) 51-60: 172 (28%) 61-70: 231 (38%) 71-80: 124 (20%) 81-90: 20 (3%) Deceased: n=30
Mastectomy vs. no mastectomy					
Domchek et al, 2010[292] Fair	Prospective cohort	To assess the relationship of RRM or RRSO with cancer outcomes.	Eligible: 2482 Analyzed: 1458 with no prior breast cancer (935 *BRCA1*, 523 *BRCA2*)	1974-2008 U.K., Europe, and North America Women from 22 centers in the PROSE consortium	NR
Evans et al, 2009[293] NA	Prospective cohort	To assess effectiveness of risk-reducing surgery in women at high risk of breast cancer, including carriers and noncarriers of *BRCA1/2* mutation.	Eligible: 550 Enrolled: 314 women with no prior breast cancer	1987-1992 Europe Multidisciplinary family history clinics established at 10 centers	Mean age of women undergoing mastectomy at Manchester site, years: 41 (range, 21-60) Age range at all sites, years: 21-72

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Design	Purpose	Sample size	Population/setting	Demographics
Skytte et al, 2011[294] Good	Prospective cohort	To compare incidence of breast cancer after RRM in healthy BRCA mutation carriers versus nonoperated mutation carriers and background population.	Eligible: 307 with mutation (201 *BRCA1*, 106 *BRCA2*)	January 1996-February 2008 Denmark Women from clinical genetics departments at multiple sites with mutation status diagnosed	Median age at entry into study, years: 36.2 (range, 17.9-86.3) Mean age at group entry, years (mastectomy vs. no mastectomy): 37.1 vs. 37.7 <40 years: 64/96 (67%) vs. 127/211 (60%) Note: age at group entry = age at mastectomy for mastectomy group and age at BRCA diagnosis for no mastectomy group
Prior Report					
Mastectomy					
Hartmann et al, 1999[290]	Retrospective cohort	To define the effect of RRM on incidence of breast cancer and risk of death from breast cancer	Eligible: 639 Analyzed: 639	1960-1993 U.S.; Mayo Clinic medical records of women who underwent RRM	Mean age at surgery, 42 (range, 18-79)
Hartmann et al, 2001[291]	Retrospective cohort	To report the effect of RRM on breast cancer risk in *BRCA1/2* carriers identified from a high-risk cohort	18 *BRCA1/2*	*BRCA1/2* mutation carriers undergoing RRM and enrolled as high-risk participants in prior study (Hartmann 1999)	Mean age at surgery, 41 (range, 20-75)
Oophorectomy					
Struewing et al, 1995[229]	Prospective cohort	To determine the incidence of post-oophorectomy carcinomatosis and quatify the effectiveness of risk-reducing surgery	Eligible: 16 families Analyzed: 12 families (390 1st-degree relatives of breast or ovarian cancer cases)	Women with high genetic risk of ovarian cancer and oophorectomy matched to high-risk women who did not undergo surgery from National Cancer Institute, Creighton University, and U.K.	NR

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Inclusion/exclusion criteria	Risk definition	Followup
Salpingo-oophorectomy or oophorectomy vs. no oophorectomy			
Domchek et al, 2010[292] Fair	Inclusion Women with *BRCA1/2* mutations, no prior ovarian cancer, no salpingo-oophorectomy at time of ascertainment, and minimum 6 months followup. Exclusion Women with cancer diagnosis within first 6 months of followup, women who had RRM prior to ascertainment excluded from all breast cancer end points, and women with occult ovarian cancer during RRSO excluded from ovarian cancer end points.	BRCA status	Patients followed until end of 2009. Median followup was 3.65 years for those who had surgery and 4.29 years for those who did not. **Oophorectomy & breast cancer outcomes:** *BRCA1* followed mean 4.7 years to censoring *BRCA2* followed mean 4.7 years to censoring **Oophorectomy & ovarian cancer outcomes:** *BRCA1* followed mean 5.6 years to censoring *BRCA2* followed mean 5.8 years to censoring
Kramer et al, 2005[185] Fair Note: only oophorectomy performed	Inclusion Female, bloodline family member from *BRCA1*-positive family, no history of breast cancer before ascertainment, no history of bilateral mastectomy, age ≥20 years by study closing date. Exclusion Breast cancer diagnosed before family ascertainment and families with variants of uncertain significance.	BRCA status	Mean followup: 16.5 years; 11,105 person-years of observation Mean followup per patient (years) *BRCA1* positive: 14.1 *BRCA1* negative: 17.6 *BRCA1* unknown: 15.8
Olson et al, 2004[296] NA Note: only oophorectomy performed	Inclusion Women age <60 years with bilateral oophorectomy during study dates. Exclusion Women who had hysterectomy alone or only had 1 ovary removed, had prophylactic mastectomy at any time, or had any history of cancer prior to surgery, aside from nonmelanoma skin cancer.	High risk ≥1 1st-degree relative with breast cancer at age <50 or 1 1st-degree relative with ovarian cancer at any age and ≥1 other 1st- or 2nd-degree relative with either diagnosis at any age. Moderate risk Only 1 1st-degree relative with breast cancer at any age. Low risk No breast or ovarian cancer family history	N/A
Mastectomy vs. no mastectomy			
Domchek et al, 2010[292] Fair	Inclusion Women with *BRCA1/2* mutations, no prior ovarian cancer, no salpingo-oophorectomy at time of ascertainment, and minimum 6 months followup. Exclusion Women with cancer diagnosis within first 6 months of followup, women who had RRM prior to ascertainment excluded from all breast cancer end points, and women with occult ovarian cancer during RRSO excluded from ovarian cancer end points.	BRCA status	Patients followed until end of 2009. Median followup was 3.65 years for those who had surgery and 4.29 years for those who did not. **Mastectomy & breast cancer outcomes:** *BRCA1* followed mean 2.7 years to censoring *BRCA2* followed mean 2.5 years to censoring

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Inclusion/exclusion criteria	Risk definition	Followup
Evans et al, 2009[293] NA	Inclusion Eligible for bilateral RRM if lifetime breast cancer risk in excess of 25% or eligible for unilateral RRM if already had a diagnosis of in situ or invasive breast cancer in the contralateral breast. Paris center offered surgery to *BRCA1/2* carriers only. Exclusion NR	Lifetime risk of breast cancer >25% based on family history with or without mutation status or diagnosis of breast cancer in contralateral breast	Followup in all women with RRM, years: Median, 7.5; Mean, 6.1; 3,334 women-years Followup in women undergoing bilateral RRM: 2,155 women-years (Manchester site, 1,274 women-years) Followup in control women: 2,438 women-years
Skytte et al, 2011[294] Good	Inclusion *BRCA1/2* mutation positive and women who did not have mastectomy or salpingo-oophorectomy prior to study. Exclusion Diagnosis of breast or ovarian cancer before BRCA testing and women who opted for risk-reducing surgery before receiving test result.	BRCA status	Median time from study entry to mastectomy: 7.7 years Total at-risk time in mastectomy group: 378.7 years Total at-risk time in no mastectomy group: 934.6 years
Prior Report			
Mastectomy			
Hartmann et al, 1999[290]	Inclusion Women with a family history of breast cancer who had bilateral RRM Exclusion Breast cancer detected in surgically treated breast; surgery for augmentation of reduction High-risk comparison group inclusion Sisters of high-risk subjects were recruited to the study	High risk ≥2 1st-degree relatives with breast cancer; 1 1st-degree relative and ≥2 2nd- or 3rd-degree relatives with breast cancer; 1 1st-degree relative with breast cancer before age 45 years and 1 other relative with breast cancer; 1 1st-degree relative with breast cancer and ≥1 relatives with ovarian cancer; 2 2nd- or 3rd-degree relatives with breast cancer and ≥1 with ovarian cancer; 1 2nd- or 3rd-degree relative with breast cancer and ≥2 with ovarian cancer; ≥3 2nd- or 3rd-degree relatives with breast cancer; 1 1st-degree relative with bilateral breast cancer; breast cancer in male family members Moderate risk Women who did not meet these criteria	Median, 14 years; with a minimum of 2 years for 99% of the subjects.
Hartmann et al, 2001[291]	Inclusion Women with *BRCA1/2* mutations who had bilateral RRM mastectomy	BRCA status	13.1 years

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Inclusion/exclusion criteria	Risk definition	Followup
Oophorectomy			
Struewing et al, 1995[229]	Inclusion: Families with ≥3 cases of ovarian cancer or ≥2 cases of ovarian cancer and ≥1 case of breast cancer before age 50. Exclusion: Families fitting criteria for Lynch Syndrome II.	Results presented by those with an affected 1st-degree relative and those with an affected 2nd-degree relative	Surgery vs. no surgery Ovarian cancer incidence 1st-degree relative: 460 vs. 1665 person-years 2nd-degree relative: 106 vs. 2123 person-years Breast cancer incidence 1st-degree relative: 484 vs. 1587 person-years 2nd-degree relative: 106 vs. 2131 person-years

Author, year Quality	Results	Conclusions	Funding source
Salpingo-oophorectomy or oophorectomy vs. no oophorectomy			
Domchek et al, 2010[292] Fair	Number of cancer cases in women with no history of breast cancer; surgery vs. no surgery Risk-reducing salpingo-oophorectomy and ovarian or primary peritoneal cancer risk Total: 6/465 (1.3%) vs. 63/1092 (5.8%); HR, 0.28 (95% CI, 0.12-0.69) *BRCA1*: 6/342 (1.8%) vs. 49/661 (7.4%); HR, 0.31 (95% CI, 0.12-0.82) *BRCA2*: 0/123 vs. 14/431 (3.2%); HR N/A Note: HR adjusted for year of birth, oral contraceptive use, and stratified by center Risk-reducing salpingo-oophorectomy and breast cancer risk Total: 39/336 (12%) vs. 223/1034 (22%); HR, 0.54 (95% CI, 0.37-0.79) *BRCA1*: 32/236 (14%) vs. 129/633 (20%); HR, 0.63 (95% CI, 0.41-0.96) *BRCA2*: 7/100 (7%) vs. 94/401 (23%); HR, 0.36 (95% CI, 18.1-82.7) Note: HR adjusted for year of birth and stratified by center Risk-reducing salpingo-oophorectomy and all-cause mortality Total: 8/447 (1.8%) vs. 60/1011 (5.9%); HR, 0.45 (95% CI, 0.21-0.95) *BRCA1*: 8/327 (2.4%) vs. 43/608 (7.1%); HR, 0.52 (95% CI, 0.24-1.14) *BRCA2*: 0/120 vs. 17/403 (4.2%); HR N/A Note: HR adjusted for year of birth and stratified by center Risk-reducing salpingo-oophorectomy and breast cancer–specific mortality Total: 2/441 (0.5%) vs. 22/973 (2.3%); HR, 0.27 (95% CI, 0.05-1.33) *BRCA1*: 2/321 (1.0%) vs. 16/581 (2.8%); HR, 0.30 (95% CI, 0.06-1.53) *BRCA2*: 0/120 vs. 6/392 (1.5%); HR N/A Note: HR adjusted for year of birth and stratified by center Risk-reducing salpingo-oophorectomy and ovarian cancer–specific mortality Total: 3/442 (0.7%) vs. 24/975 (2.5%); HR, 0.39 (95% CI, 0.12-1.29) *BRCA1*: 3/322 (0.9%) vs. 20/585 (3.4%); HR, 0.46 (95% CI, 0.08-2.72) *BRCA2*: 0/120 vs. 4/390 (1.0%); HR N/A Note: HR adjusted for year of birth, oral contraceptive use, and stratified by center	Among a cohort of women with BRCA mutations, RRSO was associated with a lower risk of ovarian cancer, first diagnosis of breast cancer, all-cause mortality, breast cancer–specific mortality, and ovarian cancer–specific mortality.	Public Health Service; University of Pennsylvania Cancer Center; Cancer Genetics Network; Marjorie Cohen Research Fund; SPORE grant from the Dana-Farber/ Harvard Cancer Center; U.S. Department of Defense; Utah Cancer Registry; Utah State Department; Nebraska State Cancer and Smoking-Related Diseases Research Program grants; Cancer Research U.K. Grant; National Cancer Institute; Dr. Olopade received funding as the Doris Duke Distinguished Clinical Scientist; Dr. Eeles received funding from the National Institute for Health Research

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Results	Conclusions	Funding source
Kramer et al, 2005[185] Fair Note: only oophorectomy performed	Number of breast cancer cases; oophorectomy vs. no oophorectomy *BRCA1* positive (n=98): 6/33 (18%) vs. 27/65 (42%); HR, 0.38 (95% CI, 0.15 to 0.97); p=0.043 *BRCA1* negative (n=353): 1/34 (2.9%) vs. 4/319 (1.3%); HR NR *BRCA1* status unknown (n=222): 0/18 vs. 5/204 (2.5%); HR NR Absolute risk reduction in women who had oophorectomy was most prominent when surgery was done at a younger age (<40 years), figure representation	In a cohort of *BRCA1* mutation carriers from multiple-case families, oophorectomy was associated with decreased risk of breast cancer; affect was strongest in younger women; oophorectomy status affects breast cancer penetrance	Intramural Research Program of National Cancer Institute; funding source not specifically reported
Olson et al, 2004[296] NA Note: only oophorectomy performed	Expected vs. observed number of cancer cases <u>Age of surgery <60 years</u> High risk (n=55): 5.4 vs. 3; RR, 0.56 (95% CI, 0.11-1.33) Moderate risk (n=193): 10.9 vs. 9; RR, 0.83 (95% CI, 0.38-1.44) <u>Age of surgery <50 years</u> High risk (n=41): 3.9 vs. 1; RR, 0.26 (95% CI, 0.001-0.99) Moderate risk (n=130): 7.7 vs. 5; RR, 0.65 (95% CI, 0.21-1.32) <u>Age of surgery <60 years and premenopausal before surgery</u> High risk (n=52): 5.1 vs. 3; RR, 0.59 (95% CI, 0.12-1.41) Moderate risk (n=186): 10.4 vs. 7; RR, 0.67 (95% CI, 0.27-1.24) <u>Age of surgery <50 years and premenopausal before surgery</u> High risk (n=40): 3.8 vs. 1; RR, 0.26 (95% CI, 0.00-1.00) Moderate risk (n=126): 7.4 vs. 3; RR, 0.41 (95% CI, 0.08-0.98)	The number of observed breast cancers in women in the cohort was lower than expected for nearly all levels of risk, and especially for those age <50 years and premenopausal prior to surgery	Fraternal Order of the Eagles and the National Cancer Institute
Mastectomy vs. no mastectomy			
Domchek et al, 2010[292] Fair	Number of cancer cases in women with no history of breast cancer; surgery vs. no surgery <u>Risk-reducing mastectomy and risk of first occurrence of breast cancer</u> Total: 0/75 vs. 34/585 (5.8%) *BRCA1*: 0/43 vs. 19/372 (5.1%) *BRCA2*: 0/32 vs. 15/213 (7.0%)	In a cohort of women with BRCA mutations, RRM was associated with a lower risk of breast cancer	Public Health Service; University of Pennsylvania Cancer Center; Cancer Genetics Network; Marjorie Cohen Research Fund; SPORE grant from Dana-Farber/Harvard Cancer Center; U.S. Department of Defense; Utah Cancer Registry; Utah State Department; Nebraska State Cancer and Smoking-Related Diseases Research Program grants; Cancer Research U.K. Grant; National Cancer Institute; Dr. Olopade funded as the Doris Duke Distinguished Clinical Scientist; Dr. Eeles received funding from the National Institute for Health Research

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Results	Conclusions	Funding source
Evans et al, 2009[293] NA	Manchester (mastectomy vs. no mastectomy): RRM: 179 vs. 0 Breast cancers expected based on life tables: 12.12 vs. 20.8 Cancers diagnosed: 0 vs. 21 All sites: RRM: 307 per Table 2 (314 per text [p. 256]) Expected cancers: 21.30 Cancers diagnosed: 0	Risk-reducing surgery is highly effective	NR
Skytte et al, 2011[294] Good	Number of breast cancer cases (incidence per person-year); mastectomy vs. no mastectomy: 3/96 (0.8%) vs. 16/211 (1.7%); HR, 0.394 (95%CI, 0.115-1.355); p=0.14 Note: 3/3 women with breast cancer in the mastectomy group and 12/16 women in no mastectomy group were *BRCA1*-positive Note: All women diagnosed with cancer in mastectomy group had also had bilateral salpingo-oophorectomy; 1 woman diagnosed with breast cancer on date of mastectomy, contributed to the "no mastectomy" group at risk time and cancer incidence Adjusting for age did not change significance (HR, 0.455; p=0.224) Effect of age was significant (p=0.008); in both groups, 1-year age difference associated with 4.2% increase in breast cancer risk Annual incidence of breast cancer after mastectomy by carrier status: 1.1% for *BRCA1* (n=67); 0 for *BRCA2* (n=29)	Study of 307 healthy *BRCA1/2* carriers suggests bilateral RRM reduces risk of breast cancer but does not completely eliminate it. Study size too small to show a significant difference	NR
Prior Report			
Mastectomy			
Hartmann et al, 1999[290]	Overall: 425 subjects were classified moderate risk, 214 subjects high risk. 95% were alive at the time of the study. 7 were diagnosed with breast cancer (4 moderate risk, 3 high risk); all cases occurred after subcutaneous mastectomy. Cancer Diagnosis: 37 in the moderate-risk group (based on Gail model estimates) and 53 in the high-risk group (based on the high-risk comparison group) were expected to develop breast cancer had they not had mastectomy. RRM reduced risk in the moderate-risk group by 89.5% (p<0.001) and in the high-risk group by 90%-94% (depending on adjusted analysis). 2 women in the high-risk group were diagnosed with ovarian cancer. Death Reduction: 10 in the moderate-risk group (based on Gail model estimates) and 31 in the high-risk group (based on the high-risk comparison group) were expected to die from breast cancer had they not had mastectomy. Death was reduced in the moderate-risk group by 100% (no deaths) (95% CI, 70-100) and in the high-risk group by 81%-94% (depending on adjusted analysis) (2 deaths).	In women with high risk of breast cancer on the basis of family history, RRM can significantly reduce the incidence of breast cancer	U.S. Department of Defense; National Cancer Insitute; Donaldson Charitable Trust
Hartmann et al, 2001[291]	Risk Reduction: Easton model (a high-penetrance model), 6.1 cases were expected; Struewing model (a low-penetrance model), 4.5 cases. Mastectomy resulted in risk reduction of 89.5% or 100% for the Easton model (95% CI, 41.4-99.7 and CI, 68-100) and 85% or 100% for the Struewing model (95% CI, 15.6-99.6 and CI, 54.1-100).	Risk-reducing mastectomy is associated with a substantial reduction in the incidence of breast cancer in known *BRCA1/2* mutation	NR

Appendix C12. Evidence Table of Risk-Reducing Surgery

Author, year Quality	Results	Conclusions	Funding source
		carriers	
Oophorectomy			
Struewing et al, 1995[229]	Surgery vs. no surgery *Preliminary Analysis from National Cancer Insititute only* <u>Ovarian cancer incidence</u> 1st-degree relative: 2/44 vs. 8/346 2nd-degree relative: 0 vs. 1 Note: Incidence includes post-oophorectomy ovarian carcinomatosis <u>Breast cancer incidence</u> 1st-degree relative: 3/44 vs. 14/346 2nd-degree relative: 0 vs. 3	Findings suggest that there is a finite risk of post-oophorectomy carcinomatosis. Preliminary analysis suggests a statistically nonsignificant protective effect of surgery for ovarian cancer	NR

Abbreviations: CI = confidence interval; HR = hazard ratio; MRI = magnetic resonance imaging; NA = not applicable; NR = not reported; RR = relative risk; RRM = risk-reducing mastectomy; RRSO = risk-reducing salpingo-oophorectomy; PROSE = Prevention and Observation of Surgical Endpoints.

Appendix C13. Evidence Table of Harms of Intensive Screening

Author, year Quality	Subcategory	Study design	Country/population/ setting	Inclusion/exclusion criteria	Risk level definition
Breast cancer screening					
Kriege et al, 2004[277] NA Dutch MRISC study	Physical harms of increased screening	Prospective cohort (breast cancer characteristics compared to registry data and women with breast cancer from another prospective cohort study)	The Netherlands Women with increased familial or genetic predisposition for breast cancer attending academic and/or cancer centers at 6 sites	Inclusion Cumulative lifetime risk of breast cancer >15% due to genetic or familial predisposition according to modified Claus tables; age at entry between 25 and 70 years (could be tested at before age 25 if family member diagnosed before age 30 years) Exclusion Women with symptoms suggestive of breast cancer or personal history of breast cancer; women proven not to have a mutation in a family with a proven mutation	Cumulative lifetime risk of breast cancer >15% due to genetic or familial predisposition according to modified Claus tables
Kriege et al, 2006[297] NA Dutch MRISC study	Physical harms of increased screening	Prospective cohort (breast cancer characteristics compared to registry data and women with breast cancer from another prospective cohort study)	The Netherlands Women with increased familial or genetic predisposition for breast cancer attending academic and/or cancer centers at 6 sites	Inclusion Cumulative lifetime risk of breast cancer >15% due to genetic or familial predisposition according to modified Claus tables, age at entry between 25 and 70 years (could be tested at before age 25 if family member diagnosed before age 30 years), no previous breast cancer or symptoms suspicious for breast cancer Exclusion Women with symptoms suggestive of breast cancer or personal history of breast cancer; women proven not to have a mutation in a family with a proven mutation	Cumulative lifetime risk of breast cancer >15% due to genetic or familial predisposition according to modified Claus tables
Leach et al, 2005[274] NAMARIBS study	Physical harms of increased screening	Prospective cohort, one-arm	U.K. Women attending 1 of 22 participating centers in the U.K. with increased breast cancer risk	Inclusion Asymptomatic women aged 35-49 years fulfilling 1 of the following: known carrier of a deleterious *BRCA1*, *BRCA2*, or TP53 mutation; FDR of someone with 1 of these deleterious mutations; strong family history of breast or ovarian cancer or both; or family history consistent with classic Li-Fraumeni syndrome. Aim was to include women whose affected FDRs had ≥60% chance of being a *BRCA1* or *BRCA2* mutation carrier or women with an annual risk of ≥0.9% Exclusion Women with previous breast cancer, those with any cancer such that prognosis was <5 years, participants who had predictive genetic testing during study and whose results were negative, women who developed cancer during study period	Known carrier of a deleterious *BRCA1*, *BRCA2*, or TP53 mutation; FDR of someone with 1 of these deleterious mutations; strong family history of breast or ovarian cancer or both; or family history consistent with classic Li-Fraumeni syndrome

Appendix C13. Evidence Table of Harms of Intensive Screening

Author, year Quality	Subcategory	Study design	Country/population/ setting	Inclusion/exclusion criteria	Risk level definition
Le-Petross et al, 2011[276] NA	Physical harms of increased screening	Retrospective analysis of prospective cohort study, one-arm	U.S. Women at increased genetic risk of breast cancer at single institution	Inclusion Women age ≥18 years, having undergone alternating screening mammography and breast MRI every 6 months at study institution, either confirmed *BRCA1/2* carriers or FDR of confirmed *BRCA1/2* carrier Exclusion Women with history of breast cancer, who had calculated lifetime risk of breast cancer >20%, or who did not undergo a screening MRI, women who used chemoprevention or underwent bilateral prophylactic mastectomy, those with metastatic disease, undergoing treatment, or high BMI preventing MRI, women lost to followup, or died during original trial	Based on BRCA status or FDR of BRCA mutation carrier
Ovarian cancer screening					
Hermsen et al, 2007[281] NA	Physical harms of increased screening	Prospective cohort, one-arm (Staging compared to 2 external comparison groups; unscreened family members with cancer, combined data from multiple studies)	The Netherlands Women with BRCA mutation screened at 6 University Family Cancer Clinics	Inclusion Women with *BRCA1/2* mutation screened at 1 of participating centers Exclusion Women with symptoms at first visit, who had only 1 visit, or who were found to have cancer at first screening visit	Based on BRCA status
Prior report					
Bourne et al, 1993[279] NA	Physical harms of increased screening	Prospective cohort, one-arm	U.K. Self-referred asymptomatic women with a close relative diagnosed with ovarian cancer	Inclusion Women age ≥25 years with ≥1 close relatives who had developed ovarian cancer; symptomless	Based on pedigree/pattern of inheritance

Appendix C13. Evidence Table of Harms of Intensive Screening

Author, year Quality	N	Demographics	Duration/followup	Screening method and interval
Breast cancer screening				
Kriege et al, 2004[277] NA Dutch MRISC study	Enrolled: 1952 Analyzed: 1909 n=358 mutation carriers (276 *BRCA1*, 77 *BRCA2*, 1 both *BRCA1/2*, 2 PTEN, and 2 TP53), n=1052 high risk, n=499 moderate risk	Mean age at entry, years: 40 (range, 19-72)	1999-2003 Median, 2.9 years (mean, 2.7; range, 0.1-3.9 years)	A) Biannual CBE B) Annual mammography C) Annual contrast enhanced MRI Note: When 1 of the examinations reported as "probably benign finding" or "need additional imaging evaluation" (BI-RADS 3 or 0), further investigation undertaken by ultrasonography ± fine needle aspiration, or mammography or repeated MRI; when 1 of the examinations reported as "suspicious abnormality" or "highly suggestive of malignancy" (BI-RADS 4 or 5), cytologic or histologic evaluation of biopsy specimen performed; when results of imaging were negative but clinical breast exam was uncertain or suspicious, additional investigations performed
Kriege et al, 2006[297] NA Dutch MRISC study	Analyzed: 1909 n=358 mutation carriers (276 *BRCA1*, 77 *BRCA2*, 1 both *BRCA1* and *BRCA2*, 2 PTEN, and 2 TP53), n=1052 high-risk, n=499 moderate risk	Mean age at entry, years: 40 (range, 19-72)	1999-2003 Median, 2.9 years (mean, 2.7; range, 0.1-3.9 years)	A) Biannual CBE B) Annual mammography C) Annual contrast enhanced MRI Note: When 1 of the examinations reported as "probably benign finding" or "need additional imaging evaluation" (BI-RADS 3 or 0), further investigation undertaken by ultrasonography ± fine needle aspiration, or mammography or repeated MRI; when 1 of the examinations reported as "suspicious abnormality" or "highly suggestive of malignancy" (BI-RADS 4 or 5), cytologic or histologic evaluation of biopsy specimen performed; when results of imaging were negative but clinical breast exam was uncertain or suspicious, additional investigations performed
Leach et al, 2005[274] NAMARIBS study	649 n=82 (13%) with known *BRCA1* mutation n=38 (6%) with known *BRCA2* mutation	Median age at entry, years: 40 (range, 31-55; only 1 woman age >50 years)	Study recruitment 1997-2003 Variable screening episodes per individual but screening continued until each women had ≥2 annual scans (in 2004)	A) Annual mammography from age 35 years (or younger if FDR developed cancer at age <35 years) B) Annual CE MRI Note: In women with equivocal results, high specificity MRI exam done 2-6 weeks later (followed by ultrasound, fine needle aspiration, localization, and tissue sampling by conventional methods, as appropriate)
Le-Petross et al, 2011[276] NA	Screened: 321 Analyzed: 73 (37 [51%] *BRCA1*, 36 [49%] *BRCA2*)	Median age at entry, years: 44 (range, 23-75)	Records from 1997-2009 Median followup, 2 years (range, 1-6 years) Mean followup from suspicious finding to diagnosis, 1.7 years (range, 1-3 years)	All women underwent: A) Mammography every 6 months B) MRI every 6 months Note: imaging was performed on an alternating basis, women had clinical breast exam every 6 months, ultrasound used to evaluate abnormal mammographic or MRI findings, biopsy as required

Appendix C13. Evidence Table of Harms of Intensive Screening

Author, year Quality	N	Demographics	Duration/followup	Screening method and interval
Ovarian cancer screening				
Hermsen et al, 2007[281] NA	883 n=683 *BRCA1*, 200 *BRCA2* 459 for analysis of screening/compliance (data available for all screening visits)	Median age, years: *BRCA1*: 40 (range, 21-76) *BRCA2*: 44 (range, 25-77)	1993-2005 1473 person-years	A) Annual serum CA-125 measurement B) Annual TVUS Starting at age 35 years or 5 years earlier than youngest diagnosed ovarian cancer in the family Note: Biannual screens were done in some centers during the study period, but this was not systematically adopted
Prior report				
Bourne et al, 1993[279] NA	1601	Mean age, years: 47 (range, 17-79)	Unclear duration 4 years	TVUS ± color flow imaging§ (screening interval NR)

Author, year Quality	Results	Funding source
Breast cancer screening		
Kriege et al, 2004[277] NA Dutch MRISC study	Based on 45 cancers, B vs. C: <u>Additional investigations:</u> Ultrasound, 889 times/627 women Fine needle aspiration, 312 times (267 times plus ultrasound, 45 times plus palpation) Biopsy, used 85 times/82 women (malignancy in 50 cases, lobular carcinoma in situ in 1 case; rate of positive histologic findings 60.0%) Unneeded additional exams*: 207 vs. 420 Unneeded biopsies: 28% (7/25*) vs. 43% (24/56†)	Grant from Dutch Health Insurance Council
Kriege et al, 2006[297] NA Dutch MRISC study	Imaging rounds of 39 evaluable invasive breast cancers, B vs. C: <u>First imaging round, with prior mammography</u> False positive rate (%): 5.5 vs. 14.0; P<0.001 False negatives (n): 12 vs. 1 <u>Subsequent imaging rounds</u> False positive rate (%): 4.6 vs. 8.2; p<0.001 False negatives (n): 12 vs. 4	Grant from Dutch Health Insurance Council
Leach et al, 2005[274] NAMARIBS study	Based on 33 screen-detected cancers: <u>Recall rates, A vs. B</u> 279 exams led to recall (40 based purely on reader's judgment, not score) 3.9% vs. 11% per woman year A plus B: 13% per woman year 245 recalls for benign findings 73% diagnosed cancer-free using noninvasive tests <u>Additional diagnostic procedures in 245 women without cancer:</u> Ultrasound, n=93 Core biopsy, n=32 Fine needle aspiration, n=47 Surgery, n=7 (3% of recalled women without cancer, 27% of recalled women with cancer) 8.5 recalls per cancer detected 0.21 benign surgical biopsies per cancer detected	Grant from U.K. Medical Research Council; MRI cost paid from subvention funding for research from U.K. National Health Service

Appendix C13. Evidence Table of Harms of Intensive Screening

Author, year Quality	Results	Funding source
	Number of women per 1000 screening episodes needing diagnostic surgical biopsy was 0.4% (7/1881) for benign lesions, 0.5% (9/1881) for malignant lesions PPV of diagnostic surgical biopsy=56% 62% (172/279) of suspicious findings on MRI resolved without invasive procedure, n=16 women had diagnostic surgery to complete diagnosis, n=91 had some form of percutaneous biopsy procedure Preoperative diagnosis of cancer made in 24/33 (73%) of screen-detected cancers	
Le-Petross et al, 2011[276] NA	13 cancers in 11 women (12 on screen, 1 on prophylatic mastectomy) 20/73 women underwent biopsy, 11 cancers diagnosed by biopsy in 10 women Overall biopsy yield for MRI was 50% (10/20) <u>False positive, A vs. B</u> 11/73 (15%) vs. 8/73 (11%) Required further imaging: 8 vs. 4 Required biopsy: 3 vs. 2 Required imaging plus biopsy: 0 vs. 2	NR
Ovarian cancer screening		
Hermsen et al, 2007[281] NA	15 cancers diagnosed in cohort 10 cancers diagnosed during followup 5 screen-detected <u>Based on 459 women with data on each visit</u>: 7 cancers diagnosed (2 prevalent, 2 interval, 3 incident) Abnormalities were found by 1 or both screening modalities in 3% (38/1116) of screening visits. Overall, abnormalities were found in 9% (40/459) of women (some due to physical complaints), resulting in 26 diagnostic operations <u>Benign‡ diagnostic surgery, A vs. B</u> 67% (4/6) vs. 100% (9/9) A+B: 55% (6/11) Note: Not all benign diagnostic surgeries were done due to abnormal screen findings; some surgeries were undertaken to follow up on abnormal findings from CA-125 measurement ± TVUS done to assess symptomatic complaints	NIHR Biomedical Research Centre at Central Manchester Foundation Trust
Prior report		
Bourne et al, 1993[279] NA	11 cancers diagnosed (6 screen-detected, 5 interval) 3.8% (61/1601) with positive screening result, referral to surgery False-positive cases: 55/61 referred cases (cancer detected in 6/61 referred cases) False-positive rate: 3.4% (95% CI, 2.6-4.5 [55/1595]) <u>Addition of color flow imaging and criterion of morphological score ≥5 or pulsatility index <1:</u> Retrospective addition (applied to positive ultrasound results) = 15 false-positive cases Prospective addition (applied at the time of ultrasound exam) = 6 false-positive cases Note: 43% of women had only 1 TVUS (prevalent screen)	NR

*Additional investigation included ultrasound ± fine needle biopsy, or repeat mammography, or repeat MRI.
†Women with BIRAD score ≥3 on mammography or MRI.
‡Surgery for final benign diagnosis.
§Color flow imaging applied prospectively to 600 ultrasound exams; retrospectively after a positive ultrasound result to the remainder.

Abbreviations: BI-RADS = Breast Imaging-Reporting and Data System; BMI = body mass index; CA-125 = cancer antigen-125; CBE = clinical breast examination; CE = contrast enhanced; FDR = first-degree relative; MARIBS = Magnetic Resonance Imaging Breast Screening; MRI = magnetic resonance imaging; MRISC = Magnetic Resonance Imaging Screening Study; NA = not applicable; NIHR = National Institute for Health Research; NR = not reported; PPV = positive predictive value; PTEN = phosphatase and tensin homolog; TP53 = tumor protein 53; TVUS = transvaginal ultrasound.

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Subcategory	Purpose	Study type	N	Country	Population/ Setting	Demographics
Brandberg et al, 2008[302] Brandberg et al, 2012[304] NA	Sexual functioning Psychological	To prospectively evaluate body image, sexuality, emotional reactions, and quality of life in a sample of women having increased risk for breast cancer before RRM, and 6 months and 1 year after.	Before and after	Eligible: NR Enrolled: 90 Analyzed: 65	Sweden	Karolinska University Hospital	Age (years): 20-29: 7/90 (8%) 30-39: 33/90 (37%) 40-49: 35/90 (39%) 50-59: 13/90 (14%) 60-69: 2/90 (2%)
Finch et al, 2011[306] NA	Sexual functioning	To examine the impact of RRSO on menopausal symptoms and sexual functioning in women who carry a *BRCA1/2* mutation.	Case-series	Eligible: NR Enrolled: 67	Canada	University Health Network	Not reported separately for women without breast cancer
Gahm et al, 2010[303] NA	Sexual functioning QOL Pain	To analyze the physical effects and to report effects on sexual functioning and health-related quality of life at least 2 years after RRM.	Cross-sectional	Eligible: NR Enrolled: 1784 (59 with RRM and 1725 included as reference sample)	Sweden	Karolinska University Hospital	Mean age of 40 years (range, 25-65)
Metcalfe et al, 2004[301] NA	Sexual functioning Psychological	To assess psychosocial functioning in a population-based series of women who have previously undergone RRM in a specified time period.	Case-series	Eligible: 122 Enrolled: 75 Analyzed: 60	Canada	Ontario hospitals in the Central East Health Information Partnership	Mean age of 43.5 years (SD, 7.8) at time of surgery and 47.8 years (SD, 8.6) at time of questionnaire
Rijnsburger et al, 2004[275] Fair	QOL	To describe the short-term effects of screening for breast cancer in high-risk women on health-related quality of life.	Prospective cohort Before and after	Eligible: 529 Enrolled: 329 Analyzed: 288	The Netherlands	MRI Screening Study conducted at 6 family cancer centers	Mean age of 40.9 years (SD, 8.9)
Spiegel et al, 2011[298] NA	Psychological	To compare women with recall examinations following MRI to those without recall examinations on breast cancer worry and anxiety.	Before and after	Eligible: 221 Enrolled: 134 Analyzed: 55	Canada	Women participating in an MRI screening trial	Mean age of 45 years (range, 25-60)
Wasteson et al, 2011[305] NA	Risk perception Psychological	To evaluate the long-term physical and psychological consequences of RRM in after 10 years.	Case-series	Eligible: NR Enrolled: 15 Analyzed: 13	Sweden	Women at Karolinska University Hospital enrolled in retrospective study	Mean age of 45 years (range: 40-57)

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Inclusion/Exclusion criteria	Risk level definition	Mutation status	Measures	Interventions
Brandberg et al, 2008[302] Brandberg et al, 2012[304] NA	Inclusion: Women who had RRM, including reconstruction Exclusion: Women with a breast cancer diagnosis	Lifetime risk definition not described 50% lifetime risk: 26/90 (28.9%) 25% lifetime risk: 8/90 (8.9%)	37/90 (41.1%) *BRCA1* 13/90 (14.4%) *BRCA2* 2/90 (2.2%) unknown mutation	Impact on areas of life measures Sexuality Activity Questionnaire (SAQ, pleasure subscale 0 to 18, discomfort subscale 0 to 6, and habit subscale 0 to 3) Body Image Scale (BIS, scale 0 to 30) Hospital Anxiety and Depression Scale (HADS, subscales 0 to 21) Swedish Short Term-36 Health Survey (SF-36, subscales 0 to 100)	A) RRM with reconstruction
Finch et al, 2011[306] NA	Inclusion: Women age 30-70 years at time of surgery who had RRSO Exclusion: Diagnosed with occult cancer at surgery or with breast cancer during the 1 year followup period	High risk due to positive genetic mutation	*BRCA1* or *BRCA2* positive	Menopause-Specific Quality of Life-Intervention (MENQOL, scale NR) Sexual Activity Questionnaire (scale NR)	RRSO
Gahm et al, 2010[303] NA	Inclusion: Women with increased risk for breast cancer who had RRM and immediate breast reconstruction Exclusion: Personal history of breast cancer	NR	NR	Pain and discomfort questionnaire (subscales 1 to 7) Sexuality questionnaire Swedish Short Term-36 Health Survey (SF-36, subscales 0 to 100) Decision Regret Scale (DRS, scale NR)	A) RRM with reconstruction B) Reference comparison group who did not have RRM
Metcalfe et al, 2004[301] NA	Inclusion: Women who had RRM at an Ontario hospital and returned the questionnaire Exclusion: Prior or current diagnosis of invasive or in situ breast cancer	Strong family history: had either 1 1st-degree or 2 2nd-degree relatives with any of the following: 1) breast cancer diagnosed <50 years; 2) ovarian cancer; or 3) male breast cancer (55.0% of population, also did not have genetic testing done) Limited family history: none of the above (23.3% of population, did not have genetic testing done)	21.7% had *BRCA1/2* mutation	Brief Symptom Inventory (BSI, scale 0 to 100) Body Image after Breast Cancer (BIBC, each subscale 1 to 5) Impact of Events Scale (IES, IES-I subscale 0 to 35 and IES-A subscale 0 to 40) Sexual activity questionnaire (pleasure subscale 0 to 18, discomfort subscale 0 to 6, habit subscale 0 to 3)	A) RRM 53/60 (88.3%) total 7/60 (11.7%) subcutaneous

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Inclusion/Exclusion criteria	Risk level definition	Mutation status	Measures	Interventions
Rijnsburger et al, 2004[275] Fair	Inclusion: Women already under intensive surveillance and women who came for the first time to the clinic Exclusion: Women with evident symptoms suspicious for breast cancer or previous breast cancer	Risk category 1: *BRCA1/2* mutation carriers (50%-85% cumulative lifetime risk) Risk category 2: 30%-50% cumulative lifetime risk Risk category 3: 15%-30% cumulative lifetime risk	35 were *BRCA1/2* mutation positive	Medical Outcomes Study 36-Item Short Form (SF-36, subscales 0 to 100) EuroQoL-5 Dimensions (EQ-5D, scale 0-1) Visual Analogue Scale (VAS, scale 0 to 100) Symptom Checklist-90 (SCL-90, scale 12-60)	A) CBE (n=287) B) CBE + mammography (n=134) C) CBE + MRI (n=109)
Spiegel et al, 2011[298] NA	Inclusion: Women participating in MRI screening trial who agreed to participate Exclusion: NR	All were mutation carriers	30/55 (54.5%) *BRCA1* 25/55 (45.5%) *BRCA2*	Hospital Anxiety and Depression Scale (HADS, subscales 0 to 21) Breast Cancer Worry Interference Scale (WIS, scores 7 to 35)	All received annual mammography, MRI, and ultrasound and semiannual CBE A) Women with recall exams (n=18) B) Women without recall exams (n=37)
Wasteson et al, 2011[305] NA	Inclusion: Women enrolled in previous retrospective study of RRM with reconstruction, agreed to participate 10 years later Exclusion: NR	Either BRCA positive or 25%-40% lifetime risk of breast cancer according to Mendelian laws and the estimated penetrance of the *BRCA1/2* mutations, or to Claus tables	3/13 (23.1%) BRCA positive by 10 year followup	Semistructured interviews focused on experiences related to RRM with reconstruction	RRM with reconstruction

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
Brandberg et al, 2008[302] Brandberg et al, 2012[304] NA	October 1997 to December 2005 1 year	**Before RRM vs. 6 months after RRM vs. 1 year after RRM** Mean scales (SE) HADS-A: 5.59 (0.55) vs. 3.80 (0.55) vs. 3.83 (0.52); p=0.0004 HADS-D: 2.53 (0.39) vs. 1.93 (0.31) vs. 1.98 (0.36); p=NS SAQ, pleasure subscale: 12.82 (0.62) vs. 12.21 (0.66) vs. 11.18 (0.56); p=0.005 SAQ, discomfort subscale: 0.56 (0.15) vs. 0.53 (0.20) vs. 0.81 (0.19); p=NS SAQ, habit subscale: 0.94 (0.06) vs. 0.82 (0.08) vs. 0.82 (0.08); p=NS Bodily pain as reported by SF-36: 81.0 (2.98) vs. 80.7 (2.84) vs. 82.6 (3.29); p=NS	Anxiety decreased after surgery, while sexual pleasure increased. All other measures did not change over time.	Swedish Cancer Society, Swedish Association for Cancer and Traffic Victims, and Stockholm County Council

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
		NS difference over time on any portion of impact on areas of life measures, any portion of BIS, and any subscales of SF-36.		
Finch et al, 2011[306] NA	October 2002 to June 2008 1 year	Women experienced a significant worsening of vasomotor symptoms ($p<0.01$) and a decrease in sexual function ($p<0.05$)	Women had worse vasomotor symptoms and decrease in sexual functioning.	Toronto Fashion Show, Kristi Piia Callum Memorial Fellowship in Ovarian Cancer Research, and University of Toronto Open Fellowship
Gahm et al, 2010[303] NA	2004-2006 Mean followup of 29 months (range, 24-49)	A vs. B **Mean SF-36 subscales (estimated from graph)** Physical functioning: 94 vs. 89; p=NS Role functioning: 86 vs. 85; p=NS Bodily pain: 87 vs. 72; p=0.002 General health: 79 vs. 77; p=NS Vitality: 68 vs. 68; p=NS Social functioning: 90 vs. 89; p=NS Role emotional: 80 vs. 85; p=NS Mental health: 80 vs. 80; p=NS **Pain and discomfort questionnaire responses after RRM** 38/55 (69%) pain in breasts 20/55 (36%) pain affected sleep 12/55 (22%) pain affected daily activities 39/55 (71%) discomfort in breasts 48/55 (87%) pain or discomfort in breasts No association between pain and age (OR, 0.99; p=0.771); pain and complication (OR, 0.60; p=0.538); or pain and reoperation (OR, 3.72; p=0.110) Pain or discomfort not related with negative effects in sexual outcomes ($p>0.05$ for both) **Postoperative complications** 11/59 (18.6%) had infections 3/59 (5.1%) required implant extraction 4/59 (6.8%) had hematoma 2/59 (3.4%) required acute operative evacuation 2/59 (3.4%) had revision of flap necrosis 35/59 (59%) had corrective surgical procedures 24/59 (41%) had procedure involving implant pockets **Sexuality questionnaire responses after RRM** 25/55 (45%) totally lost sexual sensations 22/55 (40%) substantially impaired sexual sensations 38/55 (69%) negative change in sexual importance of breasts 41/55 (75%) negative change in sexual enjoyment of breasts 32/55 (58.2%) no change in sexual intercourse Sexual attractiveness changes varied substantially	Women who had RRM had less bodily pain than the reference group, but no other differences on the SF-36. Most women who had RRM experienced pain, discomfort, and decrease in sexual enjoyment, attractiveness, and enjoyment. However, almost all women felt the choice was a good one and would make the same decision.	None

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
		Regret scale responses after RRM 52/55 (94.5%) agreed the decision was right 51/55 (92.7%) would make the same decision again 48/55 (87.3%) said it was a wise decision		
Metcalfe et al, 2004[301] NA	January 1991 to June 2000 Mean time between surgery and questionnaire of 52.2 months (SD, 32.3)	97% were satisfied or extremely satisfied with decision to have RRM **Mean scales (SD) for whole group after RRM** IES-I: 8.44 (8.11); 4/57 (7.0%) scored above clinical cut-off, of these all (100%) had a strong family history of breast cancer and 3/4 (75%) had a mother who died from breast cancer IES-A: 8.79 (8.53); 5/57 (8.8%) scored above clinical cut-off, 3/5 (60%) had a strong family history of breast cancer, 1/5 (20%) had a BRCA mutation, and 1/5 (20%) had a mother who died of breast cancer Sexual activity, pleasure: 12.25 (4.72) Sexual activity, discomfort: 1.97 (2.13) Sexual activity, habit: 1.22 (0.66) BIBC, vulnerability: 2.43 (0.81) BIBC, body concerns: 3.09 (0.99) BIBC, body stigma: 2.33 (0.89) BIBC, transparency: 2.19 (0.79) **Age <50 years vs. ≥50 years** Mean scales (SD) IES-I: 9.07 (8.57) vs. 6.31 (6.10); p=NS IES-A: 8.61 (9.03) vs. 9.38 (6.85); p=NS Sexual activity, pleasure: 12.75 (4.70) vs. 10.25 (4.56); p=NS Sexual activity, discomfort: 1.78 (2.12) vs. 2.88 (2.03); p=NS Sexual activity, habit: 1.18 (0.64) vs. 1.42 (0.79); p=NS BIBC, vulnerability: 2.38 (0.80) vs. 2.60 (0.87); p=NS BIBC, body concerns: 3.12 (1.03) vs. 2.99 (0.86); p=NS BIBC, body stigma: 2.27 (0.91) vs. 2.52 (0.81); p=NS BIBC, transparency: 2.26 (0.86) vs. 1.97 (0.46); p=NS **Postsurgical symptoms** 38 (64.4%) of women reported postsurgical symptoms: numbness (27), pain (7), tingling (7), infection (7), swelling (2), breast hardness (2), bleeding (1), organizing hematoma (1), failed reconstruction (1), breathing complications (1), thrombosis (1), pulmonary embolism (1) 18 women reported only 1 symptom, 15 women reported 2 symptoms, and 5 women reported 3 symptoms as a result of surgery. No difference in reporting of postsurgical symptoms based on time elapsed since mastectomy.	Most women were happy with their decision to have RRM. For most women, the surgery did not cause high levels of distress and there was no correlation with age.	NR

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
Rijnsburger et al, 2004[275] Fair	2000-2002 1-4 weeks after screening	A vs. B vs. C Experienced no pain after screening: 92.6% vs. 14.3% vs. 88.0%; p=NR Experienced no discomfort after screening: 91.5% vs. 30.8% vs. 54.6%; p=NR Experienced no anxiety after screening: 77.9% vs. 72.4% vs. 63.0%; p=NR **Before screening (T0) vs. day of screening (T1) vs. after screening (T2)** Mean VAS: 81.9 vs. 79.0 vs. 80.7; p<0.01 T0 vs. T1 and p<0.05 T1 vs. T2 **Before screening vs. after screening (A, B, and C groups combined) vs. reference group (Dutch general population)** Mean on SF-36 subscales; p=NS for before and after screening Physical functioning: 89.9 vs. 89.4 vs. 86.3; p<0.01 for reference group vs. before screening Role-physical: 85.7 vs. 84.1 vs. 77.6; p<0.01 for reference group vs. before screening Bodily pain: 82.4 vs. 83.0 vs. 72.8; p<0.01 for reference group vs. before screening General health perceptions: 76.4 vs. 77.3 vs. 72.2; p<0.01 for reference group vs. before screening Vitality: 67.1 vs. 68.9 vs. 64.8; p=NS Social functioning: 87.7 vs. 87.9 vs. 83.5; p<0.01 for reference group vs. before screening Role-emotional: 85.2 vs. 88.1 vs. 80.1; p<0.05 for reference group vs. before screening Mental health: 76.8 vs. 77.7 vs. 74.4; p<0.05 for reference group vs. before screening Mean SCL-90: 17.5 vs. 17.1 vs. 18.7; p<0.05 for reference group vs. before screening Mean ED-5D utility score (compared to Swedish reference group): 0.88 vs. 0.88 vs. 0.85; p<0.01 for reference group vs. before screening	Women who received MRI experienced less pain and discomfort than those who received mammography. Women in screening showed better health-related quality of life per the SF-36 than the reference group.	Health Care Insurance Board, the Netherlands
Spiegel et al, 2011[298] NA	Years NR 6 months	**Before screening vs. 4-6 weeks after screening vs. 6 months after screening** Mean HADS-A (SD): 7.15 (4.2) vs. 6.85 (4.5) vs. 6.31 (3.9); NS Mean HADS-D (SD): 2.65 (3.6) vs. 2.60 (3.5) vs. 2.60 (3.5); NS Mean WIS (SD): 10.27 (4.2) vs. 11.07 (4.9) vs. 10.44 (4.7); NS **A vs. B 4-6 weeks after screening** Mean HADS-A (SD): 8.8 (5.2) vs. 5.9 (3.9); p=0.03 Mean HADS-D (SD): 3.3 (4.3) vs. 2.2 (3.1); NS Mean WIS (SD): 13.6 (6.4) vs. 9.8 (3.5); NS **A vs. B 6 months after screening** Mean HADS-A (SD): 7.1 (3.8) vs. 5.9 (4.0); NS Mean HADS-D (SD): 3.1 (4.3) vs. 2.3 (3.1); NS Mean WIS (SD): 12.4 (6.3) vs. 9.4 (3.2); NS	Women who were recalled for examinations after screening had increased anxiety 4-6 weeks after screening, but by 6 months all scores returned to baseline levels.	Canadian Breast Cancer Research Alliance grant #012345 and private donation from Florence and Maury Rosenblatt

Appendix C14. Evidence Table of Psychological and Sexual Functioning Harms of Interventions

Author, year Quality	Duration of followup	Results	Conclusions	Funding source
Wasteson et al, 2011[305] NA	Years NR Median, 10 years (range, 9-12)	**Affects 10 years after RRM with reconstruction** 8/13 (61.5%) stated family life unchanged 4/13 (30.8%) stated positive affect on family life 5/13 (38.5%) stated negative affect on relationship with spouse (due to decreased sensation and changed body appearance) 10/13 (76.9%) considered cosmetic results positive 10/11 (90.9%) had discussed breast cancer risk with daughters	Most women stated positive affects 10 years after RRM with reconstruction.	NR

Abbreviations: BIBC = Body Image after Breast Cancer; BIS = Body Image Scale; RRM = risk-reducing mastectomy; BSI = Brief Symptom Inventory; CBE = clinical breast exam; DRS = Decision Regret Scale; EQ-5D = EuroQoL-5 Dimensions; HADS = Hospital Anxiety and Depression Scale; IES = Impact of Events Scale; MENQOL = Menopause-Specific Quality of Life-Intervention; MRI = magnetic resonance imaging; NA = not applicable; NR = not reported; NS = not significant; OR = odds ratio; RRSO = risk-reducing salpingo-oophorectomy; QOL = quality of life; SAQ = Sexual Activity Questionnaire; SCL-90 = Symptom Checklist-90; SD = standard deviation; SE = standard error; SF-36 = Short-Form 36-Item Health Survey; VAS = Visual Analogue Scale.

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NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)

Genetic/Familial High-Risk Assessment: Breast and Ovarian

Version 2.2014

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Clinical Trials: NCCN believes that the best management for any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

To find clinical trials online at NCCN Member Institutions, click here: nccn.org/clinical_trials/physician.html.

NCCN Categories of Evidence and Consensus: All recommendations are category 2A unless otherwise specified.

See NCCN Categories of Evidence and Consensus.

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NCCN Guidelines Version 2.2014 Updates
Genetic/Familial High-Risk Assessment: Breast and Ovarian

Updates in Version 2.2014 of the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian from Version 1.2014 include:

Breast and Ovarian Cancer Genetic Assessment:
BR/OV-2
- **A link to the new multi-gene testing section was added: "See Multi-Gene Testing (GENE-1)"**
- **Footnote "l" was added: "In some cases, multi-gene testing may be a preferable way to begin testing over the single-gene testing process."**

Hereditary Breast and Ovarian Cancer Syndrome:
HBOC-2
- **Genetic testing: "Consider multi-gene testing, if appropriate" was added. Also for LIFR-2 and COWD-2.**

Multi-Gene Testing
GENE-1
- **The multi-gene testing section is new to the Guidelines and includes:**
  - **An overview of multi-gene testing**
  - **Points in favor of multi-gene testing**
  - **Limitations in the use of multi-gene testing**
  - **Issues to consider in assessing a positive multi-gene test result for less characterized genes**
  - **General recommendations**

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Updates in Version 1.2014 of the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian from Version 4.2013 include:

Breast and Ovarian Cancer Genetic Assessment:

BR/OV-1

- An affected individual
  - 6th bullet was modified: "≥1 family member on same side of family with a combination of breast cancer and ≥1 of the following (especially if early onset): pancreatic cancer, ~~aggressive~~ prostate cancer (Gleason score ≥7)..." (Also for an unaffected individual)
- An unaffected individual
  - 3rd and 4th bullet, "(maternal or paternal)" was removed from "same side of family."

BR/OV-2

- Detailed medical and surgical history
  - 1st bullet was modified by adding: "Any personal cancer history *(eg, age, type, laterality)*."
- Focused physical exam was revised to include: "conducted by *qualified clinician* ~~physician or nurse~~."

Hereditary Breast and/or Ovarian Cancer Syndrome Testing Criteria:

HBOC-1

- 2nd bullet, this section for "Personal history of breast cancer + one or more of the following" was reorganized by age.
  - 2nd sub-bullet, the 3rd tertiary bullet was revised by adding: "*An unknown or* limited family history."
  - 4th sub-bullet, the 4th tertiary bullet was revised: "≥2 close blood relatives with pancreatic cancer *and*/or ~~aggressive~~ prostate cancer (Gleason score ≥7) at any age."
- 5th bullet modified by removing "aggressive" from "prostate cancer."
  - A new sub-bullet was added: "For pancreatic cancer, if Ashkenazi Jewish ancestry, only one additional affected relative is needed."
- After criteria, "If HBOC testing criteria not met" was clarified by adding: "*consider testing for other hereditary syndromes.*" Then text after this was clarified by adding, "If criteria for other *hereditary syndromes not met, then* cancer screening as per NCCN Screening Guidelines."
- Footnote "a" was combined with another footnote and revised as: "*Meeting one or more of these criteria warrants further personalized risk assessment, genetic counseling, and often genetic testing and management. The probability of mutation detection associated with these criteria will vary based on family structure.* Individuals with *unknown* or limited family history/structure, such as fewer than 2 first- or second-degree female relatives having lived beyond age 45 in either lineage, may have an underestimated probability of familial mutation *detection. The likelihood of mutation detection may be very low in families with a large number of unaffected female relatives. Clinical judgment should be used to determine the appropriateness of genetic testing.* The maternal and paternal sides should be considered independently."

HBOC-2

- Footnote "n" was modified by removing: "If all affected family members are deceased, consider testing of paraffin-derived DNA from deceased relatives, if DNA is obtainable."

HBOC-A 1 of 2

- HBOC syndrome management for women
  - 3rd bullet, the previous recommendation, "Annual mammogram and breast MRI screening starting at age 25 y, or individualized based on earliest age of onset in family," was replaced by:
    - Breast screening
      - Age 25–29 y, annual breast MRI screening (preferred) or mammogram if MRI is unavailable or individualized based on earliest age of onset in family.
      - Age >30–75 y, annual mammogram and breast MRI screening.
      - Age >75 y, management should be considered on an individual basis.
- Footnote "3" was revised by removing: "The best screening strategy for woman age 25-30 is uncertain with some data suggesting that mammogram be added to MRI only after age 30."
- Footnote "5" was modified: "Given the high rate of occult neoplasms,...*See NCCN Guidelines for Ovarian Cancer for treatment of findings.*"

HBOC-A 2 of 2

- HBOC syndrome management for men
  - 4th bullet, "Consider prostate cancer screening starting at age 40 y" was replaced by,
    - Starting at age 40:
      - Recommend prostate cancer screening for *BRCA2* carriers
      - Consider prostate cancer screening for *BRCA1* carriers
  - Footnote "8" was added: "There are only limited data to support breast imaging in men."

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Updates in Version 1.2014 of the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian from Version 4.2013 include:

Li-Fraumeni Syndrome:

LIFR-1

- Early-age-onset breast cancer criteria was revised: "Individual with breast cancer ≤35 y ~~with a negative BRCA1/BRCA2 test~~, *TP53 testing can be ordered concurrently with BRCA1/2 testing or as a follow-up test after negative BRCA1/2 testing.*"

LIFR-A

- Breast cancer risk for women
  - 3rd bullet, the previous recommendation, "Annual mammogram and breast MRI screening starting at age 20-25 y, or individualized based on earliest age of onset in family" was replaced by:
    - Breast screening
      - Age 20–29 y, annual breast MRI screening (preferred) or mammogram if MRI is unavailable or individualized based on earliest age of onset in family.
      - Age >30–75 y, annual mammogram and breast MRI screening.
      - Age >75y, management should be considered on an individual basis.
- Other cancer risks
  - 3rd bullet was modified: "Therapeutic RT for cancer should be *avoided when possible* ~~used with caution~~."
  - 6th bullet was revised as: "Discuss option to participate in novel screening approaches using technologies ~~within clinical trials when possible~~..."
  - Footnote was removed: "Given theoretical concerns with harmful effects of radiation exposure in LFS, for patients age 20-30 y, annual MRI-only screening may be sufficient based on physician's discretion."

Cowden Syndrome/PTEN Hamartoma Tumor Syndrome:

COWD-1

- "PTEN hamartoma tumor syndrome" (PHTS) was added to "Cowden syndrome."
- Footnote "c" reference was updated to: "Pilarski R, Burt R, Kohlmann W, Pho L, Shannon KM, Swisher E. Cowden syndrome and the PTEN Hamartoma Tumor Syndrome: Systematic review and revised diagnostic criteria. J Natl Cancer Inst 2013;105:1607-1616."

COWD-2

- For no known familial *PTEN* mutation
  - After test outcomes for "Not tested or no mutation found" and "Variant of unknown significance found (uninformative)," the algorithm choices are now "Meets CS/PHTS diagnostic criteria (See COWD-3)" and "Does not meet CS/PHTS diagnostic criteria (See COWD-3)."

COWD-3

- A new page titled, "Revised PTEN Hamartoma Tumor Syndrome Clinical Diagnostic Criteria" was added to the guidelines.

COWD-A

- Cowden syndrome/PHTS management for women
  - 4th bullet was modified as: "For endometrial cancer screening, encourage patient education and prompt response to symptoms ~~and participation in a clinical trial to determine the effectiveness and necessity of screening modalities~~. *Consider annual random endometrial biopsies and/or ultrasound beginning at age 30–35 y.*"
- Cowden syndrome management for men and women
  - 1st bullet was modified: "...with particular attention to ~~breast and~~ thyroid exam."
  - 2nd bullet was revised as: "~~Baseline~~ *Annual* thyroid ultrasound *starting* at age 18 y *or 5–10 y before the earliest known thyroid cancer in the family, whichever is earlier.*"
  - 3rd bullet was revised as: "~~Consider~~ Colonoscopy, starting at age 35 y, then every 5-~~10~~ y or more frequently..."
  - 4th bullet was added: "Consider renal ultrasound starting at age 40 y, then every 1–2 y."
  - 5th bullet was changed from "Consider annual dermatologic exam" to "Dermatologic management may be indicated for some patients."
  - 6th bullet was added: "Consider psychomotor assessment in children at diagnosis and brain MRI if there are symptoms."

Note: All recommendations are category 2A unless otherwise indicated.
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Breast and/or Ovarian Cancer Genetic Assessment

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CRITERIA FOR FURTHER GENETIC RISK EVALUATION[a]

An affected individual with one or more of the following:

- A known mutation in a breast cancer susceptibility gene within the family
- Early-age-onset breast cancer[b]
- Triple negative (ER-, PR-, HER2-) breast cancer
- Two breast cancer primaries[c] in a single individual
- Breast cancer at any age, and
  - ≥1 close blood relative[d] with breast cancer ≤50 y, or
  - ≥1 close blood relative[d] with epithelial ovarian[e] cancer at any age, or
  - ≥2 close blood relatives[d] with breast cancer and/or pancreatic cancer at any age
  - From a population at increased risk[f]
- ≥1 family member on same side of family with a combination of breast cancer and ≥1 of the following (especially if early onset): pancreatic cancer, prostate cancer (Gleason score ≥7), sarcoma, adrenocortical carcinoma, brain tumors, endometrial cancer, leukemia/lymphoma; thyroid cancer, dermatologic manifestations[g,h] and/or macrocephaly, hamartomatous polyps of GI tract;[h] diffuse gastric cancer[i]
- Ovarian[e] cancer
- Male breast cancer

An unaffected individual with a family history of one or more of the following:[f]

- A known mutation in a breast cancer susceptibility gene within the family
- ≥2 breast primaries in single individual[d]
- ≥2 individuals with breast primaries on the same side of family[d]
- ≥1 ovarian[e] cancer primary from the same side of family
- First- or second-degree relative with breast cancer ≤45 y
- ≥1 family member on same side of family with a combination of breast cancer and ≥1 of the following (especially if early onset): pancreatic cancer, prostate cancer (Gleason score ≥7), sarcoma, adrenocortical carcinoma, brain tumors, endometrial cancer, leukemia/lymphoma; thyroid cancer, dermatologic manifestations[g,h] and/or macrocephaly, hamartomatous polyps of GI tract;[h] diffuse gastric cancer[i]
- Male breast cancer

→ **Referral to cancer genetics professional recommended[j]** → **See Assessment (BR/OV-2)**

[a] The criteria for further risk evaluation and genetic testing are not identical. For the purposes of these guidelines, invasive and ductal carcinoma in situ breast cancers should be included. The maternal and paternal sides of the family should be considered independently for familial patterns of cancer.

[b] Clinically use age ≤50 y because studies define early onset as either ≤40 or ≤50 y.

[c] Two breast primaries includes bilateral (contralateral) disease or two or more clearly separate ipsilateral primary tumors either synchronously or asynchronously.

[d] Close blood relatives include first-, second-, and third-degree relatives. (See BR/OV-3)

[e] For the purposes of these guidelines, fallopian tube and primary peritoneal cancers are included. Ovarian/fallopian tube/primary peritoneal cancers are component tumors of Lynch syndrome/hereditary non-polyposis colorectal cancer; be attentive for clinical evidence of this syndrome. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.

[f] For populations at increased risk, requirements for inclusion may be modified (eg, women of Ashkenazi Jewish descent with breast or ovarian or pancreatic cancer at any age).

[g] For dermatologic manifestations, see COWD-1.

[h] For hamartomatous colon polyps in conjunction with breast cancer and hyperpigmented macules of the lips and oral mucosa, STK11 testing should be considered. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal- Peutz-Jeghers syndrome. Melanoma has been reported in some HBOC families.

[i] For lobular breast cancer with a family history of diffuse gastric cancer, *CDH1* gene testing should be considered.

[j] Genetic counseling is highly recommended when genetic testing is offered and after results are disclosed. A genetic counselor, medical geneticist, oncologist, surgeon, oncology nurse, or other health professional with expertise and experience in cancer genetics should be involved early in counseling patients who potentially meet criteria for an inherited syndrome.

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ASSESSMENT

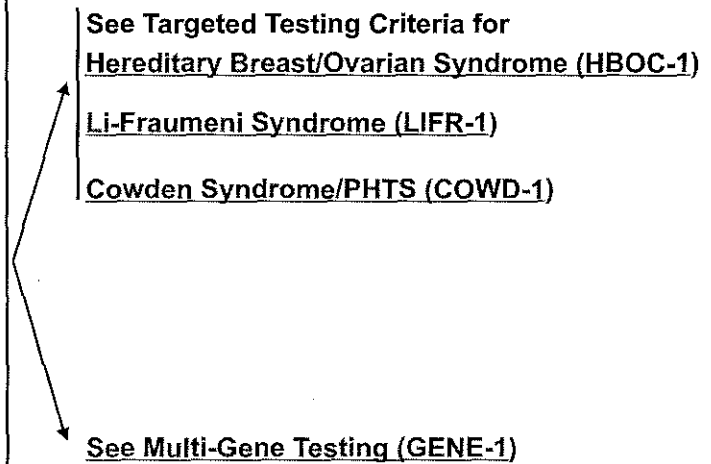
Patient needs and concerns:
- Knowledge of genetic testing for cancer risk, including benefits, risks, and limitations
- Goals for cancer family risk assessment

Detailed family history:
- Expanded pedigree to include first-, second-, and third-degree relatives (parents, siblings, children, grandparents, aunts, uncles, nieces, nephews, grandchildren, half-siblings, great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins) (See BR/OV-3)
- Types of cancer, bilaterality, age at diagnosis
- History of chemoprevention and/or risk-reducing surgery
- Medical record documentation as needed, particularly pathology reports of primary cancers

Detailed medical and surgical history:
- Any personal cancer history (eg, age, type, laterality)
- Carcinogen exposure (eg, history of radiation therapy)
- Reproductive history
- Hormone use
- Previous breast biopsies
- History of salpingo-oophorectomy

Focused physical exam (conducted by qualified clinician):
- Breast/ovarian
- Cowden syndrome/PHTS specific:
  - Dermatologic,[k] including oral mucosa
  - Head circumference
  - Thyroid (enlarged or nodular on palpation)

GENE TESTING[l]



[k]For Cowden syndrome dermatologic manifestations, see COWD-1 and for PJS dermatologic manifestations, see NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.

[l]In some cases, multi-gene testing may be a preferable way to begin testing over the single-gene testing process.

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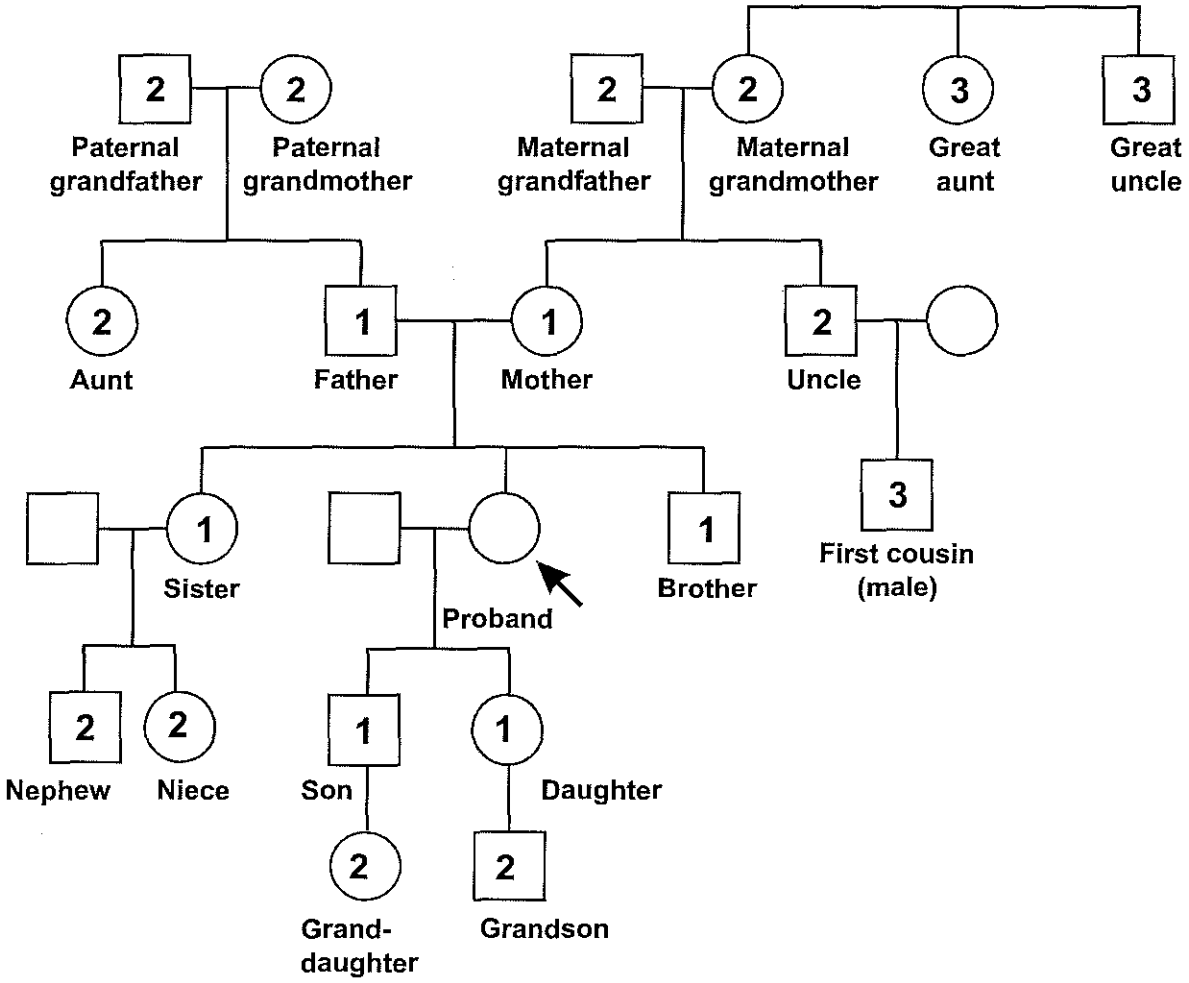
PEDIGREE: FIRST-, SECOND-, AND THIRD-DEGREE RELATIVES OF PROBAND[m]



[m]First-degree relatives: parents, siblings, and children;
second-degree relatives: grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings;
third-degree relatives: great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins.

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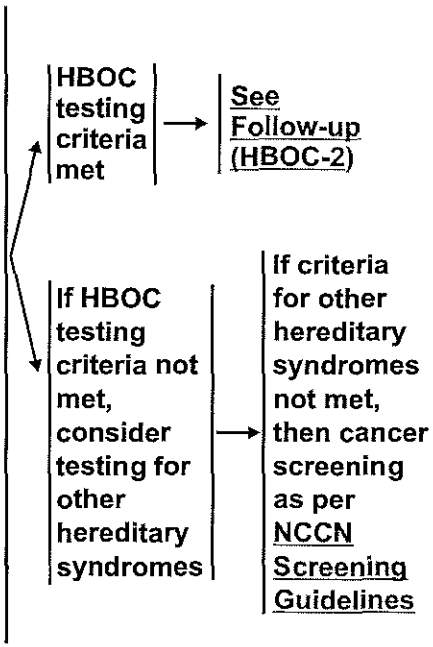
HEREDITARY BREAST AND/OR OVARIAN CANCER SYNDROME TESTING CRITERIA[a,b,c]

- **Individual from a family with a known deleterious *BRCA1/BRCA2* mutation**
- **Personal history of breast cancer[b] + one or more of the following:**
  - Diagnosed ≤45 y
  - Diagnosed ≤50 y with:
    - An additional primary[d]
    - ≥1 close blood relative[e] with breast cancer at any age
    - An unknown or limited family history[a]
  - Diagnosed ≤60 y with a:
    - Triple negative breast cancer
  - Diagnosed at any age with:
    - ≥1 close blood relative[e] with breast cancer diagnosed ≤50 y
    - ≥2 close blood relatives[e] with breast cancer at any age
    - ≥1 close blood relative[e] with epithelial ovarian[f] cancer
    - ≥2 close blood relatives[e] with pancreatic cancer and/or prostate cancer (Gleason score ≥7) at any age
    - A close male blood relative[e] with breast cancer
    - For an individual of ethnicity associated with higher mutation frequency (eg, Ashkenazi Jewish) no additional family history may be required[g]
- **Personal history of epithelial ovarian[f] cancer**
- **Personal history of male breast cancer**
- **Personal history of pancreatic cancer or prostate cancer (Gleason score ≥7) at any age with ≥2 close blood relatives[e] with breast and/or ovarian[f] and/or pancreatic or prostate cancer (Gleason score ≥7) at any age**
  - **For pancreatic cancer, if Ashkenazi Jewish ancestry, only one additional affected relative is needed**
- **Family history only (significant limitations of interpreting test results for an unaffected individual should be discussed):**
  - **First- or second-degree blood relative meeting any of the above criteria**
  - **Third-degree blood relative with breast cancer[b] and/or ovarian[f] cancer with ≥2 close blood relatives[e] with breast cancer (at least one with breast cancer ≤50 y) and/or ovarian[f] cancer**
  - **Clinical judgment should be used to determine if the patient has reasonable likelihood of a mutation, considering the unaffected patient's current age and the age of female unaffected relatives who link the patient with the affected relatives.**
  - **Testing of unaffected individuals should only be considered when an appropriate affected family member is unavailable for testing.**



[a] Meeting one or more of these criteria warrants further personalized risk assessment, genetic counseling, and often genetic testing and management. The probability of mutation detection associated with these criteria will vary based on family structure. Individuals with unknown or limited family history/structure, such as fewer than 2 first- or second-degree female relatives having lived beyond age 45 in either lineage, may have an underestimated probability of familial mutation detection. The likelihood of mutation detection may be very low in families with a large number of unaffected female relatives. Clinical judgment should be used to determine the appropriateness of genetic testing. The maternal and paternal sides should be considered independently.

[b] For the purposes of these guidelines, invasive and ductal carcinoma in situ breast cancers should be included.

[c] Patients who have received an allogeneic bone marrow transplant should not have molecular genetic testing via blood or buccal samples due to unreliable test results from contamination by donor DNA. If available, DNA should be extracted from a fibroblast culture. If this source of DNA is not possible, buccal samples can be considered, subject to the risk of donor DNA contamination.

[d] Two breast primaries includes bilateral (contralateral) disease or two or more clearly separate ipsilateral primary tumors either synchronously or asynchronously.

[e] Close blood relatives include first-, second-, and third-degree relatives on same side of family. (See BR/OV-3)

[f] For the purposes of these guidelines, fallopian tube and primary peritoneal cancers are included. Ovarian/fallopian tube/primary peritoneal cancers are component tumors of Lynch syndrome/hereditary non-polyposis colorectal cancer; be attentive for clinical evidence of this syndrome. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.

[g] Testing for Ashkenazi Jewish founder-specific mutation(s) should be performed first. Full sequencing may be considered if ancestry also includes non-Ashkenazi Jewish relatives or if other HBOC criteria are met. Founder mutations exist in other populations.

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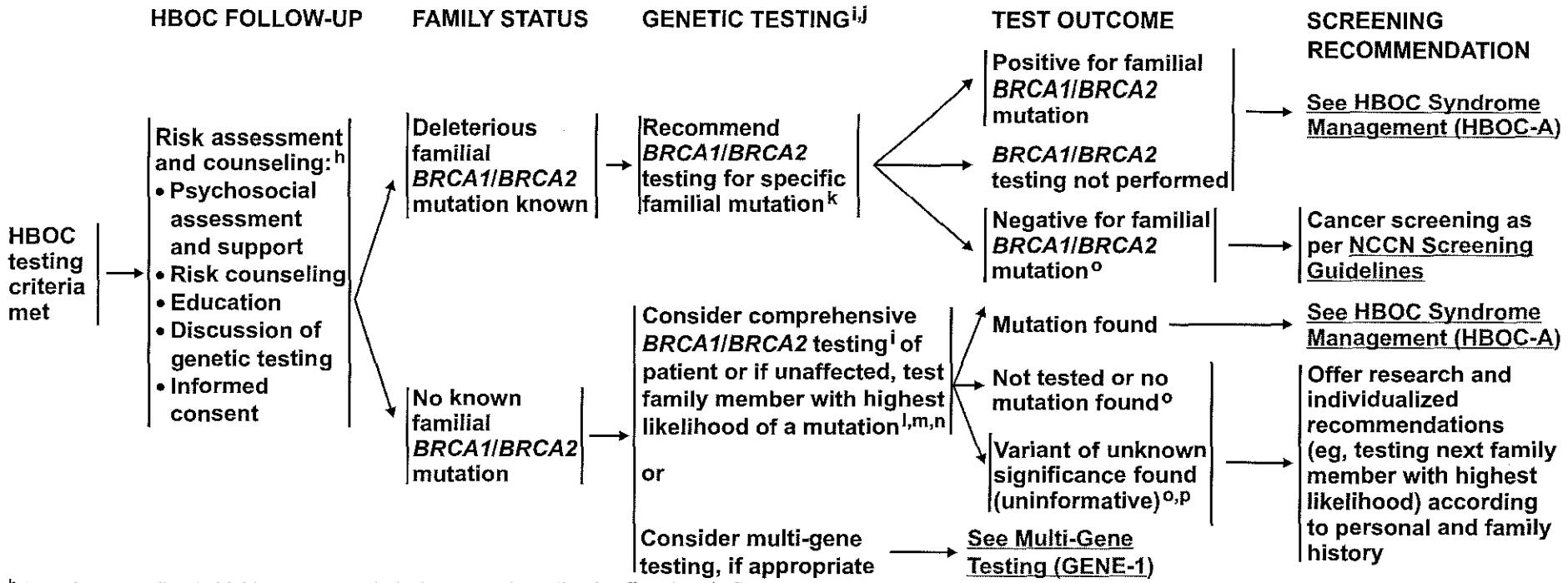

[h] Genetic counseling is highly recommended when genetic testing is offered and after results are disclosed. A genetic counselor, medical geneticist, oncologist, surgeon, oncology nurse, or other health professional with expertise and experience in cancer genetics should be involved early in counseling patients who potentially meet criteria for an inherited syndrome.

[i] Comprehensive genetic testing includes full sequencing of *BRCA1/BRCA2* and testing for large genomic rearrangements.

[j] Genetic testing for familial *BRCA1/2* in children <18 y is generally not recommended.

[k] If of Ashkenazi Jewish descent, in addition to the specific familial mutation, test for all three founder mutations.

[l] Testing of unaffected family members when no affected member is available should be considered. Significant limitations of interpreting test results should be discussed.

[m] If more than one family member affected, first consider: youngest age at diagnosis, bilateral disease, multiple primaries, ovarian cancer, and most closely related to the proband/patient. If no living family member with breast or ovarian cancer, consider testing first- or second-degree family members affected with cancers thought to be related to *BRCA1/BRCA2* (eg, prostate, pancreas, melanoma).

[n] For both affected and unaffected individuals of Ashkenazi Jewish descent with no known familial mutation, first test for the three common mutations. Then, if negative for the three mutations and ancestry also includes non-Ashkenazi Jewish relatives or other HBOC criteria are met, consider comprehensive genetic testing. For both affected and unaffected individuals who are non-Ashkenazi Jewish and who have no known familial mutation, comprehensive genetic testing is the approach, if done.

[o] If no mutation found, consider other hereditary breast/ovarian cancer syndromes such as Li-Fraumeni (LIFR-1) and/or Cowden syndrome (COWD-1) or multi-gene testing (GENE-1). For additional information on other genetic mutations associated with breast/ovarian cancer risk for which genetic testing is clinically available, see ADDIT-1.

[p] Testing family members for a variant of unknown significance should not be used for clinical purposes. Consider referral to research studies that aim to define functional impact of variant.

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HBOC SYNDROME MANAGEMENT (1 of 2)

WOMEN

- **Breast awareness[1] starting at age 18 y.**
- **Clinical breast exam, every 6–12 mo,[2] starting at age 25 y.**
- **Breast screening[3]**
  - **Age 25–29 y, annual breast MRI[4] screening (preferred) or mammogram if MRI is unavailable or individualized based on earliest age of onset in family.**
  - **Age ≥30–75 y, annual mammogram and breast MRI[4] screening.**
  - **Age >75 y, management should be considered on an individual basis.**
- **Discuss risk-reducing mastectomy**
  - **Counseling may include a discussion regarding degree of protection, reconstruction options, and risks.**
- **Recommend risk-reducing salpingo-oophorectomy,[5] ideally between 35 and 40 y, and upon completion of child bearing, or individualized based on earliest age of onset of ovarian cancer in the family.**
  - **Counseling includes a discussion of reproductive desires, extent of cancer risk, degree of protection for breast and ovarian cancer, management of menopausal symptoms, possible short-term hormone replacement therapy to a recommended maximum age of natural menopause, and related medical issues.**
- **Address psychosocial, social, and quality-of-life aspects of undergoing risk-reducing mastectomy and/or salpingo-oophorectomy.**
- **For those patients who have not elected risk-reducing salpingo-oophorectomy, consider concurrent transvaginal ultrasound (preferably day 1–10 of menstrual cycle in premenopausal women) + CA-125 (preferably after day 5 of menstrual cycle in premenopausal women)[6] every 6 mo starting at age 30 y or 5–10 y before the earliest age of first diagnosis of ovarian cancer in the family.**
- **Consider chemoprevention options for breast and ovarian cancer, including discussing risks and benefits[7] (See NCCN Guidelines for Breast Cancer Risk Reduction).**
- **Consider investigational imaging and screening studies, when available (eg, novel imaging technologies, more frequent screening intervals) in the context of a clinical trial.**

Continued on next page

[1] Women should be familiar with their breasts and promptly report changes to their health care provider. Periodic, consistent breast self exam (BSE) may facilitate breast self awareness. Premenopausal women may find BSE most informative when performed at the end of menses.

[2] Randomized trials comparing clinical breast exam versus no screening have not been performed. Rationale for recommending clinical breast exam every 6–12 mo is the concern for interval breast cancers.

[3] The appropriateness of imaging modalities and scheduling is still under study. Lowry KP, et al. Annual screening strategies in BRCA1 and BRCA2 gene mutation carriers: a comparative effectiveness analysis. Cancer 2012;118:2021-2030.

[4] High-quality breast MRI limitations include having: a need for a dedicated breast coil, the ability to perform biopsy under MRI guidance, experienced radiologists in breast MRI, and regional availability. Breast MRI is performed preferably days 7–15 of menstrual cycle for premenopausal women.

[5] Given the high rate of occult neoplasms, special attention should be given to sampling and pathologic review of the ovaries and fallopian tubes. (See Discussion for details.) See NCCN Guidelines for Ovarian Cancer for treatment of findings. See the College of American Pathologists, Protocol for the Examination of Specimens from Patients with Carcinoma of the Ovary.

[6] There are data that show that annual transvaginal ultrasound and CA-125 are not effective strategies for screening for ovarian cancer in high-risk women. There are limited data regarding the effectiveness of a six-month screening interval. Thus, until such data are available it is reasonable to consider this approach in high-risk women, especially in the context of a clinical research setting.

[7] Data suggest that oral contraceptives (OCs) reduce ovarian cancer risk in *BRCA* mutation carriers. The risk/benefit ratio is uncertain because of contradictory evidence about OCs increasing breast cancer risk; however, OC use for contraception is acceptable. Other chemoprevention options for breast cancer include tamoxifen and raloxifene; however, only limited data with these agents are available in patients with *BRCA* mutations. (See Discussion for details.)

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HBOC SYNDROME MANAGEMENT (2 of 2)

MEN

- Breast self-exam training and education starting at age 35 y
- Clinical breast exam, every 6–12 mo, starting at age 35 y
- Consider baseline mammogram at age 40 y;[8] annual mammogram if gynecomastia or parenchymal/glandular breast density on baseline study.
- Starting at age 40 y:
  - Recommend prostate cancer screening for *BRCA2* carriers
  - Consider prostate cancer screening for *BRCA1* carriers

MEN AND WOMEN

- Education regarding signs and symptoms of cancer(s), especially those associated with *BRCA* gene mutations.
- No specific guidelines exist for pancreatic cancer and melanoma, but screening may be individualized based on cancers observed in the family.[9]

RISK TO RELATIVES

- Advise about possible inherited cancer risk to relatives, options for risk assessment, and management.
- Recommend genetic counseling and consideration of genetic testing for at-risk relatives.

REPRODUCTIVE OPTIONS

- For patients of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including pre-implantation genetic diagnosis. Discussion should include known risks, limitations, and benefits of these technologies. See Discussion for details.
- For *BRCA2* mutation carriers, risk of a rare (recessive) Fanconi anemia/brain tumor phenotype in offspring should be discussed if both partners carry a *BRCA2* mutation.[10]

[8]There are only limited data to support breast imaging in men.
[9]Consider full-body skin exam for melanoma and investigational protocols for pancreatic cancer.
[10]Offit K, Levran O, Mullaney B, et al. Shared genetic susceptibility to breast cancer, brain tumors, and Fanconi anemia. J Natl Cancer Inst 2003;95:1548-1551.

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LI-FRAUMENI SYNDROME TESTING CRITERIA

- **Individual from a family with a known *TP53* mutation**
- **Classic Li-Fraumeni syndrome (LFS) criteria:[a]**
  - **Combination of an individual diagnosed age <45 y with a sarcoma[b]**
    AND
    A first-degree relative diagnosed age <45 y with cancer
    AND
    An additional first- or second-degree relative in the same lineage with cancer diagnosed age <45 y, or a sarcoma at any age
- **Chompret criteria:[c,d]**
  - **Individual with a tumor from LFS tumor spectrum (eg, soft tissue sarcoma, osteosarcoma, brain tumor, breast cancer, adrenocortical carcinoma, leukemia, lung bronchoalveolar cancer) before 46 years of age, AND at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) before the age of 56 years or with multiple primaries at any age**
    OR
  - **Individual with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum with the initial cancer occurring before the age of 46 years**
    OR
  - **Individual with adrenocortical carcinoma or choroid plexus carcinoma[d,e] at any age of onset, regardless of the family history**
- **Early-age-onset breast cancer:**
  - **Individual with breast cancer ≤35 y, *TP53* testing can be ordered concurrently with *BRCA1/2* testing or as a follow-up test after negative *BRCA1/2* testing**

FOLLOW-UP

LFS testing criteria met[f] → **See Follow-up (LIFR-2)**

LFS testing criteria not met → **Individualized recommendations according to personal and family history**

Cancers associated with LFS include but are not limited to:
- **Premenopausal breast cancer**
- **Bone and soft tissue sarcomas**
- **Acute leukemia**
- **Brain tumor**
- **Adrenocortical carcinoma**
- **Choroid plexus carcinoma**
- **Colon cancer**
- **Early onset of other adenocarcinomas or other childhood cancers**

[a] Li FP, Fraumeni JF, Jr., Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988;48:5358-5362.

[b] To date, there have been no reports of Ewing sarcoma, GIST, desmoid tumor, or angiosarcoma in *TP53* mutation carriers.

[c] Chompret A, Abel A, Stoppa-Lyonnet D, et al. Sensitivity and predictive value of criteria for p53 germline mutation screening. J Med Genet 2001;38:43-47.

[d] Tinat J, Bougeard G, Baert-Desurmont S, et al. 2009 version of the Chompret criteria for Li Fraumeni syndrome. J Clin Oncol 2009;27:e108-9.

[e] Gonzalez KD, Noltner KA, Buzin CH, et al. Beyond Li Fraumeni Syndrome: Clinical characteristics of families with p53 germline mutations. J Clin Oncol 2009;27:1250-1256.

[f] Patients who have received an allogeneic bone marrow transplant should not have molecular genetic testing via blood or buccal samples due to unreliable test results from contamination by donor DNA. If available, DNA should be extracted from a fibroblast culture. If this source of DNA is not possible, buccal samples can be considered, subject to the risk of donor DNA contamination.

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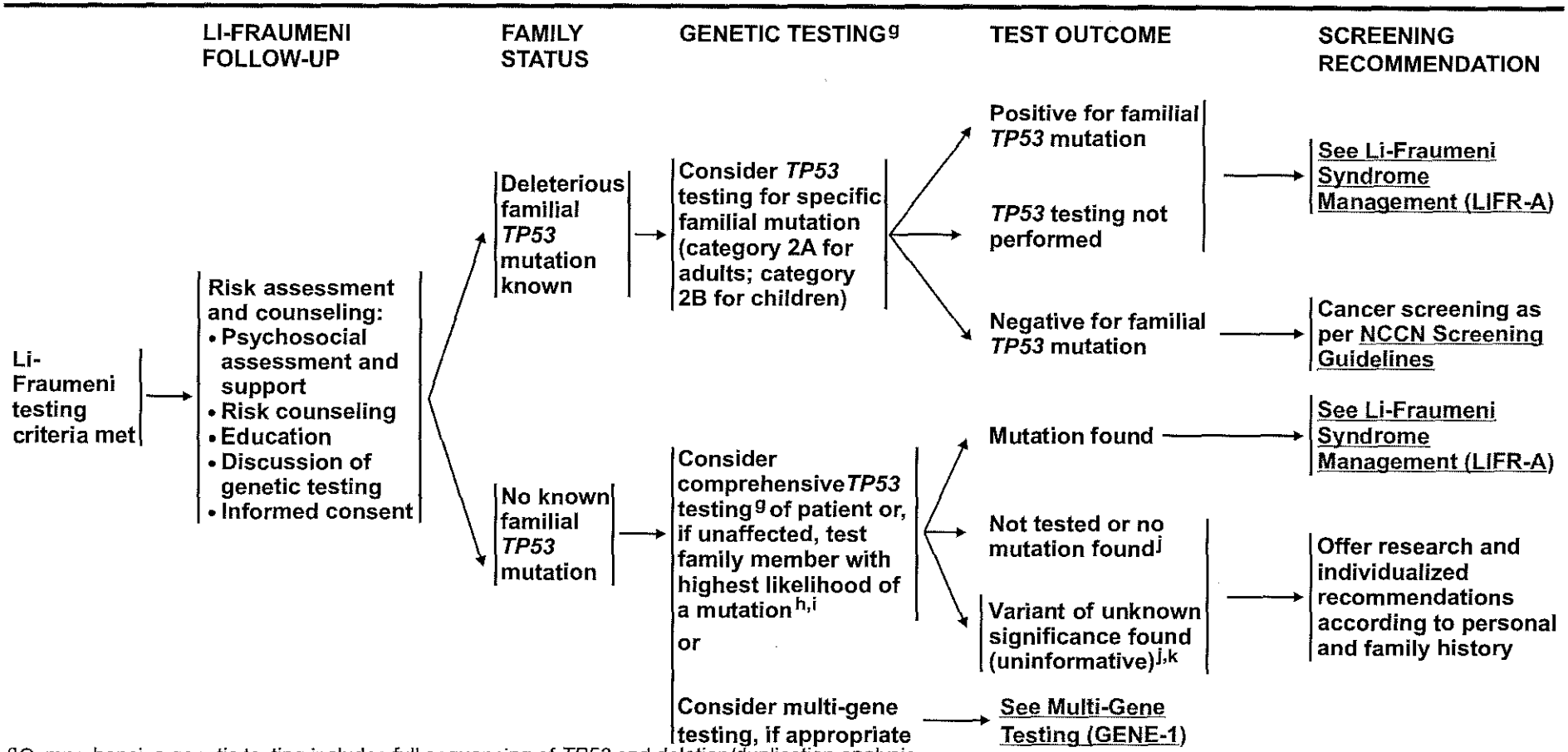

[g]Comprehensive genetic testing includes full sequencing of *TP53* and deletion/duplication analysis.

[h]Youngest age at diagnosis, bilateral disease, multiple primaries, or sarcoma at age <45 y.

[i]Testing of unaffected family members when no affected member is available may be considered. Significant limitations of interpreting test results should be discussed.

[j]If no mutation is found, consider other hereditary breast cancer syndromes such as HBOC (HBOC-1) and/or Cowden syndrome (COWD-1) or multi-gene testing (GENE-1). For additional information on other genetic mutations associated with breast/ovarian cancer risk for which genetic testing is clinically available, see ADDIT-1.

[k]Testing family members for a variant of unknown significance should not be used for clinical purposes. Consider referral to research studies that aim to define functional impact of variant.

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LI-FRAUMENI SYNDROME MANAGEMENT

BREAST CANCER RISK FOR WOMEN

- **Breast awareness[1] starting at age 18 y.**
- **Clinical breast exam, every 6–12 mo, starting at age 20–25 y or 5–10 y before the earliest known breast cancer in the family (whichever comes first).**
- **Breast screening[2]**
  - **Age 20–29 y, annual breast MRI[3] screening (preferred) or mammogram if MRI is unavailable or individualized based on earliest age of onset in family.**
  - **Age >30–75 y, annual mammogram and breast MRI[3] screening**
  - **Age >75 y, management should be considered on an individual basis.**
- **Discuss risk-reducing mastectomy and counsel regarding degree of protection, degree of cancer risk, and reconstruction options.**
- **Address psychosocial, social, and quality-of-life aspects of undergoing risk-reducing mastectomy.**

OTHER CANCER RISKS

- **Address limitations of screening for many cancers associated with LFS. Because of the remarkable risk of additional primary neoplasms, screening may be considered for cancer survivors with LFS and a good prognosis from their prior tumor(s).**
- **Annual comprehensive physical exam with high index of suspicion for rare cancers and second malignancies in cancer survivors: include careful skin and neurologic examinations.**
- **Therapeutic RT for cancer should be avoided when possible.**
- **Consider colonoscopy every 2–5 y starting no later than 25 y.**
- **Pediatricians should be apprised of the risk of childhood cancers in affected families.**
- **Discuss option to participate in novel screening approaches using technologies, such as whole-body MRI, abdominal ultrasound, and brain MRI.[4]**
- **Additional surveillance based on individual family histories.**
- **Education regarding signs and symptoms of cancer.**

Continued on next page

[1]Women should be familiar with their breasts and promptly report changes to their health care provider. Periodic, consistent breast self exam (BSE) may facilitate breast self awareness. Premenopausal women may find BSE most informative when performed at the end of menses.

[2]The appropriateness of imaging modalities and scheduling is still under study.

[3]High-quality breast MRI limitations include having: a need for a dedicated breast coil, the ability to perform biopsy under MRI guidance, experienced radiologists in breast MRI, and regional availability. Breast MRI is performed preferably days 7–15 of menstrual cycle for premenopausal women.

[4]A surveillance study has been published that utilizes these screening approaches (Villani A, Tabori U, Schiffman J, et al. Lancet Oncol 2011;12:559-567). See Discussion.

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LI-FRAUMENI SYNDROME MANAGEMENT

REPRODUCTIVE OPTIONS

- **For patients of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including pre-implantation genetic diagnosis. Discussion should include known risks, limitations, and benefits of these technologies. See Discussion for details.**

RISK TO RELATIVES

- **Advise about possible inherited cancer risk to relatives, options for risk assessment, and management.**
- **Recommend genetic counseling and consideration of genetic testing for at-risk relatives.**

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NCCN Guidelines Version 2.2014
Cowden Syndrome/PHTS

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Discussion

COWDEN SYNDROME/PTEN HAMARTOMA TUMOR SYNDROME TESTING CRITERIA[a,b]

- Individual from a family with a known *PTEN* mutation
- Individual meeting clinical diagnostic criteria[c] for CS/PHTS
- Individual with a personal history of:
  - Bannayan-Riley-Ruvalcaba syndrome (BRRS) or
  - Adult Lhermitte-Duclos disease (cerebellar tumors) or
  - Autism spectrum disorder and macrocephaly or
  - Two or more biopsy-proven trichilemmomas or
  - Two or more major criteria (one must be macrocephaly) or
  - Three major criteria, without macrocephaly or
  - One major and ≥3 minor criteria[d] or
  - ≥4 minor criteria
- At-risk individual[e] with a relative with a clinical diagnosis of CS/PHTS or BRRS for whom testing has not been performed
  - The at-risk individual must have the following:
    - Any one major criterion or
    - Two minor criteria

FOLLOW-UP

- CS/PHTS testing criteria met[f] → See Follow-up (COWD-2)
- CS/PHTS testing criteria not met → Individualized recommendations according to personal and family history

Major criteria:
- Breast cancer
- Endometrial cancer
- Follicular thyroid cancer
- Multiple GI hamartomas or ganglioneuromas
- Macrocephaly (megalocephaly) (ie, ≥97%, 58 cm in adult women, 60 cm in adult men)[g]
- Macular pigmentation of glans penis
- Mucocutaneous lesions[h]
  - One biopsy-proven trichilemmoma
  - Multiple palmoplantar keratoses
  - Multifocal or extensive oral mucosal papillomatosis
  - Multiple cutaneous facial papules (often verrucous)

Minor criteria:[i]
- Autism spectrum disorder
- Colon cancer
- ≥3 esophageal glycogenic acanthoses
- Lipomas
- Mental retardation (ie, IQ ≤75)
- Papillary or follicular variant of papillary thyroid cancer
- Thyroid structural lesions (eg, adenoma, nodule(s), goiter)
- Renal cell carcinoma
- Single GI hamartoma or ganglioneuroma
- Testicular lipomatosis
- Vascular anomalies (including multiple intracranial developmental venous anomalies)

[a] These are testing criteria, clinical diagnostic criteria on COWD-3.

[b] If two criteria involve the same structure/organ/tissue, both may be included as criteria.

[c] Pilarski R, Burt R, Kohlmann W, Pho L, Shannon KM, Swisher E. Cowden syndrome and the PTEN Hamartoma Tumor Syndrome: Systematic review and revised diagnostic criteria. J Natl Cancer Inst 2013;105:1607-1616.

[d] If an individual has two or more major criteria, such as breast cancer and non-medullary thyroid cancer, but does not have macrocephaly, one of the major criteria may be included as one of the three minor criteria to meet testing criteria.

[e] An at-risk individual can be defined as a first-degree relative of an affected individual and/or proband. If a first-degree relative is unavailable or unwilling to be tested, more distant relatives should be offered testing.

[f] Patients who have received an allogeneic bone marrow transplant should not have molecular genetic testing via blood or buccal samples due to unreliable test results from contamination by donor DNA. If available, DNA should be extracted from a fibroblast culture. If this source of DNA is not possible, buccal samples can be considered, subject to the risk of donor DNA contamination.

[g] Roche AF, Mukherjee D, Guo SM, Moore WM. Head circumference reference data: Birth to 18 years. Pediatrics 1987;79:706-712.

[h] The literature available on mucocutaneous lesions is not adequate to accurately specify the number or extent of mucocutaneous lesions required to be a major criterion for CS/PHTS. Clinical judgement should be used.

[i] Insufficient evidence exists in the literature to include fibrocystic disease of the breast, fibromas, and uterine fibroids as diagnostic criteria.

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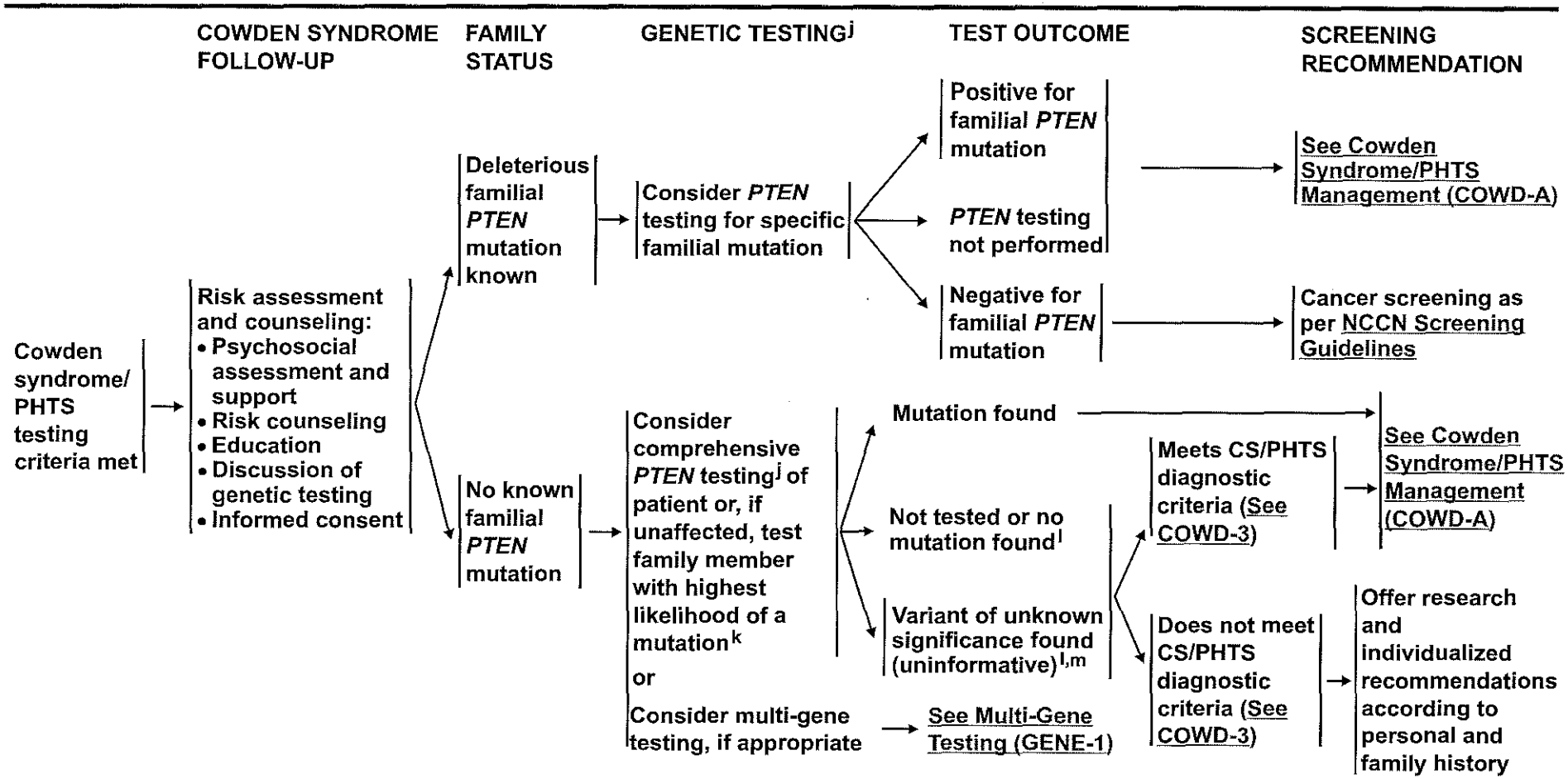

[j]Comprehensive genetic testing should include full sequence analysis, deletion/duplication analyses, and promoter analysis.

[k]Testing of unaffected family members when no affected member is available may be considered. Significant limitations of interpreting test results should be discussed.

[l]If no mutation is found, consider other hereditary breast cancer syndromes such as HBOC (HBOC-1) and/or Li-Fraumeni syndrome (LIFR-1) or multi-gene testing (GENE-1). For additional information on other genetic mutations associated with breast/ovarian cancer risk for which genetic testing is clinically available, see ADDIT-1.

[m]Testing family members for a variant of unknown significance should not be used for clinical purposes. Consider referral to research studies that aim to define functional impact of variant.

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REVISED PTEN HAMARTOMA TUMOR SYNDROME CLINICAL DIAGNOSTIC CRITERIA[n]

MAJOR CRITERIA:
- Breast cancer
- Endometrial cancer (epithelial)
- Thyroid cancer (follicular)
- Gastrointestinal hamartomas (including ganglioneuromas, but excluding hyperplastic polyps; ≥3)
- Lhermitte-Duclos disease (adult)
- Macrocephaly (≥97 percentile: 58 cm for females, 60 cm for males)
- Macular pigmentation of the glans penis
- Multiple mucocutaneous lesions (any of the following):
  - Multiple trichilemmomas (≥3, at least one biopsy proven)
  - Acral keratoses (≥3 palmoplantar keratotic pits and/or acral hyperkeratotic papules)
  - Mucocutaneous neuromas (≥3)
  - Oral papillomas (particularly on tongue and gingiva), multiple (≥3) OR biopsy proven OR dermatologist diagnosed

MINOR CRITERIA:
- Autism spectrum disorder
- Colon cancer
- Esophageal glycogenic acanthoses (≥3)
- Lipomas (≥3)
- Mental retardation (ie, IQ ≤75)
- Renal cell carcinoma
- Testicular lipomatosis
- Thyroid cancer (papillary or follicular variant of papillary)
- Thyroid structural lesions (eg, adenoma, multinodular goiter)
- Vascular anomalies (including multiple intracranial developmental venous anomalies)

Operational diagnosis in an individual (either of the following):
1. Three or more major criteria, but one must include macrocephaly, Lhermitte-Duclos disease, or gastrointestinal hamartomas; or
2. Two major and three minor criteria.

Operational diagnosis in a family where one individual meets revised PTEN hamartoma tumor syndrome clinical diagnostic criteria or has a PTEN mutation:
1. Any two major criteria with or without minor criteria; or
2. One major and two minor criteria; or
3. Three minor criteria.

[n] Pilarski R, Burt R, Kohlman W, Pho L, Shannon KM, Swisher E. Cowden syndrome and the PTEN Hamartoma Tumor Syndrome: Systematic review and revised diagnostic criteria. J Natl Cancer Inst 2013;105:1607-1616.

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COWDEN SYNDROME/PHTS MANAGEMENT

WOMEN

- **Breast awareness[1] starting at age 18 y.**
- **Clinical breast exam, every 6–12 mo, starting at age 25 y or 5–10 y before the earliest known breast cancer in the family.**
- **Annual mammography and breast MRI screening starting at age 30–35 y or individualized based on earliest age of onset in family.[2,3]**
- **For endometrial cancer screening,[4] encourage patient education and prompt response to symptoms. Consider annual random endometrial biopsies and/or ultrasound beginning at age 30–35 y.**
- **Discuss risk-reducing mastectomy and hysterectomy[5] and counsel regarding degree of protection, extent of cancer risk, and reconstruction options.**
- **Address psychosocial, social, and quality-of-life aspects of undergoing risk-reducing mastectomy and/or hysterectomy.**

MEN AND WOMEN

- **Annual comprehensive physical exam starting at age 18 y or 5 y before the youngest age of diagnosis of a component cancer in the family (whichever comes first), with particular attention to thyroid exam.**
- **Annual thyroid ultrasound starting at age 18 y or 5–10 y before the earliest known thyroid cancer in the family, whichever is earlier.**
- **Colonoscopy, starting at age 35 y, then every 5 y or more frequently if patient is symptomatic or polyps found.**
- **Consider renal ultrasound starting at age 40 y, then every 1–2 y**
- **Dermatologic management may be indicated for some patients**
- **Consider psychomotor assessment in children at diagnosis and brain MRI if there are symptoms.**
- **Education regarding the signs and symptoms of cancer.**

RISK TO RELATIVES

- **Advise about possible inherited cancer risk to relatives, options for risk assessment, and management.**
- **Recommend genetic counseling and consideration of genetic testing for at-risk relatives.**

REPRODUCTIVE OPTIONS

- **For women of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including pre-implantation genetic diagnosis. Discussion should include known risks, limitations, and benefits of these technologies. See Discussion for details.**

[1] Women should be familiar with their breasts and promptly report changes to their health care provider. Periodic, consistent breast self exam (BSE) may facilitate breast self awareness. Premenopausal women may find BSE most informative when performed at the end of menses.

[2] The appropriateness of imaging modalities and scheduling is still under study.

[3] High-quality breast MRI limitations include having: a need for a dedicated breast coil, the ability to perform biopsy under MRI guidance by experienced radiologists in breast MRI, and regional availability. Breast MRI is preferably preformed on days 7–15 of a menstrual cycle for premenopausal women.

[4] There are limited data regarding the lifetime risk of endometrial cancer in CS/PHTS. Surveillance screening and surgical intervention should be on an individual basis.

[5] Oophorectomy is not indicted for CS/PHTS alone but may be indicated for other reasons.

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EXAMPLES OF ADDITIONAL GENETIC MUTATIONS ASSOCIATED WITH BREAST/OVARIAN CANCER RISK

- **Hereditary Diffuse Gastric Cancer Syndrome**
  - ***CDH1* gene**
  - **Diffuse gastric cancer – 67%–83% risk**
  - **Lobular cancer of the breast – 39%–52% risk**

- **Peutz-Jeghers Syndrome (See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal for more information)**
  - ***STK11/LKB1* gene**
  - **Breast cancer – 44%–50% risk**
  - **Ovarian cancer – 18%–21% risk (ovarian sex cord tumors are the most common)**

- **Lynch Syndrome (See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal for more information)**
  - **Mismatch Repair (MMR) genes – *MLH1, MSH2, MSH6, PMS2***
  - ***EPCAM* gene deletion**
  - **Ovarian cancer – 9% risk**
  - **Breast cancer – conflicting data regarding increased risks**

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MULTI-GENE TESTING

Overview of multi-gene testing

- The recent introduction of multi-gene testing for hereditary forms of cancer has rapidly altered the clinical approach to testing at-risk patients and their families. Based on next-generation sequencing technology, these tests simultaneously analyze a set of genes that are associated with a specific family cancer phenotype or multiple phenotypes. Multi-gene testing includes "intermediate" penetrant genes of unclear actionability, and may reveal missense variants of unknown significance. It is for these and other reasons that they are ideally offered in the context of professional genetic expertise for pre- and post- test counseling.
- The decision to use multi-gene testing for patient care should be no different than the rationale for testing a single gene known to be associated with the development of a specific type of cancer. Testing is focused on identifying a mutation known to be clinically actionable- that is, whether the management of an individual patient is altered based on the presence or absence of a mutation. However, as the genes on the multi-gene tests may differ in penetrance, cancer pathogenicity, and the availability of specific prevention and screening guidelines, testing and post multi-gene testing management will require consultation with genetic professionals experienced in cancer susceptibility. The benefits, limitations, and management recommendations should all be reviewed by the provider and discussed with the patient when considering the use of multi-gene testing for cancer susceptibility.
- The focus of this guideline is on testing for germline (inherited) mutations. However, genetic testing for mutations in tumors is also available. While this tumor testing is aimed at identifying somatic mutations that could direct treatment, it is possible to incidentally identify germline mutations in the patient. Providers need to be aware of this and discuss the implications with the patient before ordering tumor testing, because it may have implications for the patient and his or her family members.[1]

- Examples of clinical situations where multi-gene testing may be helpful and more cost-effective include:
  - A 35-year-old woman diagnosed with breast cancer, thyroid nodules, two lipomas, and a single hemangioma. The patient meets NCCN testing criteria for *BRCA1*, *BRCA2*, *TP53*, and *PTEN*. In addition, consideration would be given to testing other genes such as *ATM* and *PALB2*.
  - A 49-year-old woman diagnosed with both ovarian and colon cancer. The patient meets testing criteria for *BRCA1*, *BRCA2*, and Lynch syndrome (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*).
  - A 45-year-old woman diagnosed with ovarian cancer and a family history of colon cancer in a maternal uncle. If the patient is negative for mutations in *BRCA1* and *BRCA2*, consideration should be given to testing the Lynch syndrome genes, and, as data accumulate, possibly others as well as ovarian cancer genes such as *RAD51D*, *RAD 51C*, and *BRIP1*.

Continued
References on GENE-5

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MULTI-GENE TESTING

Points in favor of multi-gene testing include:

- Using a broad and unbiased testing approach will increase the likelihood of finding mutations in patients who do not meet specific personal and family history criteria because:
  1. Multiple genes may contribute to the breast/ovarian phenotype, and there is often overlap in the cancers associated with mutations in different genes.
  2. Atypical family presentations or limited family structure can make it difficult to use family history alone to determine the most appropriate gene(s) to test.
  3. Patients with a new mutation could have a negative or minimal family history of cancer.
  4. Studies suggest that as high as 17% of *BRCA*-negative, high-risk breast cancer families, and as high as 24% of unselected ovarian cancer cases, will have a mutation identified on multi-gene testing.[2-4] Although the clinical actionability of all mutations is not clearly known, there is the potential to identify mutations in more patients/families with multi-gene testing than by *BRCA1/BRCA2* alone.[4]
- The higher mutation detection rate may reduce the number of high-risk families with uninformative (negative) tests, and may increase the number of patients who can be provided with tailored surveillance, risk reduction options, and testing of at-risk family members.
- Multi-gene testing is generally more cost- and time-effective than sequentially testing more than 2–3 single genes associated with the phenotype. For example, it is recommended that young women diagnosed with breast cancer be tested for mutations in *BRCA1*, *BRCA2*, and *TP53*. Using a multi-gene test could reduce the cost of testing for these genes individually.
- Multi-gene tests may also reveal more than one pathogenic mutation. In some cases this may alter recommendations for proband screening and family member gene testing for both mutations and follow-up risk reduction recommendations.

Limitations in use of multi-gene testing may include:

- Technical:
  - Commercially available tests may differ significantly in the number of genes analyzed, turn-around time, cost, insurance coverage, analysis/annotation of variants of uncertain significance, depth of gene coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication, and other factors. Thus, choosing the appropriate laboratory and tests should consider these factors:
    1. There is an increased likelihood of finding variants of unknown significance since more genes are being analyzed.[5,6] This possibility should be included in pre-test counseling with the patient.
    2. Clinical laboratories may differ on their determination of whether a particular variant should be classified as benign, likely benign variant, pathogenic, or likely pathogenic variant. As there are no clinically validated algorithms for classification, members of the same family who undergo testing by different laboratories may thus receive differing result interpretations for the same mutation. These complex test results are best addressed by a genetic specialist.
- In some circumstances mutations may be missed using next-generation sequencing, which would be detectable using traditional single-gene analytic methods.
- Clinical:
  - There are limited data regarding the degree of cancer risk associated with some of the genes on the current multi-gene tests.
  - There is a lack of clear guidelines on risk management for carriers of mutations in some of these genes. This could lead to extra surveillance, surgeries, and utilization of resources downstream that may be considered unnecessary once further data exist. In many cases, management will continue to be based on personal and family history rather than mutation status.
  - It is possible that mutations may be identified in more than one gene in the same patient, making management recommendations more difficult to determine.
  - The potential added turnaround time to receipt of test results inherent in most multi-gene testing can make them an unsuitable starting point for patients needing rapid results (ie, for surgical decision-making or other treatment decisions.)

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Continued
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MULTI-GENE TESTING

Issues to Consider in Assessing a Positive Multi-Gene Test Result for Less Characterized Genes:

- A key consideration in developing a management plan for a patient with a positive result from a multi-gene test is whether the gene mutation(s) is/are clinically actionable. Not all genes included in available multi-gene tests are necessarily clinically actionable given current data.
- The cancer risks and clinical implications of some genes included on multi-gene tests have not been fully determined, and thus consensus management recommendations may not be available. In these cases, management recommendations should be based on careful review of the available literature and the patient's family history. In many cases the information from testing for moderate penetrance genes may not change risk management guidelines compared to that recommended based on patient's family history alone. Specific issues to consider include:
  - The strength of the evidence supporting each reported cancer risk associated with a particular gene.
  - Studies done only on higher-risk families should be carefully assessed because of the inherent selection bias, which could inflate the estimated cancer risks for that gene or mutation.
  - Cancer risk data may vary by age group, and age-specific risks are unknown for many of the genes on the panels.
  - As is the case with high-risk genes, it is possible that the risks associated with moderate-risk genes may not be entirely due to that gene alone, given the possibility of gene/gene or gene/environment interactions. Therefore, there may be difficulty in using a known mutation to assign risk for relatives.
  - The strength of the family history must be taken into consideration with moderate-risk genes, since the presence of higher numbers of cancers related to the mutated gene may indicate the presence of adverse or protective modifying genetic or environmental factors in the family.
  - Patient should be made aware that results interpretation is likely to change over time, as medical science advances.
  - Although mutations in most genes included on currently available multi-gene tests cause autosomal-dominantly inherited diseases, a few are associated with autosomal-recessive inheritance. For these genes consideration should be given to testing the partner for mutations in the same gene if it would inform reproductive decision-making and/or risk assessment and management. This should be included in all documentation for notification of family members.
  - There may be gaps in the available data sets in the published studies, and therefore a conclusion about risk for a particular type of cancer may be unknown. This is currently true, for example, for ovarian cancer risk for some genes in multi-gene tests.
  - Patients with positive or uncertain results on panel testing should be encouraged to participate in family studies and/or research registries when available.

Continued
References on GENE-5

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MULTI-GENE TESTING

GENERAL RECOMMENDATIONS

Provider:

1. **Because of their complexity hereditary cancer multigene tests should be ordered in consultation with a cancer genetics professional.[a]**
2. **As in other genetic testing, an affected family member should be tested first, whenever possible.**
3. **Multi-gene testing may be more cost- and time-effective in certain cases than sequentially testing more than 2–3 single genes associated with a phenotype.**
4. **Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used for each patient, and in which labs they were performed.**
5. **Multi-gene tests vary in technical specifications (eg, depth of gene coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis). Tests should be chosen that maximize the likelihood of identifying mutations in the genes of interest and that will likely alter patient management.**
6. **Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.**

Laboratories:

1. **The classification of variants of uncertain significance remains an ongoing issue. The methods and data sources used to classify genetic variants can differ significantly by laboratory. Genetic testing laboratories should be transparent in the manner by which they classify variants.**
2. **Since variant classification is greatly facilitated by public availability of data, laboratories should make their data available.**
3. **Laboratories have differing policies regarding whether or not to notify providers for interpretation changes of a previously identified variant. Because the notification of variant changes is an important aspect of care, there is an urgent need for standardized procedures across laboratories for handling such notifications.**
4. **Other next-generation sequencing methods, such as whole genome sequencing (WGS) and whole exome sequencing (WES), may become useful testing strategies in the future. At present, however, there is no evidence for the general use of these tests for cancer susceptibility testing.**

References on GENE-5

[a] Genetic counseling is highly recommended when genetic testing is offered and after results are disclosed. A genetic counselor, medical geneticist, oncologist, surgeon, oncology nurse, or other health professional with expertise and experience in cancer genetics should be involved early in counseling patients who potentially meet criteria for an inherited syndrome.

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MULTI-GENE TESTING

REFERENCES

1. Bombard Y, Robson M, Offit K. Revealing the incidentalome when targeting the tumor genome. JAMA 2013;310:795-796.
2. Walsh T, Lee MK, Casadei S, et al. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. Proc Natl Acad Sci 2010;107:12629-12633.
3. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA 2006;295:1379-1388.
4. Walsh T, Casadei S, Lee MK, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci 2011;108:18032-18037.
5. Rainville IR, Rana HQ. Next-generation sequencing for inherited breast cancer risk: counseling through the complexity. Curr Oncol Rep 2014;16:371.
6. Cragun D, et al. Panel-based testing for inherited colorectal cancer: a descriptive study of clinical testing performed by a US laboratory. Clin Genet epub 20 Mar 2014.

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NCCN Guidelines Version 2.2014
Genetic/Familial High-Risk Assessment: Breast and Ovarian

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Discussion

This discussion is being updated to correspond with the newly updated algorithm. Last updated 08/08/13

NCCN Categories of Evidence and Consensus

Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All recommendations are category 2A unless otherwise noted.

Discussion update in progress

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NCCN Guidelines Version 2.2014 Genetic/Familial High-Risk Assessment: Breast and Ovarian

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Overview

All cancers develop as a result of mutations in certain genes, such as those involved in the regulation of cell growth and/or DNA repair,[1,2] although not all of these mutations are inherited from a parent. For example, sporadic mutations can occur in somatic/tumor cells only, and de novo mutations can occur for the first time in a germ cell (i.e., egg or sperm) or in the fertilized egg itself during early embryogenesis. However, family studies have long documented an increased risk of several forms of cancer among first-degree relatives (i.e., parents, siblings, and children) and second-degree relatives (i.e., grandparents, aunts or uncles, grandchildren, and nieces or nephews) of affected individuals. These individuals may have an increased susceptibility to cancer as the result of one or more gene mutations present in parental germline cells; cancers developing in these individuals may be classified as hereditary or familial cancers.

Hereditary cancers are often characterized by mutations associated with a high probability of cancer development (i.e., a high penetrance genotype), vertical transmission through either mother or father, and an association with other types of tumors.[3,4] They often have an early age of onset, and exhibit an autosomal dominant inheritance pattern (i.e., occur when the individual has a mutation in only one copy of a gene). Familial cancers share some but not all features of hereditary cancers. For example, although familial breast cancers occur in a given family more frequently than in the general population, they generally do not exhibit the inheritance patterns or onset age consistent with hereditary cancers. Familial cancers may be associated with chance clustering of sporadic cancer cases within families, genetic variation in lower penetrance genes, a shared environment, or combinations of these factors.[5-8]

Assessment of an individual's risk of familial or hereditary cancer is based on a thorough evaluation of the family history. With respect to hereditary cancers, advances in molecular genetics have identified a number of genes associated with inherited susceptibility to breast and/or ovarian cancers (e.g., *BRCA1, BRCA2, PTEN, TP53, CDH1*) and provided a means of characterizing the specific gene mutation or mutations present in certain individuals and families exhibiting an increased risk of cancer. The field of cancer genetics has implications for all aspects of cancer management of individuals with hereditary or familial cancers, including prevention, screening, and treatment.

The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Breast and Ovarian were developed with an acute awareness of the preliminary nature of much of our knowledge regarding the clinical application of the rapidly emerging field of molecular genetics, and with an appreciation for the need for flexibility when applying these guidelines to individual families. Furthermore, it should be emphasized that these guidelines were not developed as a substitute for professional genetic counseling. Rather, they are intended to serve as a resource for healthcare providers to identify individuals who may benefit from cancer risk assessment and genetic counseling, to provide genetic counselors with an updated tool for the assessment of individual breast cancer and ovarian cancer risk and to guide decisions related to genetic testing, and to facilitate a multidisciplinary approach in the management of individuals at increased risk of hereditary breast and/or ovarian cancer. Although cancers other than breast and ovarian cancers are associated with these hereditary syndromes, the main focus of this NCCN Guidelines is on the management of breast and ovarian cancer risk in these individuals. During the last few years, a number of genetic aberrations that may contribute to increased risks for development of

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breast and/or ovarian cancers have been identified. The current NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian focus specifically on assessment of mutations in the genes *BRCA1/BRCA2, TP53 and PTEN*, and recommended approaches to genetic testing/counseling and management strategies in individuals with these genetic mutations.

A glossary of genetic terms is included in Table 1 for reference.

Hereditary Breast or Breast/Ovarian Cancer Syndromes

Breast cancer is the most prevalent type of cancer in women in the U.S. and the second leading cause of cancer death in women.[9] In the U.S., approximately 234,580 new cases of breast cancer and 40,030 deaths are estimated in 2013 (estimated figures include both genders).[9] Up to 10% of breast cancers are due to specific mutations in single genes that are passed down in a family.[6,8] Specific patterns of hereditary breast/ovarian cancers are linked to mutations in the *BRCA1* or *BRCA2* genes.[10,11] In addition, two very rare hereditary cancer syndromes exhibiting an increased risk of breast cancer are Li-Fraumeni syndrome and Cowden syndrome, which are related to germline mutations in the *TP53* and *PTEN* genes, respectively.[12,13] Similar to the *BRCA 1/2* genes, the *TP53* and *PTEN* genes encode for proteins involved in processes related to tumor suppression, such as DNA repair and cell cycle regulation. Hereditary diffuse gastric cancer (HDGC) is another rare hereditary syndrome that is also associated with development of lobular breast cancer. This syndrome arises from mutation(s) in the *CDH1* (cadherin 1, type 1, E-cadherin [epithelial]) gene which encodes for a tumor suppressor gene product.[14] In an analysis of 4 predominantly gastric cancer pedigrees from Newfoundland with a specific *CDH1* mutation, the cumulative risk of female lobular breast cancer by the age of 75 was estimated to be as high as 52%.[15,16] Furthermore, germline *CDH1* mutations may be associated with lobular breast cancer in the absence of diffuse gastric cancer.[17]

These hereditary syndromes share several features beyond elevation of breast cancer risk. These syndromes arise from germline gene mutations that are not within sex-linked genes; hence, the mutations can be inherited from either parent. The syndromes are associated with breast cancer onset at an early age and development of other types of cancer, and exhibit an autosomal dominant inheritance pattern (see Table 1). Offspring of an individual with one of these hereditary syndromes have a 50% chance of inheriting the mutation. In addition, individuals with these hereditary syndromes share increased risks of multiple cases of early onset disease as well as bilateral disease. The gene mutations associated with these hereditary syndromes are considered to be highly penetrant, although a subsequent alteration in the second copy of the gene without the hereditary mutation is believed to be necessary for the initiation of cancer development (i.e., 2-hit hypothesis).[18,19] In addition, the manifestations (i.e., expression) of these hereditary syndromes are often variable in individuals within a single family (e.g., age of onset, tumor site, and number of primary tumors). The risk of developing cancer in individuals with one of these hereditary syndromes depends upon numerous variables including the gender and age of the individual.

Hereditary Breast/Ovarian Cancer Syndrome

The overall prevalence of disease-related mutations in *BRCA1* and *BRCA2* genes has been estimated as 1 in 300 and 1 in 800, respectively.[20,21] Currently, hundreds of unique mutations have been identified in both *BRCA1* and *BRCA2* genes. However, a number of founder effects (see Table 1) have been observed in certain populations, wherein the same mutation has been found in multiple,

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unrelated families and can be traced back to a common ancestor. Among the Ashkenazi Jewish population, for example, the frequency of 187delAG and 5385insC mutations in *BRCA1* and the 6174delT mutation in *BRCA2* approximates 1 in 40.[6,22] Certain founder mutations have also been identified in other populations.[20,23-28] It has been estimated that over 90% of early onset cancers in families with both breast and ovarian cancers are caused by mutation(s) in the *BRCA1* or *BRCA2* genes.[29] Hence, the degree of clinical suspicion for a *BRCA* mutation in a single individual with both breast and ovarian cancer or someone with a family history of both breast and ovarian cancer should be very high.

Both the *BRCA1* and *BRCA2* genes encode for proteins involved in tumor suppression. The *BRCA1* gene is located on chromosome 17 and is believed to be involved in both DNA repair and in the regulation of cell-cycle checkpoints in response to DNA damage. However, the molecular mechanism through which *BRCA1* functions to preserve genomic stability remains unclear.[30] The *BRCA2* gene, located on chromosome 13, is involved in repair of replication-mediated double-strand DNA breaks.[31,32]

Mutations in the *BRCA1* or *BRCA2* genes can be highly penetrant (for definition, see Table 1) although the probability of cancer development in carriers of *BRCA1* or *BRCA2* mutations is variable, even within families with the same mutation.[33-35] Estimates of penetrance range from 41% to 90% lifetime risk for breast cancer, with an increased risk of contralateral breast cancer.[36-42] In addition, female carriers of these genes have an estimated 8% to 62% lifetime risk for ovarian cancer, depending upon the population studied.[37,54,38-44] In a meta-analysis (2007) of published data that evaluated *BRCA1* and *BRCA2* penetrance, estimates for mean cumulative risks of breast cancer and ovarian cancer by age 70 years for *BRCA1* mutation carriers were 57% and 40%, respectively.[38] The corresponding estimates for *BRCA2* mutation carriers were 49% and 18%, respectively. In a recent prospective analysis of risk estimates from individuals with *BRCA1* and *BRCA2* mutations in the UK (N=1887), estimates for mean cumulative risks of breast cancer and ovarian cancer by age 70 years for *BRCA1* mutation carriers were 60% and 59%, respectively.[41] The corresponding estimates for *BRCA2* mutation carriers were 55% and 16.5%, respectively. Among the patients diagnosed with unilateral breast cancer (n=651), the mean cumulative risks for contralateral breast cancer by age 70 years were estimated to be 83% for *BRCA1* carriers and 62% for *BRCA2* carriers.[41] At present, it is unclear whether penetrance is related to the specific mutation identified in a family or whether additional factors, either genetic or environmental, affect disease expression. It is generally accepted, however, that carriers of mutations in *BRCA1* or *BRCA2* genes have an excessive risk for both breast and ovarian cancer that warrants consideration of more intensive screening and preventive strategies.

Some histopathologic features have been reported to occur more frequently in breast cancers characterized by a *BRCA1/2* mutation. For example, several studies have shown that *BRCA1* breast cancer is more likely to be characterized as ER-,PR-negative, and HER2-negative (i.e., "triple negative").[45-50] Studies have reported *BRCA1* mutations in 9% to 28% of patients with triple-negative breast cancer.[50-56] In addition, it appears that among patients with triple-negative disease, *BRCA* mutation carriers were diagnosed at a younger age compared with non-carriers.[53,57] A recent study in a large cohort of patients with triple-negative breast cancer (N=403) reported a median age of diagnosis of 39 years among carriers of *BRCA1* mutations (n=65).[52] Patients in this population-based study were unselected for family history or age. Among the group of patients with early onset (age

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at diagnosis <40 years) triple-negative breast cancer (n=106), the incidence of *BRCA1* mutations was 36%; among those diagnosed before age 50 years (n=208), the incidence was 27%. For patients with triple-negative breast cancer with a family history of breast and/or ovarian cancer (n=105), *BRCA1* mutations were found in 48%.[52] An increased incidence of *BRCA* mutations was reported in triple-negative breast cancer cases from at-risk populations. Among Ashkenazi Jewish women with breast cancer unselected for family history (N=451), triple-negative disease was observed in 14% and *BRCA* founder mutations were found in 11% of patients.[58] Among the subgroup with triple-negative breast cancer (n=65), the incidence of *BRCA* mutations was 39% (*BRCA1* mutation in 30%; *BRCA2* mutation in 9%).[58] Although many of the mutation studies in triple-negative breast cancer have reported on the association with *BRCA1* mutations, several reports have also suggested the role of *BRCA2* mutations in triple-negative breast cancer. The incidence of *BRCA2* mutations range from 4% to 17% in studies of triple-negative breast cancer cases unselected for age or family history.[51,58,59]

An increased frequency of other malignancies has been reported in families with mutations in the *BRCA1* or *BRCA2* gene.[39,60,61] Germline *BRCA1* and *BRCA2* mutations have been associated with an increased risk of prostate cancer in numerous reports.[39,60-67] In particular, *BRCA2* mutations have been associated with 2- to 6-fold increase in risk of prostate cancer,[62-64,67,68] while increased risks were not observed for *BRCA1* mutation carriers in some studies.[62-64,68] Prostate cancer with germline *BRCA* mutations appear to have a more aggressive phenotype (e.g., more frequently associated with Gleason score ≥8) than tumors from non-carrier patients.[69,70] A recent study in a large cohort of patients with prostate cancer from Spain (N=2019) showed that the group of patients with *BRCA* mutations had significantly higher rates of aggressive prostate cancer (Gleason score ≥8), nodal involvement and distant metastasis compared with non-carriers.[69] Moreover, cause-specific survival outcome was significantly poorer in *BRCA* mutation carriers compared with non-carriers (median survival 8.6 years vs. 15.7 years; P=0.015). Subgroup analysis by mutation type showed poor outcomes in patients with *BRCA2* mutations (n=61); the role of *BRCA1* mutations was not well defined, possibly due to the small patient size (n=18) and limited follow-up in this subgroup.[69] Prostate cancer in patients with *BRCA2* mutations has also been associated with a higher histologic grade in other studies.[62,63] In addition, analyses of data obtained from cancer registries and treatment center databases showed that *BRCA2* mutation carriers with prostate cancer had more aggressive or rapidly progressive disease, and significantly decreased survival compared with patients who were *BRCA1* mutation carriers or non-carriers.[71-73] In a study of patients with prostate cancer from a population-based cancer registry in Iceland (N=596), patients with *BRCA2* mutations had significantly decreased median survival compared with non-carriers (having wild type *BRCA2*) patients (2 years vs. 12 years; P<0.001).[73] Moreover, in a study of patients with prostate cancer using data obtained from cancer center databases (N=301), patients with *BRCA2* mutations had significantly decreased median survival compared with patients with *BRCA1* mutations (4 years vs. 8 years; P<0.01).[71] *BRCA2* mutation carriers have also been reported to have a higher risk of pancreatic cancer and melanoma.[60,61,67,74,75] Both *BRCA1* and *BRCA2* mutations have been associated with increased propensity for developing pancreatic cancer.[67,75-78] In an analysis of samples taken from patients with familial pancreatic cancer (kindreds in which ≥3 family members had pancreatic cancer, at least 2 of which were first-degree relatives), *BRCA2* mutations were detected in 17% of patient samples.[78] Among the Ashkenazi Jewish population, *BRCA2*

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mutations have been identified in about 4% of patients with pancreatic cancer.[74,79]

Some data related to the risk of cancers in this population at some sites other than the breast/ovary are contradictory.[80] For example, it has been suggested that the increased risk of endometrial cancer observed in some *BRCA1* or *BRCA2* mutation carriers is mainly due to the use of tamoxifen therapy by these women rather than the presence of a gene mutation.[81]

Germline mutations in *BRCA1* and *BRCA2* are responsible for 5% to 10% of epithelial ovarian cancers (i.e., ovarian cancer developing on the surface of the ovary).[82] Increased risks of cancers of the fallopian tube and primary peritoneal cancer are also observed in this population. In the setting of an invasive ovarian cancer diagnosis, as many as 15% of unselected individuals will have a germline *BRCA1* or *BRCA2* mutation.[42,83] However, it has been reported that about half of families showing a genetic predisposition to ovarian cancer do not have identifiable mutations in *BRCA1/2* genes.[84] Hence, other gene mutations predisposing to ovarian cancer are likely to exist.[85] Of note, ovarian cancer is a component tumor of Lynch syndrome which is associated with germline mutations in mismatch repair genes.[86] Interestingly, results from a prospective study suggest that women from families at increased risk of hereditary breast cancer without site-specific *BRCA* mutations are not at increased risk for ovarian cancer, although these results may have been confounded by the ethnic characteristics and size of the study population.[87]

It is interesting to note that several recent studies have reported more favorable survival outcomes among *BRCA1/2* mutation carrier patients with ovarian cancer compared with non-carrier patients.[88-93] In a case-control study of patients with epithelial ovarian cancer (N=66), patients with *BRCA1/2* mutations had improved outcomes compared with patients with non-hereditary ovarian cancer, including significantly longer median survival from time of diagnosis (101 months vs. 35 months; $P<0.002$).[92] In a large case-control study of Jewish patients with epithelial invasive ovarian cancer (N=779), patients with *BRCA1/2* mutations had significantly longer median survival compared with non-carrier patients (54 months vs. 38 months; $P=0.002$).[91] Results from a recent pooled analysis from 26 observational studies that included invasive epithelial ovarian cancer cases from *BRCA1/2* mutation carriers (n=1213) and non-carriers (n=2666) showed favorable survival outcomes for patients with *BRCA1/2* mutations.[89] The 5-year survival rate for non-carriers, *BRCA1* carriers and *BRCA2* carriers was 36%, 44%, and 52%, respectively. The survival advantage compared with non-carriers was significant for both the *BRCA1* carriers (hazard ratio=0.78; 95% CI, 0.68-0.89; $P<0.001$) and *BRCA2* mutation carriers (hazard ratio=0.61; 95% CI, 0.50-0.76; $P<0.001$).[89] In a recent population-based case-control study of women with invasive epithelial (nonmucinous) ovarian cancer (N=1001) from the Australian Ovarian Cancer Study Group, *BRCA1/2* mutation carriers had improved survival outcomes compared with non-carriers in terms of median progression-free survival (20 months vs. 16 months; not statistically significant) and median survival (62 months vs. 55.5 months; $P=0.031$).[88] Moreover, *BRCA* mutation carriers appeared to be more responsive to cytotoxic chemotherapy (regardless of class of agent) compared with non-carrier patients. Outcomes appeared to be most favorable for *BRCA2* mutation carriers; in the subgroup of patients with *BRCA2* mutations (n=53), the median survival was 70 months.[88] In an observational study of patients with high-grade serous ovarian cancer (N=316), patients with *BRCA2* mutations had significantly favorable survival outcomes (hazard ratio=0.33; 95% CI, 0.16–0.69; $P=0.003$; 5-year rate: 61% vs. 25%) and progression-free survival (hazard ratio=0.40; 95% CI, 0.22–0.74;

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P=0.004; 3-year rate: 44% vs. 16%) compared with non-carrier patients (having wild type *BRCA*).[93] Additionally, *BRCA2* mutations were associated with significantly higher response rates (compared with non-carriers or with *BRCA1* mutation carriers) to primary chemotherapy. In contrast, *BRCA1* mutations were not associated with prognosis or improved chemotherapy response.[93]

The histology of ovarian cancers in carriers of a *BRCA1* or *BRCA2* mutation is more likely to be characterized as serous adenocarcinoma and high grade compared with ovarian cancers in non-mutation carriers, although endometrioid and clear cell ovarian cancers have also been reported in the former population.[82,94-97] In studies of women with *BRCA1/2* mutations who underwent risk reduction salpingo-oophorectomy (RRSO), occult gynecological carcinomas were identified in 4.5%-9% of cases based on rigorous pathological examinations of the ovaries and fallopian tubes.[98-100] Tubal intraepithelial carcinoma (TIC) is thought to represent an early precursor lesion for serous ovarian cancers, and TIC (with or without other lesions) was detected in 5% to 8% of cases from patients with *BRCA1/2* mutations who underwent RRSO.[98,101,102] The fimbriae or distal tube was reported to be the predominant site of origin for these early malignancies found in patients with *BRCA1/2* mutations.[98,102,103] Although TIC appeared to present more frequently among *BRCA1/2* mutation carriers compared with non-carriers undergoing RRSO,[102,103] TIC has also been documented among patients with serous carcinomas unselected for family history or *BRCA* mutation status.[104] Because TIC was identified in individuals who underwent surgery for risk reduction (for *BRCA1/2* mutation carriers) or other gynecological indications, the incidence and significance of these early lesions within the general population is unclear. Hence, at the present time, there is no justifiable role for *BRCA* testing for cases based solely on the finding of TIC during pathology evaluation for gynecological indications.

Male carriers of a *BRCA* gene mutation also have a greater risk for cancer susceptibility.[61] In one study of 26 high-risk families with at least one case of male breast cancer, 77% demonstrated a *BRCA2* mutation.[29] Among male patients with breast cancer who were not selected on the basis of family history, 4% to 14% tested positive for a germline *BRCA2* mutation.[105-108] In a recent series of male breast cancer cases (N=115; primarily from cancer registry data), *BRCA2* mutations were detected in 16% of cases; the incidence of *BRCA2* mutations was 40% among patients selected for family history of breast cancer and 13% among those unselected for family history.[107] For males with a *BRCA2* mutation, the cumulative lifetime risk of breast cancer has been estimated at 7% to 8%.[109,110] In contrast, for men without such a mutation, the lifetime risk of breast cancer has been estimated at approximately 0.1% (1 in 1,000).[107,111]

The NCCN panel recommends that individuals from a family with a known deleterious *BRCA1* or *BRCA2* mutation be considered for testing (see Guidelines section on HBOC Syndrome Testing Criteria).

In individuals from a family without a known deleterious *BRCA* mutation, testing should be considered for those individuals who meet the testing criteria discussed below. In evaluating risks based on family history factors, the maternal and paternal sides should be considered independently. For the testing criteria mentioned below, "close relatives" pertain to first-, second- or third-degree blood relatives on the same side (either maternal or paternal side) of the family. Individuals with a limited family history (e.g., having fewer than 2 first- or second-degree relatives or female relatives surviving beyond 45 years of age on either the maternal or paternal side) may have an underestimated probability

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of a familial gene mutation. The panel recommends that patients with a personal history of breast cancer *in addition to* one or more of the following criteria be considered for *BRCA1/BRCA2* testing:

- Diagnosed at age 45 years or younger;
- Having 2 breast primaries (bilateral tumors or 2 or more clearly separate ipsilateral tumors, occurring synchronously or asynchronously) with the first breast cancer diagnosed at age 50 years or younger;
- Diagnosed at 50 years or younger with 1 or more close relative with breast cancer at any age (or with a limited family history);
- Diagnosed with triple-negative breast cancer at age 60 years or younger;
- Diagnosed at any age with 1 or more close relative with breast cancer diagnosed at age 50 years or younger;
- Diagnosed at any age with 2 or more close relatives with breast cancer at any age; diagnosed at any age with 1 or more close relative with epithelial ovarian cancer diagnosed at any age;
- Diagnosed at any age with 2 or more close relatives with pancreatic cancer or aggressive prostate cancer (Gleason score ≥7) at any age; or
- Having a close male relative with breast cancer at any age.

In patients with a personal history of breast cancer and with an ethnic background associated with higher mutation frequency (e.g., Ashkenazi Jewish heritage), no additional family history may be needed to meet testing criteria. In addition, the NCCN panel recommends testing for patients with a personal history of the following:

- Epithelial ovarian cancer diagnosed at any age;
- Male breast cancer diagnosed at any age; or
- Pancreatic cancer or aggressive prostate cancer (Gleason score ≥7) diagnosed at any age, with 2 or more close relatives with breast cancer and/or ovarian cancer and/or pancreatic or aggressive prostate cancer diagnosed at any age.

In unaffected individuals with a family history only (i.e., no personal history of breast or ovarian cancer), significant limitations of interpreting test results should be discussed prior to any testing. Moreover, testing of unaffected individuals should only be considered when an appropriate affected family member is unavailable for testing. Clinical judgement should be used to evaluate each unaffected individual for his/her likelihood of carrying the mutation based on factors such as the unaffected individual's current age and the age of unaffected female relatives who link the individual with an affected close relative.

Individuals not meeting testing criteria, including those with an increased risk of familial breast cancer, should be followed according to the recommendations in the NCCN Guidelines for Breast Cancer Screening and Diagnosis.

Li-Fraumeni Syndrome

Li-Fraumeni syndrome (LFS) is a rare hereditary cancer syndrome associated with germline *TP53* gene mutations.[13] It has been estimated to be involved in only about 1% of hereditary breast cancer cases,[112] although results from a recent study suggest that germline *TP53* gene mutations may be more common than previously believed.[113] The tumor suppressor gene, *TP53*, is located on chromosome 17,[114,115] and the protein product of the *TP53* gene (i.e., p53) is located in the cell nucleus and binds directly to DNA. It has been called the "guardian of the genome" and plays important roles in controlling the cell cycle and apoptosis.[114-116] Germline mutations in the *TP53* gene have been observed in over 50% (and in over 70% in some studies) of families

DISCUSSION
Updates in progress

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meeting the classic definition of LFS (see Guidelines section on Li-Fraumeni Syndrome Testing Criteria).[13,113,117] Additional studies are needed to investigate the possibility of other gene mutations in families meeting these criteria not carrying germline *TP53* mutations.[118]

LFS, a highly penetrant cancer syndrome associated with a high lifetime risk of cancer, is characterized by a wide spectrum of neoplasms occurring at a young age. It is associated with soft-tissue sarcomas, osteosarcomas (although Ewing's sarcoma is less likely to be associated with LFS), premenopausal breast cancer, acute leukemia, and cancer of the colon, adrenal cortex, and brain tumors.[13,113,116,119-124] Sarcoma, breast cancer, adrenocortical tumors and certain brain tumors have been referred to as the "core" cancers of LFS since they account for the majority of cancers observed in individuals with germline mutations in the *TP53* gene, and in one study, at least one of these cancers was found in one or more members of all families with a germline *TP53* gene mutation.[113] Interestingly, recent retrospective studies have reported a very high frequency of HER2-positive breast tumors (67%–83% of evaluated breast tumors) among patients with germline *TP53* mutations, which suggest that amplification of HER2 may arise in conjunction with *TP53* mutations.[125,126] This association between HER2-positive breast cancer and germline *TP53* mutations warrants further investigation, as such patients may potentially benefit from chemoprevention therapies that incorporate HER2-targeted agents.

Individuals with LFS often present with certain cancers (e.g., soft-tissue sarcomas, brain tumors, and adrenocortical carcinomas) in early childhood,[121] and have an increased risk of developing multiple primary cancers during their lifetimes.[127] Results of a segregation analysis of data collected on the family histories of 159 patients with childhood soft tissue sarcoma showed carriers of germline *TP53* mutations to have estimated cancer risks of approximately 60% and 95% by age 45 and 70 years, respectively.[128] Although similar cancer risks are observed in men and women with LFS when gender-specific cancers are not considered, female breast cancer is commonly associated with the syndrome.[113] It is important to mention that estimations of cancer risks associated with LFS are limited to at least some degree by selection bias since dramatically affected kindreds are more likely to be identified and become the subject of further study.

A number of different sets of criteria have been used to help identify individuals with LFS. For the purposes of the NCCN Guidelines, 2 sets of these criteria are used to facilitate the identification of individuals who are candidates for *TP53* gene mutation testing.

Classic LFS criteria, based on a study by Li and Fraumeni involving 24 LFS kindreds, include the following: member of a kindred with a known *TP53* mutation; combination of an individual diagnosed at age 45 years or younger with a sarcoma, and a first-degree relative diagnosed with cancer at age 45 years or younger, and an additional first- or second-degree relative in the same lineage with cancer diagnosed at age younger than 45 years or a sarcoma at any age (see Guidelines section on Li-Fraumeni Syndrome Testing Criteria). Classic LFS criteria have been estimated to have a high positive predictive value (estimated at 56%) as well as a high specificity, although the sensitivity is relatively low (estimated at 40%).[113] Thus, it is not uncommon for individuals with patterns of cancer outside of these criteria to be carriers of germline *TP53* mutations.[124,129] Classic LFS criteria make up one set of criteria included in the Guidelines to guide selection of individuals for *TP53* gene mutation testing (see Guidelines section on Li-Fraumeni Syndrome Testing Criteria).

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Other groups have broadened the classic LFS criteria to facilitate identification of individuals with LFS,[119,130-132] One set of these less strict criteria proposed by Birch and colleagues shares many of the features of classic LFS criteria, although a larger range of cancers are included.[113,119] Uncommonly, individuals with de novo germline *TP53* mutations (no mutation in either biological parent) have been identified.[113,120,133] These cases would not be identified as *TP53* testing candidates based upon classic LFS criteria due to requirement of a family history. This issue is circumvented, in part, by the criteria for *TP53* testing proposed by Chompret and colleagues, which recommends testing for patients with multiple primary tumors of at least 2 "core' tumor types (i.e., sarcoma, breast cancer, adrenocortical carcinoma, brain tumors) diagnosed at age <36 years or patients with adrenocortical carcinoma diagnosed at any age, regardless of family history (see Guidelines section on Li-Fraumeni Syndrome Testing Criteria).[131] The Chompret criteria have an estimated positive predictive value of 20% to 35%,[113,131] and when incorporated as part of *TP53* testing criteria in conjunction with classic LFS criteria, have been shown to improve the sensitivity to 95% (i.e., the Chompret criteria added to classic LFS criteria detected 95% of patients with *TP53* mutations).[113] The Chompret criteria are the second set of criteria included in the NCCN Guidelines. Although not part of the original published criteria set forth by Chompret et al., the panel recommends adding lung bronchoalveolar cancer and leukemia as one of the core tumor types (for inclusion in criterion 1 and 2 of the Chompret criteria) and also recommends testing individuals with choroid plexus carcinoma diagnosed at any age and regardless of family history (for inclusion in criterion 3), based upon reports of high incidence of *TP53* mutations found in patients with this rare form of brain tumor.[113,120,134,135] The above inclusion of lung bronchoalveolar cancer and leukemia as one of the core tumors and recommendation for testing for individuals with choroid plexus carcinoma (i.e., updated Chompret criteria) was recently proposed by Tinat et al,[135] and is supported by the NCCN Guidelines panel. The panel also supports the broader age cut-offs proposed by Tinat et al, based upon a study in a large number of families, which detected germline *TP53* mutations in affected individuals with later tumor onsets.[134,135]

Women with early-onset breast cancer (age of diagnosis ≤35 years), with or without family history of core tumor types, are another group for whom *TP53* gene mutation testing may be considered. Several recent studies have investigated the likelihood of a germline *TP53* mutation in this population.[113,134,136-139] In a study of *TP53* mutations evaluated at a single reference laboratory, Gonzalez et al. found that all women younger than 30 years of age with breast cancer who had a first- or second-degree relative with at least one of the core cancer types (n=5), had germline *TP53* mutations.[113] In a recent analysis of data of patients with early-onset breast cancer (age of diagnosis <30 years) tested for *TP53* mutation at a single institution (N=28), 6 patients (33%) were found to have *TP53* mutations.[140] Among the patients who were tested, a *TP53* mutation was found in approximately 8% who did not meet traditional LFS criteria for testing. In another recent study in patients with *BRCA1/BRCA2* mutation-negative early-onset breast cancer (age of diagnosis ≤35 years) tested for *TP53* mutation at a single institution (N=83), approximately 5% were found to have *TP53* mutations.[138] Deleterious *TP53* mutations were identified in 3 of 4 patients (75%) with a family history of at least 2 LFS-associated tumors (breast cancer, bone or soft tissue sarcoma, brain tumors or adrenocortical cancer) and in 1 of 17 patients (6%) with a family history of breast cancer only.[138] Among women <30 years of age with breast cancer and without a family history, the incidence of *TP53* mutations has been reported at 3% to 8%.[113,137,139,140] Other studies have found an even lower incidence of

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germline *TP53* gene mutations in this population. For example, Bougeard et al reported that only 0.7% of unselected women with breast cancer before age 33 were carriers of a germline *TP53* mutation.[134] Furthermore, Ginsburg and colleagues found no germline *TP53* mutations in 95 unselected women with early-onset breast cancer who previously tested negative for *BRCA* mutations.[136]

Finally, a member of a family with a known *TP53* mutation is considered to be at sufficient risk to warrant gene mutation testing, even in the absence of any other risk factors. Individuals not meeting testing criteria should be followed according to recommendations tailored to his/her personal cancer history and family history.

Cowden Syndrome

Cowden syndrome, a rare hereditary cancer syndrome, was first described in 1963 and named after the Cowden family, the first family documented with signs of the disease.[141] The incidence of Cowden syndrome has been reported to be 1 in 200,000, although it is likely to be underestimated due to difficulties associated with making a clinical diagnosis of the disease.[142,143] Cowden syndrome is an autosomal dominant disorder associated with germline mutations in the *PTEN* ("*phosphatase* and *ten*sin homologue deleted on chromosome 10"[144]) tumor suppressor gene located on chromosome 10q23; the gene is thought to be involved in cell cycle arrest and apoptosis, and other cell survival pathways.[12] It is considered to be part of the spectrum of PTEN hamartoma tumor syndromes (PHTS), which also includes Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome, and Proteus-like syndrome.[12,145,146] Additional clinical syndromes related to germline mutations in *PTEN* include Lhermitte-Duclos disease and autism spectrum disorders with macrocephaly, both of which have been associated with Cowden syndrome.[12,146,147] The estimated penetrance of *PTEN* mutation is high, at approximately 80%.[148] Hamartomas, a common manifestation of these syndromes, are benign tumors resulting from an overgrowth of normal tissue.

Cowden syndrome is associated with multiple hamartomatous and/or cancerous lesions in various organs and tissues, including the skin, mucous membranes, breast, thyroid, endometrium and brain.[12,149] This syndrome is the only PHTS disorder associated with a documented predisposition to malignancies; hence, it is included in these Guidelines. However, it has been suggested that patients with other PTHS diagnoses associated with *PTEN* mutations should be assumed to have Cowden-associated cancer risks. In a study of patients meeting diagnostic criteria for Cowden syndrome (N=211; identified from published literature and records from a single institution), the cumulative lifetime risk of any cancer was 89%.[150] *PTEN* mutations had been identified in 97 of 105 patients (92%) who underwent testing. The cumulative lifetime cancer risks for all evaluable patients (n=210) were 81% for female breast cancer, 21% for thyroid cancer, 19% for endometrial cancer, 15% for renal cancer, and 16% for colorectal cancer.[150] In a recent prospective study that evaluated genotype-phenotype associations between *PTEN* mutations and cancer risks, a large number of patients meeting modified (relaxed) International Cowden Consortium criteria (N=3399) were enrolled and tested for *PTEN* mutations.[151] Deleterious germline mutations in *PTEN* were identified in 368 patients (11%). Calculation of age-adjusted standardized incidence ratios (SIRs) using cancer incidence data from the SEER database showed elevated SIRs among individuals with *PTEN* mutations for breast cancer (25), thyroid cancer (51), endometrial cancer (43), colorectal cancer (10), renal cancer (31), and melanoma (8.5). The estimated cumulative lifetime cancer risks were 85% for breast, 35% for thyroid, 28% for endometrial, 9% for colorectal, 34% for

Discussion Updates in Progress

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renal and 6% for melanoma.[151] In another recent study in individuals with PHTS found to have deleterious germline *PTEN* mutations (N=154; detailed information available in n=146), age- and gender-adjusted SIRs were elevated for female breast cancer (39), endometrial cancer (49), female thyroid cancer (43), male thyroid cancer (199.5), female melanoma (28), and male melanoma (39).[152] The cumulative lifetime risks in these individuals were 77% for female breast cancer and 38% for thyroid cancer. The cumulative lifetimes risk for any cancer was 85% overall, and women with PHTS were found to have a 2-fold greater cancer risk compared with men with PHTS.[152]

Women diagnosed with Cowden syndrome have a high risk of benign fibrocystic breast disease and their lifetime risk of breast cancer has been estimated at 25% to 50% with an average age of 38 to 46 years at diagnosis.[12,149,153] Recent studies (as discussed above) have reported a higher cumulative lifetime risk of breast cancer (77–85%) in individuals with Cowden syndrome or *PTEN* mutations.[150-152] There have been only 2 cases of breast cancer reported in men with Cowden syndrome.[12] Thyroid disease, including benign multinodular goiter, adenomatous nodules, and follicular adenomas have been reported to occur in up to approximately 70% of individuals with Cowden syndrome[154] and the lifetime risk of thyroid cancer (follicular or papillary) has been estimated at 3% to 10%.[12,155] A higher cumulative lifetime risk of thyroid cancer (21–38%) was reported in several recent studies in individuals with Cowden syndrome or *PTEN* mutations (as discussed earlier).[150-152] As in many other hereditary cancer syndromes, affected individuals are more likely to develop bilateral and multifocal cancer in paired organs.[148] Although not well defined, women with Cowden syndrome may have a 5% to 10% risk of endometrial cancer,[12,156] and an increased risk of uterine fibroids. Recent studies showed a higher lifetime risk of endometrial cancer (19–28%) in women with Cowden syndrome or *PTEN* mutations.[150,151] As discussed earlier, increased lifetime risks for colorectal cancer (9–16%), renal cancer (15–34%) and melanoma (6%) were also reported recently in individuals with Cowden syndrome or *PTEN* mutations.[150,151] In addition, brain tumors and vascular malformations affecting any organ are occasionally seen in individuals with Cowden syndrome, although the risks for developing these conditions are not well defined.[12] It is important to note, however, that most of the data on the frequencies of the clinical features of Cowden syndrome are from compilations of case reports of relatively young individuals who may have subsequently developed additional signs of the disease (i.e., new cancerous lesions), and these data are also likely to be confounded by selection bias.[12] Furthermore, a considerable number of these studies were published prior to the establishment in 1996 of the International Cowden Consortium operational diagnostic criteria for the syndrome which were based on published data and the expert opinion of individuals representing a group of centers mainly in North America and Europe.[12,157]

Classic features of Cowden syndrome include mucocutaneous papillomatous papules, palmoplantar keratoses, and trichilemmomas (i.e., benign tumors derived from the outer root sheath epithelium of a hair follicle).[12,158] Most individuals with Cowden syndrome exhibit characteristic mucocutaneous lesions by their twenties, and such lesions have been reported to occur in 99% of individuals with Cowden syndrome, a syndrome showing nearly complete penetrance.[82,145] The presence of 2 or more trichilemmomas has been reported to be pathognomonic for Cowden syndrome.[159,160] However, since most of this evidence is from the older literature, it is possible that the association between these 2 entities is somewhat overestimated.[12] There are reports of individuals with a solitary trichilemmoma who do not have Cowden syndrome.[159,160] Nevertheless, due to the strong association

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between these lesions and Cowden syndrome and the difficulty in clinically distinguishing between a trichilemmoma and another mucocutaneous lesion, it is important that a diagnosis of trichilemmoma is histologically confirmed.

It has historically been reported that about 40% individuals with Cowden syndrome have gastrointestinal polyps (often colonic), although more recent data suggest that this risk may be 80% or higher. Indeed, a recent analysis of *PTEN* mutation carriers reported gastrointestinal polyps in 93% of patients.[161] Most of the polyps are hamartomatous, although ganglioneuromas (i.e., rare, benign peripheral nervous system tumors) have also been reported to occur.[12,162] However, early-onset (age <50 years) colorectal cancer has been reported in 13% of patients with *PTEN* mutation-associated Cowden syndrome, suggesting that routine colonoscopy may be warranted in this population.[161]

Adult Lhermitte-Duclos disease (LDD) and autism spectrum disorder characterized by macrocephaly are strongly associated with Cowden syndrome.[145,148,150,163] A rare, slow growing, benign hamartomatous lesion of the brain, LDD is a dysplastic gangliocytoma of the cerebellum.[12,150] In a study of individuals meeting the diagnostic criteria for Cowden syndrome, the cumulative lifetime risk of LDD was reported to be 32%.[150] The preponderance of evidence supports a strong association between adult-onset LDD and the presence of a *PTEN* gene mutation,[148] although exceptions have been reported.[164] In addition, there is a relatively large body of evidence to support that 10% to 20% of individuals with autism spectrum disorder and macrocephaly carry germline *PTEN* mutations.[147,165-168] Macrocephaly (defined as head circumference greater than the 97th percentile)[169] is a common finding in patients with Cowden syndrome. It has been estimated that approximately 80% of individuals with this syndrome will exhibit this clinical finding.[12]

The BRRS variant of PHTS has been characterized by the presence of multiple lipomas, gastrointestinal hamartomatous polyps, macrocephaly, hemangiomas, developmental delay, and in males, pigmented macules on the glans penis,[170] although formal diagnostic criteria have not been established for this syndrome. *PTEN* gene mutations testing in individuals characterized with BRRS have been reported in approximately 60% of these patients.[171] Further, in another study, 10% of patients with BRRS for whom a *PTEN* gene mutation test was negative were shown to be carriers of large *PTEN* gene deletions.[163]

The *PTEN* mutation frequency in individuals meeting International Cowden Consortium criteria for Cowden syndrome has been estimated at about 80%.[12,171] However, a recent evaluation of data based on samples analyzed at a single academic pathology laboratory (N=802 evaluable) reported a much lower frequency (34%) of *PTEN* mutations among individuals meeting diagnostic criteria[143] for Cowden syndrome.[145] The authors concluded that the current Consortium diagnostic criteria are not as sensitive in identifying individuals with *PTEN* mutations as previously estimated. The International Cowden Consortium criteria have been updated several times since 1996[12,143,145,172,173] and they have largely served as the basis for the list of *PTEN* mutation testing criteria included in the NCCN Guidelines. On the basis of literature reports and expert consensus, the panel has recently revised both the list of criteria associated with this genetic syndrome as well as the combinations of criteria that establish which individuals are candidates for *PTEN* gene mutation testing (see Guidelines section on Cowden Syndrome Testing Criteria). Similar to earlier versions, criteria are grouped into 3 general categories. A patient is considered for *PTEN* gene mutation testing based on whether he/she meets certain criteria or combinations of criteria from these 3 categories. The first criteria category includes individuals meeting diagnostic criteria for Cowden

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syndrome[172]; or a personal history of BRRS, adult LDD, autism spectrum disorder with macrocephaly, or 2 or more biopsy proven trichilemmomas. Any individual presenting with one or more of these diagnoses warrants *PTEN* testing. Previously, some of the criteria from this group have sometimes been referred to as "pathognomonic" although it is unlikely that any of these conditions can stand alone as a definitive diagnostic criterion of Cowden syndrome. Another criterion which can be considered to be sufficient to warrant *PTEN* gene mutation testing is a family history which includes the presence of a known deleterious *PTEN* mutation.

The next category of criterion represents "major" features associated with Cowden syndrome.[143,146,172,174] The major criteria include the presence of breast cancer, macrocephaly (i.e., megalocephaly),[169] endometrial cancer, follicular thyroid cancer, multiple gastrointestinal hamartomas or ganglioneuromas, macular pigmentation of glans penis, and certain mucocutaneous lesions that are often observed in patients with Cowden syndrome (i.e., one biopsy proven trichilemmoma, multiple palmoplantar keratoses, multiple or extensive oral mucosal papillomatosis, multiple cutaneous facial papules). With respect to decisions related to the presence of mucocutaneous lesions, the panel did not consider the available literature to be adequate to accurately specify the number or extent of these lesions required for the condition to be defined as a major criterion for Cowden syndrome, and clinical judgment is needed when evaluating such lesions. An individual exhibiting 2 or more major criteria where one of these is macrocephaly meets the testing threshold. An individual with 3 or more major criteria (without macrocephaly) are also considered to meet the threshold for testing. In addition, individuals exhibiting 1 major criterion with 3 or more minor criteria (discussed below) also meet the testing threshold; if an individual exhibits 2 or more major criteria (e.g., breast cancer and follicular thyroid cancer) but does not have macrocephaly, then one of the major criteria may be included as one of the 3 minor criteria to meet the testing threshold.

The final category of criteria represents features with a "minor" association with Cowden syndrome.[143,146,172,174] These include autism spectrum disorder (without macrocephaly), colon cancer, esophageal glycogenic acanthosis (3 or more), lipomas, mental retardation, papillary or follicular variant of papillary thyroid cancer, thyroid structural lesions other than follicular thyroid cancer (e.g., adenoma, nodules, goiter), renal cell carcinoma, a single gastrointestinal hamartoma or ganglioneuroma, testicular lipomatosis, or vascular anomalies (including multiple intracranial developmental venous anomalies). The panel felt that evidence from the literature was insufficient to include fibrocystic breast disease, fibromas or uterine fibroids as part of the testing criteria. An individual would need to exhibit 4 or more minor criteria or as discussed above, 3 or more minor and one major criterion to meet testing criteria (see Guidelines section on Cowden Syndrome Testing Criteria and the Discussion section below on Risk Assessment, Counseling, and Management: Cowden Syndrome).

Lastly, an at-risk individual (first-degree relative of an affected individual) with one or more major criterion or 2 or more minor criteria, along with a relative diagnosed with Cowden syndrome or BBRS (for whom testing has not been performed), would also meet the threshold for *PTEN* testing. Individuals not meeting testing criteria should be followed according to recommendations tailored to his/her personal cancer history and family history.

Other Genetic Lesions Associated with Breast/Ovarian Cancer

Although the highly penetrant *BRCA1* and *BRCA2* gene mutations (together with genomic rearrangements in *BRCA* and other high-

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penetrance mutations such as *TP53* or *PTEN* mutations discussed above) are thought to account for a large proportion of familial breast cancers, other breast and/or ovarian cancer susceptibility genes have been identified. For instance, germline mutations in *CDH1* are associated with hereditary diffuse gastric cancer and lobular breast cancer, and studies have reported a cumulative lifetime risk of breast cancer of 39% to 52% among women who carry *CDH1* mutations.[15,175] Germline mutations in *STK11* is associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by gastrointestinal polyps, mucocutaneous pigmentation, and elevated risk of gastrointestinal cancers as well as breast or ovarian cancers. Further information on Peutz-Jeghers syndrome can be found in the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal. Other breast and/or ovarian cancer susceptibility genes with lower or moderate penetrance have been identified in recent years, and include mutations in *CHEK2, PALB2, BRIP1, RAD51C*, among others.[176-179] In a study of breast cancer patients in the U.S. with strong family history of breast or ovarian cancer but who tested negative for *BRCA1* or *BRCA2* mutations, 12% were found to have large genomic rearrangements (deletion or duplication) in *BRCA*, and 5% had *CHEK2* mutations.[178] Deleterious *CHEK2* mutations have been reported to occur with a higher frequency in Northern and Eastern European countries compared with North America.[176,177,180,181] The cumulative lifetime risk of breast cancer in women with *CHEK2* mutations and familial breast cancer has been estimated to range from approximately 28% to 37%.[182,183] In the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian, the panel specifically focuses on assessment of known high-penetrance mutations (i.e., *BRCA1, BRCA2, TP53 and PTEN*) and recommendations for genetic testing, counseling and management strategies in individuals with these mutations. A comprehensive review of other lower or moderate-penetrance susceptibility genes is beyond the scope of the Guidelines, but has been reviewed in a recent publication.[176]

With recent advances in genomic sequencing technologies, it is now possible to test for multiple breast and/or ovarian cancer susceptibility genes in parallel using multigene or multiplex panels.[176,179,184] Although multigene sequencing approaches may be resource efficient in terms of time and costs, several issues must be addressed before multiplex testing panels can be incorporated as part of standard clinical practice. At the present time, no consensus exists on recommendations for optimal management or surveillance approaches for carriers of lower or moderate penetrance genes, and no data are available to address cancer risk assessments in individuals who are found to carry multiple gene mutations with moderate penetrance.[184] Importantly, additional genetic counseling approaches must be vetted and developed in order to adequately address the limitations and implications associated with interpretation of multiplex testing results.

Initial Risk Assessment

For a patient concerned about or suspected of having a hereditary propensity to breast and/or ovarian cancer, an initial risk evaluation should be performed in order to determine if a formal risk assessment should be undertaken (see Guidelines section on Criteria for Further Genetic Risk Evaluation). The first step in this preliminary assessment is a broad and flexible evaluation of the personal and family history of the individual with respect to breast and/or ovarian cancer.[185,186] The magnitude of the risk increases with the number of affected relatives in the family, the closeness of the relationship, and is affected by the age at which the affected relative was diagnosed.[187,188] The younger the age at diagnosis, the more likely it is that a genetic component is present. When assessing a family history for a hereditary pattern, the equal

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likelihood of paternal or maternal transmission of a gene that predisposes to breast cancer must also be kept in mind.

If an individual or a close family member of that individual meets any one of the criteria presented in the NCCN Guidelines (see Guidelines section on Criteria for Further Genetic Risk Evaluation), that individual may be at increased risk for breast and/or ovarian cancer, and a referral for genetic assessment is recommended. The maternal and paternal sides of the family should be considered independently for familial patterns of cancer.

For individuals potentially meeting established criteria for one or more of the hereditary cancer syndromes, genetic testing should be considered along with appropriate pre-test counseling. A genetic counselor and/or a medical geneticist should be involved in this process. Those not meeting criteria for testing who are still considered at increased risk of familial breast cancer are also likely to benefit from appropriate risk-reduction strategies (e.g., a change in the frequency of, or modalities used for, breast cancer screening).[5] The panel recommends that these individuals follow recommendations in the NCCN Guidelines for Breast Cancer Screening and Diagnosis.

Formal Risk Assessment and Genetic Counseling

Risk Assessment

Cancer genetic risk assessment and genetic counseling is a multi-step process of identifying and counseling individuals at risk for familial or hereditary cancer.

Cancer genetic risk assessment involves use of pedigree analysis with available risk assessment models to determine whether a family history is suggestive of sporadic, familial, or hereditary cancer. Risk assessment includes both an evaluation of an individual's absolute risk of breast and/or ovarian cancer as well as an estimation of the likelihood that the individual has a heritable genetic mutation in his/her family. Genetic risk assessment is a dynamic process and can change if additional relatives are diagnosed with cancer.

Statistical models based on personal and family history characteristics have been developed to estimate a person's interval and lifetime risks of developing breast cancer. For example, the Claus tables may be useful in providing breast cancer risk estimates for white women without a known cancer-associated gene mutation who have one or two first- or second-degree female relatives with breast cancer.[189] In addition, decision models developed to estimate the likelihood that a *BRCA1/2* mutation is present include BRCAPRO[190,191] and the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA)[190]; A lifetime risk of breast cancer of 20% to 25% or greater as assessed by models based largely on family history has been used in some guidelines to identify a woman as being at high risk of breast cancer. For example, this risk threshold was used in updates to the American Cancer Society (ACS) guidelines on breast screening which incorporates magnetic resonance imaging (MRI).[192,193]

First-degree relatives of individuals with a known deleterious gene mutation in *BRCA1/2*, *TP53* or *PTEN* genes are considered to have a 50% risk of carrying that mutation.

Evaluation of Patient's Needs and Concerns

The first step in evaluating a individual's risk for hereditary breast cancer is to assess her/his concerns and reasons for seeking counseling and to guarantee that her/his personal needs and priorities will be addressed in the counseling process. Several studies have documented a highly exaggerated perception of risk among women with a family history of breast cancer who seek cancer risk counseling.[194]

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This is a situation that can interfere with the adoption of appropriate health behaviors. In addition, the patient's knowledge about the benefits, risks, and limitations of genetic testing should be assessed as well as the patient's goals. A positive, supportive interaction with the counseling team is an important determinant of ultimate satisfaction with the counseling process and of adherence to recommended health behaviors.

Detailed Family History

A detailed family history is the cornerstone of effective genetic counseling. An examination of family history involves development of an expanded pedigree collected beginning with the health of the proband (index case) and proceeding outward to include first-, second-, and third-degree relatives on both the maternal and paternal sides. Standardized pedigree nomenclature should be used.[195,196] Unaffected family members, both living and deceased, are also included, as their histories also provide information about the magnitude of genetic risk.

Information collected includes cancer diagnoses by primary site, age at diagnosis, bilaterality (when appropriate), and current age or age at death. Whenever possible, cancer diagnoses in the family are verified by obtaining medical records, pathology reports, or death certificates. This is particularly important in the case of a report of an "abdominal" cancer in a female relative—a situation in which cancers of the cervix, uterus, ovary, and/or colon is often confused. It is also important to know the ancestry/ethnicity of the individual.

Other medical conditions that may be associated with or predispose an individual to breast and/or ovarian cancer should also be noted. Family history data are then graphically represented on a pedigree that follows standard nomenclature to illustrate family relationships and disease information. Factors that limit the informativeness of the pedigree are small family size, a small number of individuals of the susceptible gender for sex-limited cancers, reduced penetrance, early deaths in family members (which precludes the possibility that they will develop adult diseases), prophylactic surgeries that remove an organ from subsequent risk of cancer (e.g., hysterectomy for uterine fibroids in which the ovaries are also removed), adoptions, and inaccurate or incomplete information on family members.[5,197]

A recent prospective registry study of 306 women diagnosed with breast cancer at < 50 years of age, who had no first- or second-degree relatives with breast or ovarian cancer, showed that those individuals with a limited family history (defined as fewer than 2 first- or second-degree female relatives or fewer than 2 female relatives surviving beyond age 45 years in either lineage) may have an underestimated probability of a *BRCA1/2* gene mutation based on models dependent on family history.[198]

Medical and Surgical History

The collection of a detailed medical and surgical history from the proband allows the counselor to estimate the contribution of other risk factors that may interact with or modify family history to determine the risk of breast cancer. A history of previous breast biopsies, especially those in which the pathology revealed atypical hyperplasia or lobular carcinoma in situ (LCIS), is associated with an increased risk of breast cancer.[199,200] Pathologic verification of these diagnoses is encouraged. History of salpingo-oophorectomy and potential exposure to carcinogens (e.g., radiation therapy) should also be included in the patient's assessment. When taking the medical history, the clinician should also be alert to the physical manifestations of Cowden syndrome, especially skin conditions (see below under Focused Physical Examination).

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Reproductive variables are important determinants of risk for both breast and ovarian cancer, suggesting a significant contribution of hormones to the etiology of these cancers. This possible link is supported by the increased breast cancer risk seen among women who have had prolonged exposure to exogenous estrogens and progestins and the reduction in risk for ovarian cancer observed among women who report using oral contraceptives.[201-204]

Focused Physical Examination

A physical examination performed by a physician or nurse should be part of the risk assessment. Particular attention should be paid to organs/areas of the body known to be affected in individuals with specific hereditary breast and/or ovarian syndromes. For example, certain patterns of mucocutaneous manifestations are associated with Cowden syndrome, as discussed earlier; a focused physical examination for Cowden syndrome should include a comprehensive dermatologic examination (including oral mucosa), evaluation of head circumference (to determine presence of macrocephaly) and palpation of the thyroid (see section above on Cowden Syndrome).

Genetic Counseling

Genetic counseling is a critical component of the cancer risk assessment process. Counseling for hereditary breast and/or ovarian cancer uses a broad approach to place genetic risk in the context of other related risk factors, thereby customizing counseling to the experiences of the individual. The purpose of cancer genetic counseling is to educate individuals about the genetic, biological, and environmental factors related to the individual's cancer diagnosis and/or risk of disease to help them derive personal meaning from cancer genetic information, to and empower them to make educated, informed decisions about genetic testing, cancer screening, and cancer prevention. Individuals need to understand the relevant genetic, medical, and psychosocial information and be able to integrate this information before they can make an informed decision. The presentation of information is most effective when tailored to the age and education of the person undergoing counseling, and that individual's personal exposure to the disease, level of risk, and social environment.[7]

Pre-test counseling is an essential element of the genetic counseling process in the event that genetic testing for a gene mutation associated with a hereditary cancer syndrome is under consideration.[7] The foundation of pre-test genetic counseling is based on the principle of informed consent. Pre-test counseling should include a discussion of why the test is being offered and how test results may impact medical management, cancer risks associated with the gene mutation in question, the significance of possible test results (see section on Genetic Testing, below), the likelihood of a positive result, technical aspects and accuracy of the test, economic considerations, risks of genetic discrimination, psychosocial aspects, confidentiality issues, as well as other topics.[7] A discussion of confidentiality issues should include an explanation of the federal Genetic Information Nondiscrimination Act (GINA) enacted in 2008 which prohibits health insurers and employers from discrimination on the basis of genetic test results.[205]

Post-test counseling must also be performed and includes disclosure of results, a discussion of the significance of the results, an assessment of the impact of the results on the emotional state of the individual, a discussion of the impact of the results on the medical management of the individual, and how and where the patient will be followed. In addition, identification of a gene mutation associated with a hereditary predisposition to breast and/or ovarian cancer in an individual necessitates a discussion of possible inherited cancer risk to relatives

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and the importance of informing family members about test results.[7] It may also be appropriate to offer genetic testing to both parents of an individual who tests positive for one of these gene mutations (i.e., *BRCA1/2*, *PTEN*, *TP53*) when the lineage is in question.

Genetic Testing

The selection of appropriate candidates for genetic testing is based on the personal and familial characteristics that determine the individual's prior probability of being a mutation carrier, and on the psychosocial degree of readiness of the person to receive genetic test results. The potential benefits, limitations, and risks of genetic testing are also important considerations in the decision-making process. Many women feel that they are already doing everything they can to minimize their risk of developing breast cancer, and others fear the emotional toll of finding out that they are a mutation carrier, especially if they have children who would be at risk of inheriting the mutation. For those who choose not to proceed with testing, the counseling team tailors recommendations for primary and secondary prevention based on the individual's personal and family history.

In the statement on Genetic Testing for Cancer Susceptibility from the American Society of Clinical Oncology (ASCO) updated in 2003, genetic testing is recommended when there is: (i) a personal or family history suggesting genetic cancer susceptibility (ii) the test can be adequately interpreted and (iii) the results will aid in the diagnosis or influence the medical or surgical management of the patient or family members at hereditary risk of cancer.[206] These recommendations were reiterated in the latest 2010 ASCO update on Genetic and Genomic Testing for Cancer Susceptibility with respect to testing individuals for gene mutations known to cause hereditary breast and/or ovarian cancer(s).[207]

As part of pre-test counseling, the counselor reviews the distinctions between true-positive, true-negative, indeterminate (or uninformative), and inconclusive (or variants of unknown significance) test results (see Table 2), as well as the technical limitations of the testing process. A clear distinction is made between the probability of being a mutation carrier and the probability of developing cancer. The probabilistic nature of genetic test results and the potential implications for other family members must also be discussed. Individuals who have received allogeneic hematopoietic stem cell transplantation (HSCT) should not have molecular genetic testing performed on blood samples, as these blood cells would represent donor-derived DNA. In such cases, DNA of the individual being tested should be extracted from a fibroblast culture, if available. If this is not possible, buccal cells may be considered as an alternative source for DNA; however, a study has reported that over time, buccal epithelial cells are replaced by donor-derived cells in allogeneic HSCT recipients.[208,209] Therefore, genetic testing using buccal swab samples may be limited given this known risk of donor DNA contamination.

The genetic testing strategy is greatly facilitated when a deleterious mutation has already been identified in another family member. In that case, the genetic testing laboratory can limit the search for mutations in additional family members to the same location in the gene. In most cases, an individual testing negative for a known familial gene mutation predisposing to breast cancer can be followed with routine breast screening. Individuals who meet testing criteria but do not undergo gene testing should be followed as if a gene mutation (i.e., *BRCA*, *PTEN*, or *TP53* gene mutation) is present, if they have a close family member who is a known carrier of the deleterious mutation.

For the majority of families in whom mutation status is unknown, it is best to consider testing an affected family member first, especially a

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family member with early-onset disease, bilateral disease, or multiple primaries, because that individual has the highest likelihood for a positive test result. Unless the affected individual is a member of an ethnic group for which particular founder gene mutations are known, comprehensive genetic testing (i.e., full sequencing of the genes and detection of large gene rearrangements) should be performed.

For individuals with family histories consistent with a pattern of hereditary breast and/or ovarian cancer on both the maternal and paternal sides, the possibility of a second deleterious mutation in the family should be considered, and full sequencing may be indicated.

In the situation of an unaffected individual with a family history only, the testing of the unaffected individual (or of unaffected family members) should only be considered when no affected family member is available for testing. In such cases, the unaffected individual or unaffected close relative with the highest likelihood of testing positive for the mutation should be tested. A negative test result in such cases, however, is considered indeterminate (see Table 2) and does not provide the same level of information as when there is a known deleterious mutation in the family. Thus, one should be mindful that when testing unaffected individuals (in the absence of having tested affected family members), significant limitations may exist in interpreting the test results.

In the case of hereditary breast/ovarian cancer (i.e., *BRCA* mutation), if no family member with breast or ovarian cancer is living, consideration can be given to testing first- or second-degree family members affected with cancers thought to be related to the deleterious mutation in question (e.g., prostate or pancreatic cancer).

Another counseling dilemma is posed by the finding of a variant or mutation of unknown significance (see Table 2), a mutation that may actually represent a benign polymorphism unrelated to an increased breast cancer risk or may indicate an increased breast cancer risk. The individual must be counseled in such a situation, because additional information about that specific mutation will be needed before its significance can be understood. These patients should be considered for referral to research studies that aim to define the functional impact of the gene variant.

Finally, it is important to mention that certain large genomic rearrangements are not detectable by a primary sequencing assay, thereby necessitating supplementary testing, in some cases.[210-213] For example, there are tests that detect rare, large cancer-associated rearrangements of DNA in the *BRCA1* and *BRCA2* genes that are otherwise not detected by direct sequencing of the *BRCA1/2* genes. Therefore, the NCCN Guidelines panel emphasizes the need for comprehensive testing, which encompasses full *BRCA1/2* sequencing and detection of large gene rearrangements.

Risk Assessment, Counseling, and Management: Hereditary Breast/Ovarian Cancer Syndrome

Detailed in the NCCN Guidelines is a set of specific risk assessment criteria which form part of the decision-making process in evaluating whether an individual suspected of being carriers of a *BRCA1/2* mutation should be considered for genetic testing (see Guidelines section on Hereditary Breast and/or Ovarian Cancer Syndrome Testing Criteria). Following risk assessment and counseling, genetic testing should be considered for individuals for whom hereditary breast/ovarian cancer syndrome testing criteria are met. Testing is generally not recommended in children under the age of 18 years.

Individuals from a family with a known deleterious *BRCA1* or *BRCA2* mutation should be tested for the specific familial mutation. For

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individuals from a family without a known *BRCA1* or *BRCA2* mutation (and who meet testing criteria), genetic testing should be comprehensive, including full sequencing of *BRCA1* and *BRCA2*, and testing for large genomic rearrangements. Individuals from a family with a known deleterious *BRCA1/BRCA2* mutation who test positive for the familial mutation, or for whom *BRCA1/BRCA2* mutation testing is not performed, should follow the screening recommendations outlined under the Guidelines section on HBOC Syndrome Management (and discussed below).

Individuals of Ashkenazi Jewish descent with no known familial *BRCA1/BRCA2* mutations should first be tested for the three known founder mutations; if the tests are negative for founder mutations, and if the individual's ancestry also included non-Ashkenazi ethnicity (or if other *BRCA1/BRCA2* testing criteria are met), comprehensive genetic testing should be considered. Comprehensive genetic testing (i.e., full sequence of *BRCA1/2* and testing for large gene rearrangements) is also recommended for individuals from other ethnic groups who meet testing criteria. In cases where testing of unaffected individuals are being considered, an affected family member with the highest likelihood of carrying the *BRCA1/BRCA2* mutation should be tested first. If more than one family member is affected, members with the following factors should be considered for testing first: youngest age at diagnosis; having bilateral disease or multiple primaries; or having ovarian cancer. If no living family member with breast or ovarian cancer exists, consider testing first- or second-degree family members affected with cancer thought to be related to deleterious *BRCA1/BRCA2* mutations (e.g., prostate cancer, pancreatic cancer, melanoma). As previously discussed, testing of unaffected individuals should only be considered when an appropriate affected family member is not available for testing; importantly, the significant limitations of interpreting testing results for an unaffected individual should be discussed prior to testing. Individuals from a family with no known deleterious *BRCA1/BRCA2* mutation who test positive for a mutation should follow the screening recommendations outlined under the Guidelines section on HBOC Syndrome Management (and discussed below). Those who test negative for *BRCA1/BRCA2* mutations can be considered for risk assessment/genetic testing for other hereditary breast/ovarian cancer syndromes such as Li-Fraumeni syndrome or Cowden syndrome, if testing criteria for these syndromes are met. For individuals who have not been tested or for those in whom variants of unknown significance are found (uninformative testing results), participation in a research program or individualized recommendations based on personal history and family history should be offered.

Counseling issues specific for both female and male carriers of a *BRCA1/2* mutation include the increased incidence of pancreatic cancer and melanoma. In addition, the risks to family members of individuals with a known *BRCA1/2* gene mutation (see Discussion sections on Risk Assessment and Genetic Testing) should also be discussed as well as the importance of genetic counseling for these individuals. Counseling issues pertaining specifically to male breast cancer have also been described, and include an increased risk of prostate cancer in male carriers of a *BRCA1/2* mutation.[214-216]

Recommendations for the medical management of hereditary breast/ovarian cancer syndrome are based on an appreciation of the early onset of disease, the increased risk of ovarian cancer, and the risk for male breast cancer in *BRCA1/2* carriers. An individual with a known deleterious *BRCA1/2* mutation in a close family member who does not undergo gene testing should be followed according to the same screening/management guidelines as a carrier of a *BRCA1/2* mutation. An individual from a family with a known deleterious *BRCA1/2* mutation

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who tests negative for the familial mutation should be followed according to the recommendations in the NCCN Guidelines for Breast Cancer Screening and Diagnosis. In situations where an individual (or family member) from a family with no known familial *BRCA1/2* mutations undergoes genetic testing, and no mutation is found, testing for other hereditary breast syndromes should be considered if testing criteria are met (see sections on Li-Fraumeni Syndrome Testing Criteria and Cowden Syndrome Testing Criteria).

Screening Recommendations

The emphasis on initiating screening considerably earlier than standard recommendations is a reflection of the early age of onset seen in hereditary breast/ovarian cancer.[217] For a woman who is a carrier of a *BRCA1/2* mutation, training in breast awareness with regular monthly practice should begin at age 18 years, and semiannual clinical breast examinations should begin at age 25 years. The woman should have annual mammograms and breast MRI screening (to be performed on day 7-15 of menstrual cycle for premenopausal women) beginning at age 25 years or on an individualized timetable based on the earliest age of cancer onset in family members.[192,217-220]

Mammography has served as the standard screening modality for detection of breast cancer during the last few decades. False-negative mammography results have been correlated with factors such as presence of *BRCA1/2* mutation and high breast tissue density,[221-224] both of which may occur more frequently among younger women. Rapidly growing or aggressive breast tumors—also more common among younger women—have also been associated with decreased sensitivity of mammographic screening methods.[221,225] Prospective studies on comparative surveillance modalities in women at high risk for familial breast cancer (i.e., confirmed *BRCA1/BRCA2* mutation or suspected mutation based on family history) have consistently reported higher sensitivity of MRI screening (77–94%) compared with mammography (33–59%) in detecting breast cancers; false-positive rates were higher with MRI in some reports, resulting in a slightly lower or similar specificity with MRI screening (81–98%) compared with mammography (92–100%).[217-219,226-228] The sensitivity with ultrasound screening (33–65%) appeared similar to that of mammography in this high-risk population.[217,226-228] In a recent prospective screening trial (conducted from 1997–2009) that evaluated the performance of annual MRI and mammography in women (age 25–65 years; N=496) with confirmed *BRCA1/BRCA2* mutation, sensitivity with MRI was significantly higher compared with mammography during the entire study period (86% vs. 19%; $P<0.0001$).[229] Sensitivity with MRI was higher during the early years (1997–2002; 74% vs. 35%) as well as the later years of the study (2003–2009; 94% vs. 9%). Factors such as age, mutation type or invasiveness of the tumor did not significantly influence the relative sensitivity of the 2 screening modalities. Importantly, the large majority (97%) of cancers detected by MRI screening were early stage tumors.[229] Among previously unaffected women diagnosed with invasive breast cancer during the study (n=28), 1 patient had died due to the cancer and 3 additional patients died due to other causes; the annual breast cancer-specific mortality rate was 0.5%. At a median follow up of 8 years from diagnosis, none of the surviving patients (n=24) has developed distant recurrence.[229] All of the studies discussed above evaluated a screening strategy that was conducted on an annual basis, and many of the studies included individuals without confirmed *BRCA1/BRCA2* mutation status. A recent retrospective study evaluated a different screening interval, using alternating mammography and MRI screening every 6 months in women with confirmed *BRCA1/BRCA2* mutation (N=73).[230] After a median follow up of 2 years, 13 breast cancers were detected among 11 women; 12 of the tumors were

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detected by MRI screening but not by mammography obtained 6 months earlier. The sensitivity and specificity with MRI screening was 92% and 87%, respectively.[230]

The optimal surveillance approach in women at high risk for familial breast cancer remains uncertain, especially for women between the ages of 25 and 30 years. Although earlier studies have reported an unlikely association between radiation exposure from mammography and increased risk of breast cancer in carriers of *BRCA1/BRCA2* mutation,[231,232], a recent report from a large cohort study suggested an increased risk in women exposed to radiation at a young age.[233] A retrospective cohort study (from the GENE-RAD-RISK study) showed that exposure to diagnostic radiation (including mammography) prior to age 30 years was associated with increased risk of breast cancer in women with *BRCA1/BRCA2* mutation (N=1993).[233] Thus, one of the potential benefits of incorporating MRI modalities into surveillance strategies may include minimizing the radiation risks associated with mammography, in addition to the higher sensitivity of MRI screening in detecting tumors. The use of MRI, however, may potentially be associated with higher false-positive results and higher costs relative to mammography. The appropriate imaging modalities and surveillance intervals are still under investigation. In a recent report based on a computer simulation model that evaluated different annual screening strategies in *BRCA1/BRCA2* mutation carriers, a screening approach that included annual MRI starting at age 25 years combined with alternating digital mammography/MRI starting at age 30 years was shown to be the most effective strategy when radiation risks, life expectancy and false-positive rates were considered.[234] Future prospective trials are needed to evaluate the different surveillance strategies in individuals at high risk for familial breast cancer. Annual MRI as an adjunct to screening mammogram and clinical breast examination for women aged 25 years or older with a genetic predisposition for breast cancer is supported by guidelines from the ACS.[192]

Post-test counseling in women with confirmed *BRCA1/BRCA2* mutation (or highly suspected of having the mutation based on presence of known deleterious mutation in the family) includes discussion of risk-reducing mastectomy and/or salpingo-oophorectomy. Counseling for these risk-reducing surgeries may include discussion of extent of cancer risk reduction/protection, risks associated with surgeries, reconstructive options, management of menopausal symptoms, and discussion of reproductive desires. It is important to address the psychosocial and quality-of-life aspects of undergoing risk-reducing surgical procedures.

For women who have not elected ovarian cancer risk-reducing surgery, concurrent transvaginal ultrasound and CA-125 determination should be considered every 6 months, starting at age 30 years or 5 to 10 years earlier than the earliest age of first diagnosis of ovarian cancer in the family, for the early detection of ovarian cancer (see Guidelines section on HBOC Syndrome Management). Although there are retrospective data indicating that annual ovarian screening using transvaginal ultrasound and measurement of serum CA-125 levels is neither an effective strategy for the early detection of ovarian tumors nor a reasonable substitute for a bilateral risk-reduction salpingo-oophorectomy,[235,236] the data are limited regarding the effectiveness of these screening interventions when used every 6 months. Investigational imaging and screening studies may be considered for this population.

Men testing positive for a *BRCA1/2* mutation should have a semiannual clinical breast examination, and undergo training in breast self-examination with regular monthly practice starting at age 35 years.

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Baseline mammography should be considered at age 40 years, followed by annual screening with mammography for those men with gynecomastia or parenchymal/glandular breast density on baseline study. In addition, screening for prostate cancer starting at age 40 years should be considered. Involvement in population screening guidelines for prostate cancer is recommended. For both men and women testing positive for a *BRCA1/2* mutation, a full body skin exam for melanoma screening and investigational protocols for pancreatic cancer screening should be considered. Although no specific screening guidelines exist for these tumor types, individualized screening approaches may be provided according to personal or family history of cancer.

Risk Reduction Surgery

Bilateral Total Mastectomy

Retrospective analyses with median follow-up periods of 13-14 years have indicated that bilateral risk reduction mastectomy (RRM) decreased the risk of developing breast cancer by at least 90% in moderate- and high-risk women and in known *BRCA1/2* mutation carriers.[237,238] Results from smaller prospective studies with shorter follow-up periods have provided support for concluding that RRM provides a high degree of protection against breast cancer in women with a *BRCA1/2* mutation.[239,240]

The NCCN Guidelines panel supports discussion of the option of RRM for women on a case-by-case basis. Counseling regarding the degree of protection offered by such surgery and the degree of cancer risk should be provided.

It is important that the potential psychosocial effects of RRM are addressed, although these effects have not been well studied.[241] Multidisciplinary consultations are recommended prior to surgery and should include the discussions of the risks and benefits of surgery, and surgical breast reconstruction options. Immediate breast reconstruction is an option for many women following RRM, and early consultation with a reconstructive surgeon is recommended for those considering either immediate or delayed breast reconstruction.[242]

Bilateral Salpingo-oophorectomy

Women with a *BRCA1/2* mutation are at increased risk for both breast and ovarian cancers (including fallopian tube cancer and primary peritoneal cancer).[243,244] Although the risk of ovarian cancer is generally considered to be lower than the risk of breast cancer in a *BRCA1/2* mutation carrier,[36,37,245] the absence of reliable methods of early detection and the poor prognosis associated with advanced ovarian cancer have lent support for the performance of bilateral risk reduction salpingo-oophorectomy (RRSO) after completion of childbearing in these women. In the studies of Rebbeck et al, the mean age at diagnosis of ovarian cancer was 50.8 years for *BRCA1/2* carriers.[246]

The effectiveness of RRSO in reducing the risk of ovarian cancer in carriers of a *BRCA1/2* mutation has been demonstrated in a number of studies. For example, results of a meta-analysis involving 10 studies of *BRCA1/2* mutation carriers showed an approximately 80% reduction in the risk of ovarian or fallopian cancer following RRSO.[247] In a large prospective study of women who carried deleterious *BRCA1* or *BRCA2* mutations (N=1079), RRSO significantly reduced the risk of *BRCA1*-associated gynecological tumors (including ovarian, fallopian tube or primary peritoneal cancers) by 85% compared with observation, during a 3-year follow-up period (hazard ratio=0.15; 95% CI, 0.04-0.56; *P*=0.005).[248] However, a 1%-4.3% residual risk of a primary peritoneal carcinoma has been reported in some studies.[99,246,247,249-251]

RRSO is also reported to reduce the risk of breast cancer in carriers of a *BRCA1/2* mutation by approximately 50%.[246,247,251,252] In the case-

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control international study by Eisen et al., a 56% (odds ratio=0.44; 95% CI, 0.29-0.66; *P*<0.001) and a 43% (odds ratio=0.57; 95% CI, 0.28-1.15; *P*=0.11) breast cancer risk reduction (adjusted for oral contraceptive use and parity) was reported following RRSO in carriers of a *BRCA1* and a *BRCA2* mutation, respectively.[252] Hazard ratios of 0.47 (95% CI, 0.29-0.77)[246] and 0.30 (95% CI, 0.11-0.84; *P*=0.022)[250] were reported in two other studies comparing breast cancer risk in women with a *BRCA1/2* mutation who had undergone RRSO with carriers of these mutations who opted for surveillance only. These studies are further supported by a recent meta-analysis which found similar reductions in breast cancer risk of approximately 50% for *BRCA1* and *BRCA2* mutation carriers following RRSO,[247] although results of a prospective cohort study suggest that RRSO may be associated with a greater reduction in breast cancer risk for *BRCA2* mutation carriers compared with *BRCA1* mutation carriers.[248]

Reductions in breast cancer risk for carriers of a *BRCA1/2* mutation undergoing RRSO may be associated with decreased hormonal exposure following surgical removal of the ovaries. Greater reductions in breast cancer risk were observed in women with a *BRCA1* mutation who had a RRSO at age 40 years or younger (odds ratio=0.36, 95% CI, 0.20-0.64) relative to *BRCA1* carriers aged 41-50 years who had this procedure (odds ratio=0.50, 95% CI, 0.27-0.92).[252] A nonsignificant reduction in breast cancer risk was found for women aged 51 or older although only a small number of women were included in this group.[252] However, results from Rebbeck et al also suggest that RRSO after age 50 is not associated with a substantial decrease in breast cancer risk.[251] Due to the limited data, an optimal age for RRSO is difficult to specify.

The NCCN Guidelines panel recommends RRSO for women with a known *BRCA1/2* mutation, ideally between ages 35 and 40 years and upon completion of child bearing or at an individualized age based on earliest age of ovarian cancer diagnosed in the family. Peritoneal washings should be performed at surgery, and pathologic assessment should include fine sectioning of the ovaries and fallopian tubes.[100,101] The protocol published by the College of American Pathologists (2009) can be consulted for details on specimen evaluation.[253]

Other topics which should be addressed with respect to RRSO include the increased risk of osteoporosis and cardiovascular disease associated with premature menopause, as well as the potential effects of possible cognitive changes, accelerated bone loss, and vasomotor symptoms on quality of life.

It has been reported that short-term hormone replacement therapy (HRT) in women undergoing RRSO does not negate the reduction in breast cancer risk associated with the surgery.[254] In addition, results of a recent case-control study of *BRCA1* mutation carriers showed no association between use of HRT and increased breast cancer risk in postmenopausal *BRCA1* mutation carriers.[255] However, caution should be used when considering use of HRT in mutation carriers following RRSO, given the limitations inherent in nonrandomized studies.[256,257]

Chemoprevention

The use of selective estrogen receptor modulators (i.e., tamoxifen, raloxifene) has been proven to reduce the risk of invasive breast cancer in postmenopausal women considered at high risk for developing breast cancer.[258-263] However, only limited data are available on the specific use of these agents in patients with *BRCA* mutations. As previously discussed, patients with *BRCA* mutations who are diagnosed with breast cancer have elevated risks for developing contralateral breast tumors. In one of the largest prospective series of *BRCA* mutation carriers evaluated, the mean cumulative lifetime risks for contralateral breast cancer were estimated to be 83% for *BRCA1* carriers and 62%

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for *BRCA2* carriers.[41] Patients with *BRCA* mutations who have intact contralateral breast tissue (and who do not undergo oophorectomy or receive chemoprevention) have an estimated 40% risk of contralateral breast cancer at 10 years.[264] Case control studies from the Hereditary Breast Cancer Clinical Study Group reported that the use of tamoxifen protected against contralateral breast cancer with an odds ratio of 0.38 (95% CI, 0.19–0.74) to 0.50 (95% CI, 0.30–0.85) among *BRCA1* mutation carriers and 0.42 (95% CI, 0.17–1.02) to 0.63 (95% CI, 0.20–1.50) among *BRCA2* carriers.[265,266] This translates to an approximately 45% to 60% reduction in risk of contralateral tumors among *BRCA* mutation carriers with breast cancer. The data were not consistent with regards to the protective effects of tamoxifen in the subset of *BRCA* mutation carriers who also underwent oophorectomy. In addition, no data were available on the estrogen receptor status of the tumors. An evaluation of the subset of healthy individuals with a *BRCA1/2* mutation in the Breast Cancer Prevent Trial revealed that breast cancer risk was reduced by 62% in those with a *BRCA2* mutation receiving tamoxifen relative to placebo (risk ratio=0.38; 95% CI, 0.06–1.56).[267] However, tamoxifen use was not associated with a reduction in breast cancer risk in those with a *BRCA1* mutation.[267] These findings may be related to the greater likelihood for development of estrogen receptor-negative tumors in *BRCA1* mutation carriers relative to *BRCA2* mutation carriers. However, this analysis was limited by the very small number of individuals with a *BRCA1/2* mutation (n=19; 7% of study population). Recently, common single-nucleotide polymorphisms were identified in genes (*ZNF423* and *CTSO* genes) that are involved in estrogen-dependent regulation of *BRCA1* expression.[268] These gene variants were associated with alterations in breast cancer risk during treatment with selective estrogen receptor modulators, and may eventually pave the way for predicting the likelihood of benefit with these chemopreventive approaches in individual patients.

With respect to the evidence regarding the effect of oral contraceptives on cancer risks in women with known *BRCA1/2* gene mutations, case-control studies have demonstrated that oral contraceptives reduced the risk of ovarian cancer by 45%-50% in *BRCA1* mutation carriers and by 60% in *BRCA2* mutation carriers[269,270]; moreover, risks appeared to decrease with longer duration of oral contraceptive use.[270] In a recent meta-analysis conducted in a large number of *BRCA1/2* mutation carriers with (n=1503) and without (n=6315) ovarian cancer, use of oral contraceptives significantly reduced the risk of ovarian cancer by approximately 50% for both the *BRCA1* mutation carriers (summary relative risk [SRR]=0.51; 95% CI, 0.40–0.65) and *BRCA2* mutation carriers (SRR=0.52; 95% CI, 0.31–0.87).[271]

Studies on the effect of oral contraceptive use on breast cancer risk among *BRCA1/2* mutation carriers have reported conflicting data. In one case-control study, use of oral contraceptives was associated with a modest but statistically significant increase in breast cancer risk among *BRCA1* mutation carriers (odds ratio=1.20; 95% CI, 1.02-1.40), but not among *BRCA2* mutation carriers.[272] Among *BRCA1* mutation carriers, breast cancer risks with oral contraceptives were significantly associated with ≥5 years of oral contraceptive use (odds ratio=1.33; 95% CI,1.11–1.60), breast cancer diagnosed before age 40 (odds ratio=1.38; 95% CI,1.11–1.72), and use of oral contraceptives before 1975 (odds ratio=1.42; 95% CI, 1.17–1.75).[272] In another case-control study, oral contraceptive used for at least 1 year was not significantly associated with breast cancer risks in either *BRCA1* or *BRCA2* mutation carriers.[273] However, among *BRCA2* mutation carriers, use of oral contraceptives for at least 5 years was associated with a significantly increased risk for breast cancer (odds ratio=2.06; 95% CI, 1.08-3.94); results were similar when only the cases with oral contraceptives use on or after 1975 were considered.[273] Other case-control studies have

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reported no significant associations with oral contraceptives use (especially with the use of low-dose formulations after 1975) and risks for breast cancer in *BRCA1/2* mutation carriers.[274,275] In fact, in one study, the use of low-dose oral contraceptives for at least 1 year was associated with significantly decreased risks for breast cancer among *BRCA1* mutation carriers (odds ratio=0.22; 95% CI, 0.10–0.49; $P<0.001$), though not for *BRCA2* mutation carriers.[275] Differences in the study design employed by these case-control studies make it difficult to compare outcomes between studies, and likely accounts for the conflicting results. The study design might have differed with regards to factors such as the criteria for defining the 'control' population for the study (e.g., non-*BRCA1/2* mutation carriers vs. mutation carriers without a cancer diagnosis), consideration of family history of breast or ovarian cancer, baseline demographics of the population studied (e.g., nationality, ethnicity, geographic region, age groups), age of onset of breast cancer, and formulations or duration of oral contraceptives used. In a meta-analysis conducted in a large number of *BRCA1/2* mutation carriers with (n=2855) and without (n=2954) breast cancer, use of oral contraceptives was not found to be significantly associated with breast cancer risks in either the *BRCA1* mutation carriers (SRR=1.09; 95% CI, 0.77–1.54) or the *BRCA2* mutation carriers (SRR=1.15; 95% CI, 0.61–2.18).[271]

Reproductive Options

The outcomes of genetic testing can have profound impact on family planning decisions for individuals of reproductive age who are found to be carriers of *BRCA1/2* mutations. For example, in cases where both partners carry a *BRCA2* mutation, there may be a high risk for the offspring to develop a rare Fanconi anemia/brain tumor phenotype (recessive disorder).[215] Counseling for reproductive options such as prenatal diagnosis, preimplantation genetic diagnosis (PGD) and assisted reproduction may therefore be warranted for couples expressing concern over the *BRCA* mutation carrier status of their future offspring. Such counseling should include a comprehensive discussion of the potential risks, benefits, and limitations of reproductive options.

Prenatal diagnosis involves postimplantation genetic analysis of an early embryo, utilizing chorionic villi or amniotic fluid cell samples; genetic testing is typically conducted between week 12 and week 16 of gestation, and testing results may potentially lead to a couple's decision to terminate pregnancy.[216,276] During the past 2 decades, PGD has emerged as an alternative method of genetic testing in early embryos. PGD involves the testing of 1 or 2 cells from embryos in very early stages of development (i.e., 6 to 8 cells) after in vitro fertilization (IVF). This procedure allows for the selection of unaffected embryos to be transferred to the uterus,[216,276] and may, therefore, offer the advantage of avoiding potential termination of pregnancy. However, procedures such as PGD are not without limitations as it may still require a confirmatory prenatal diagnosis depending upon a couple's medical needs or requests. Moreover, the PGD process requires the use of IVF regardless of the fertility status of the couple (i.e., also applies to couples without infertility issues), and IVF may not always lead to a successful pregnancy. Lastly, the technology or expertise may not be readily available in a couple's geographical location. Various factors, both medical and personal, must be weighed in the decision to utilize prenatal diagnosis or PGD. Medical considerations may include factors such as the age of onset of the hereditary cancer, penetrance, severity or associated morbidity and mortality of the cancer, and availability of effective cancer risk reduction methods or effective treatments.[216,276] Although the use of prenatal diagnosis or PGD is relatively well established for severe hereditary disorders with very high penetrance,

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their use in conditions associated with lower penetrance (e.g., hereditary breast or ovarian cancer syndrome) remains somewhat controversial from both an ethical and regulatory standpoint. Personal considerations for the decision to utilize prenatal diagnosis or PGD may include individual ethical beliefs, value systems, cultural and religious beliefs, as well as social and economic factors. Based on results from surveys administered to women at high risk for hereditary breast or ovarian cancer, 50% to 75% of respondents felt that PGD was an acceptable option for high-risk individuals,[277,278] yet only about 14% to 33% would consider undergoing PGD themselves.[277,279] A survey in high-risk men (N=228; carriers of *BRCA* mutation; or having a partner or first-degree relative with *BRCA* mutation) showed that 80% of these men were unaware of PGD; after being informed of the definition of PGD, 34% indicated that they would consider the option of using PGD.[280] Importantly, these surveys suggested that the majority of high-risk women and men have little or no knowledge of PGD,[278,280,281] highlighting the need for better awareness and education regarding potential reproductive options. Successful births have been reported with the use of PGD and IVF in *BRCA1/2* mutation carriers,[282,283] but data in the published literature are still very limited. In addition, data pertaining to long-term safety or outcomes of PDG and assisted reproduction in *BRCA* mutation carriers are not yet available.

Risk Assessment, Counseling, and Management: Li-Fraumeni Syndrome

The approach to families with other hereditary breast cancer syndromes, such as LFS, reflects that of hereditary breast/ovarian cancer in many ways. However, there are some syndrome-specific differences with regard to assessment and management. In the case of LFS, there are multiple associated cancers, both pediatric and adult, that should be reflected in the expanded pedigree (see Guidelines section on Li-Fraumeni Syndrome Testing Criteria). Cancers associated with LFS include but are not limited to premenopausal breast cancer, bone and soft tissue sarcomas, acute leukemia, brain tumor, adrenocortical carcinoma, unusually early onset of other adenocarcinomas, or other childhood cancers.[113,127] Verification of these sometimes very rare cancers is particularly important.

Following risk assessment and counseling, genetic testing should be considered in individuals for whom testing criteria are met. This recommendation is category 2A for adults and 2B for children. The NCCN Guidelines panel also suggests consideration of *TP53* mutation testing in those with early onset breast cancer (≤35 years of age) for whom *BRCA1/2* testing result is negative, especially if there is a family history of LFS related cancers. The NCCN Guidelines panel recommends comprehensive testing, which should include full sequencing and analysis of gene deletion/duplication. In the absence of additional family history, early breast cancer alone is associated with a low likelihood of mutation identification. Individuals who have tested positive for a *TP53* mutation may have greater distress than anticipated, so provisions for supportive interventions should be provided. An individual with a known deleterious *TP53* mutation in a close family member who does not undergo testing should be followed according to the same recommendations as a carrier of a *TP53* mutation (see Guidelines section on Li-Fraumeni Syndrome Management). In situations where an individual (or family member) from a family with no known familial *TP53* mutation undergoes genetic testing, and no mutation is found, testing for other hereditary breast syndromes should be considered if testing criteria are met (see sections on Hereditary Breast and/or Ovarian Cancer Syndrome Testing Criteria and Cowden Syndrome Testing Criteria). As previously discussed in the *BRCA1/BRCA2* testing section above, testing of unaffected individuals

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should only be considered when an appropriate affected family member is not available for testing; importantly, the significant limitations of interpreting testing results for an unaffected individual should be discussed prior to testing.

Management of LFS should address the limitations of screening for the many cancers associated with this syndrome. For those at risk for breast cancer, training and education in breast self-examination should start at age 18 years, with the patient performing regular self-examination on a monthly basis. For members of families with LFS, it is recommended that breast cancer surveillance by clinical breast examination, every 6 to 12 months, begin between the ages of 20 and 25 years (or 5 to 10 years before the earliest known breast cancer in the family, whichever is earlier) because of the very early age of breast cancer onset seen in these families. Annual mammograms and breast MRI screening should begin at ages 20 to 25 years or be individualized, based on earliest age of onset in the family. For relatively young patients (age 20 to 30 years), only an annual breast MRI screening may be considered (given the theoretical risks of radiation exposure with mammography in these patients) based on the physician's discretion. Although there are no data regarding risk reduction surgery in women with LFS, options for risk reducing mastectomy should be discussed on a case-by-case basis (see Discussion section on Bilateral Total Mastectomy for HBOC). Counseling for risk-reducing surgeries may include discussion of extent of cancer risk reduction/protection, risks associated with surgeries, and reconstructive options. It is also important to address the psychosocial and quality-of-life aspects of undergoing risk-reducing surgical procedures.

Many of the other cancers associated with germline mutations in *TP53* do not lend themselves to early detection. Thus, additional recommendations are general and include annual comprehensive physical examinations starting at age 20 to 25 years among family members who have survived one cancer when there is a high index of suspicion for second malignancies (Guidelines section on Li-Fraumeni Syndrome Management). Clinicians should address screening limitations for other cancers associated with LFS. The option to participate in clinical trials evaluating novel screening approaches using technologies such as whole-body MRI, abdominal ultrasound and brain MRI should also be discussed if such trials are available. Colonoscopy should be considered every 2 to 5 years, starting at no later than 25 years. Education regarding signs and symptoms of cancer is important. Patients should be advised about the risk to relatives, and genetic counseling for relatives is recommended. Annual physical examination is recommended for cancer survivors with a high index of suspicion for rare cancers and second malignancies. Pediatricians should be made aware of the risk of childhood cancers in affected families. For couples expressing the desire that their offspring not carry a familial *TP53* mutation, options for prenatal diagnosis should be discussed (for discussion on known risks, limitations, and benefits of such technologies, see section above on Reproductive Options under Risk Assessment, Counseling, and Management: Hereditary Breast and/or Ovarian Cancer Syndrome).

A recent prospective observational study incorporated a clinical surveillance protocol for asymptomatic *TP53* mutation carriers from eight families affected by LFS.[284] In this study, 18 of the 33 asymptomatic mutation carriers agreed to undergo surveillance while the remainder of the carriers did not. The surveillance protocol included both biochemical methods and imaging techniques, such as annual brain MRI for brain tumor surveillance (both children and adults); annual rapid total-body MRI (both children and adults) and ultrasound of abdomen and pelvis every 6 months (for adults only) for soft

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tissue/bone sarcoma surveillance; colonoscopy every 2 years beginning at age 40 years (or 10 years before earliest known colon cancer in the family); ultrasound of abdomen and pelvis every 3-4 months, complete urinalysis every 3-4 months, blood test every 4 months for adrenocortical carcinoma surveillance (children only); and complete blood counts and blood tests every4 months for leukemia/lymphoma surveillance (both children and adults); for surveillance of breast cancers, the protocol was similar to the NCCN Guidelines for LFS management.[284] Using this surveillance protocol, asymptomatic tumors were detected in 7 of the patients; after a median follow-up time of 24 months, all 7 of these carriers were alive. Ten individuals in the non-surveillance group developed high-grade, advanced stage tumors; only 2 of these individuals were alive at the end of follow up. The 3-year overall survival rate was significantly higher for the surveillance group compared with the non-surveillance group (100% vs. 21%; P=0.016).[284] Although this was a small study in a limited number of patients, the clinical surveillance protocol employed was feasible and detected asymptomatic tumors in about 40% of individuals with *TP53* mutations. The protocol may represent an emerging option for surveillance/management of at-risk individuals from families with LFS; further evaluation of this protocol is warranted.

Only very limited data exists on the use of prenatal diagnostics/genetic testing for *TP53* mutations in families with LFS.[285,286] Counseling for reproductive options such as prenatal diagnosis, preimplantation genetic diagnosis (PGD) and assisted reproduction may be warranted for couples expressing concern over the mutation carrier status of their future offspring. Such counseling should include a comprehensive discussion of the potential risks, benefits, and limitations of reproductive options. For general discussions on the topic of reproductive options and counseling considerations, see the Discussion section above on Reproductive Options under Risk Assessment, Counseling, and Management: Hereditary Breast/Ovarian Cancer Syndrome.

Risk Assessment, Counseling, and Management: Cowden Syndrome

The assessment of individuals suspected of having Cowden syndrome incorporates both a history of the benign and malignant conditions associated with the syndrome and a targeted physical examination, including the skin and oral mucosa, breast, and thyroid gland (see Guidelines section on Cowden Syndrome Testing Criteria). The NCCN Guidelines panel has recently revised both the list of criteria associated with this genetic syndrome as well as the combinations of criteria that establish which individuals are candidates for *PTEN* gene mutation testing (see Guidelines section on Cowden Syndrome Testing Criteria and Discussion section on Cowden Syndrome). These criteria are recommended to assess the need for further risk assessment and genetic testing, but are not intended to serve as clinical diagnostic criteria. Following risk assessment and counseling, genetic testing should be considered in individuals for whom testing criteria are met. The NCCN Guidelines panel recommends comprehensive testing, which should include full sequencing, gene deletion/duplication analysis, and promoter analysis. Unlike the "pathognomonic" criteria, none of the individual major or minor criteria are considered by the NCCN Guidelines panel to be sufficient to warrant genetic testing in the absence of other clinical evidence of Cowden syndrome. However, the panel recommends genetic testing in an individual exhibiting 2 or more major criteria where one is macrocephaly, 3 or more major criteria when one is not macrocephaly, one major criterion along with 3 or more minor criteria, or in someone meeting specifications for 4 minor criteria. Furthermore, any of the major criteria can be classified as a minor criterion for the purpose of meeting the threshold required for genetic

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testing if 2 or more major criteria are present in a single individual but the individual does not have macrocephaly. The testing threshold is lower for an individual considered to be "at risk" (e.g., a first-degree relative of an individual and/or proband with a clinical diagnosis of Cowden syndrome or BRRS for whom genetic testing has not been performed). In this case, any one major criterion or 2 minor criteria are considered to be sufficient for genetic testing to be recommended. Recommendations for individuals not meeting these testing criteria should be individualized according to personal and family history.

An individual with a known deleterious *PTEN* mutation in a close family member who does not undergo gene testing should be followed according to the same guideline as a carrier of a *PTEN* mutation (see Guidelines section on Cowden Syndrome Management). In situations where an individual (or family member) from a family with no known familial *PTEN* mutation undergoes genetic testing, and no mutation is found, testing for other hereditary breast syndromes should be considered if testing criteria are met (see sections on Hereditary Breast and/or Ovarian Cancer Syndrome Testing Criteria and Li-Fraumeni Syndrome Testing Criteria).

Current medical management recommendations for individuals with Cowden syndrome focus on primary and secondary prevention options for breast cancer and on annual physical examinations, starting at age 18 years (or 5 years before the youngest age of diagnosis of a component cancer in the family) to detect skin changes and to monitor the thyroid gland for abnormalities. A baseline thyroid ultrasound should be performed at age 18 years and considered annually thereafter for both men and women with Cowden syndrome. Annual dermatological examination should also be considered. In addition, colonoscopy should be considered starting at age 35 years, performed every 5 to 10 years or more frequently in cases where the patient is symptomatic or polyps are found. Education regarding the signs and symptoms of cancer is important; patients should also be advised about the risk to relatives, and genetic counseling is recommended for at-risk relatives.

Women should begin regular monthly breast self-examinations at age 18 years and have a semiannual clinical breast examination, beginning at age 25 years or 5-10 years earlier than the earliest known breast cancer in the family. Women should also have an annual mammogram and breast MRI screening starting at ages 30-35 years, or 5 to 10 years earlier than the earliest known breast cancer in the family. Although there are no data regarding risk reduction surgery in women with Cowden syndrome, the option of risk-reduction mastectomy and hysterectomy should be discussed on a case-by-case basis (see Discussion section on Bilateral Total Mastectomy). Oophorectomy is not indicated for Cowden syndrome alone, but may be indicated for other reasons. Counseling for risk-reducing surgeries may include discussion of extent of cancer risk reduction/protection, risks associated with surgeries, reconstructive options and reproductive desires. It is also important to address the psychosocial and quality-of-life aspects of undergoing risk-reducing surgical procedures. The panel recommends patient education regarding the symptoms of endometrial cancer including the necessity of a prompt response to such symptoms. Women diagnosed with Cowden syndrome should consider participation in a clinical trial to determine the effectiveness and necessity of endometrial cancer screening. No published data exists on the use of prenatal diagnostics/genetic testing for *PTEN* mutations in families with Cowden syndrome. However, for couples expressing the desire that their offspring not carry a familial *PTEN* mutation, options for prenatal diagnosis, preimplantation genetic diagnosis (PGD) and assisted reproduction can be discussed. Such counseling should include a comprehensive discussion of the potential risks, benefits, and

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limitations of reproductive options. For general discussions on the topic of reproductive options and counseling considerations, see the Discussion section above on Reproductive Options, under Risk Assessment, Counseling, and Management: Hereditary Breast/Ovarian Cancer Syndrome.

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Table 1. Glossary of relevant genetic terms (from the National Cancer Institute [NCI])

Autosomal dominant
Autosomal dominant inheritance refers to genetic conditions that occur when a mutation is present in one copy of a given gene (i.e., the person is heterozygous).

Autosomal recessive
Autosomal recessive inheritance refers to genetic conditions that occur only when mutations are present in both copies of a given gene (i.e., the person is homozygous for a mutation, or carries two different mutations of the same gene, a state referred to as compound heterozygosity).

de novo mutation
An alteration in a gene that is present for the first time in one family member as a result of a mutation in a germ cell (egg or sperm) of one of the parents, or a mutation that arises in the fertilized egg itself during early embryogenesis. Also called new mutation.

Familial: A phenotype or trait that occurs with greater frequency in a given family than in the general population; familial traits may have a genetic and/or nongenetic etiology.

Family history
The genetic relationships within a family combined with the medical history of individual family members. When represented in diagram form using standardized symbols and terminology, it is usually referred to as a pedigree or family tree.

Founder effect
A gene mutation observed with high frequency in a population founded by a small ancestral group that was once geographically or culturally isolated, in which one or more of the founders was a carrier of the mutant gene.

Germline
The cells from which eggs or sperm (i.e., gametes) are derived.

Kindred
An extended family.

Pedigree
A graphic illustration of family history.

Penetrance
A characteristic of a genotype; it refers to the likelihood that a clinical condition will occur when a particular genotype is present.

Proband
The individual through whom a family with a genetic disorder is ascertained. In males this is called a propositus, and in females it is called a proposita.

Sporadic cancer
This term has two meanings. It is sometimes used to differentiate cancers occurring in people who do not have a germline mutation that confers increased susceptibility to cancer from cancers occurring in people who are known to carry a mutation. Cancer developing in people who do not carry a high-risk mutation is referred to as sporadic cancer. The distinction is not absolute, because genetic background may influence the likelihood of cancer even in the absence of a specific predisposing mutation. Alternatively, sporadic is also sometimes used to describe cancer occurring in individuals without a family history of cancer.

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Table 2. Genetic test results to determine the presence of a cancer-predisposing gene

Result	*Description*
True-positive	The person is a carrier of an alteration in a known cancer-predisposing gene.
True-negative	The person is not a carrier of a known cancer-predisposing gene that has been positively identified in another family member.
Indeterminate (Uninformative)	The person is not a carrier of a known cancer-predisposing gene, and the carrier status of other family members is either also negative or unknown.
Inconclusive (Variants of unknown significance)	The person is a carrier of an alteration in a gene that currently has no known significance.

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References

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-767. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2188735.

2. Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet 1993;9:138-141. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8516849.

3. Lynch HT, Watson P, Conway TA, Lynch JF. Clinical/genetic features in hereditary breast cancer. Breast Cancer Res Treat 1990;15:63-71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2322650.

4. Pharoah PD, Day NE, Duffy S, et al. Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer 1997;71:800-809. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9180149.

5. Berliner JL, Fay AM. Risk assessment and genetic counseling for hereditary breast and ovarian cancer: recommendations of the National Society of Genetic Counselors. J Genet Couns 2007;16:241-260. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17508274.

6. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med 2008;359:2143-2153. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19005198.

7. Trepanier A, Ahrens M, McKinnon W, et al. Genetic cancer risk assessment and counseling: recommendations of the national society of genetic counselors. J Genet Couns 2004;13:83-114. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15604628.

8. Pharoah PD, Antoniou A, Bobrow M, et al. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33-36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11984562.

9. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23335087.

10. Blackwood MA, Weber BL. BRCA1 and BRCA2: from molecular genetics to clinical medicine. J Clin Oncol 1998;16:1969-1977. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9586917.

11. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002;108:171-182. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11832208.

12. Pilarski R. Cowden syndrome: a critical review of the clinical literature. J Genet Couns 2009;18:13-27. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18972196.

13. Schneider KA, Garber J. Li-Fraumeni syndrome. GeneReviews; 2013. Available at: http://www.ncbi.nlm.nih.gov/books/NBK1311/.

14. Brooks-Wilson AR, Kaurah P, Suriano G, et al. Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. J Med Genet 2004;41:508-517. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15235021.

15. Kaurah P, MacMillan A, Boyd N, et al. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA 2007;297:2360-2372. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17545690.

16. Schrader KA, Masciari S, Boyd N, et al. Hereditary diffuse gastric cancer: association with lobular breast cancer. Fam Cancer 2008;7:73-82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18046629.

17. Masciari S, Larsson N, Senz J, et al. Germline E-cadherin mutations in familial lobular breast cancer. J Med Genet 2007;44:726-731. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17660459.

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18. Oliveira C, Bordin MC, Grehan N, et al. Screening E-cadherin in gastric cancer families reveals germline mutations only in hereditary diffuse gastric cancer kindred. Hum Mutat 2002;19:510-517. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11968083.

19. Simon R, Zhang X. On the dynamics of breast tumor development in women carrying germline BRCA1 and BRCA2 mutations. Int J Cancer 2008;122:1916-1917. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18098285.

20. ACOG Practice Bulletin No. 103: Hereditary breast and ovarian cancer syndrome. Obstet Gynecol 2009;113:957-966. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19305347.

21. Whittemore AS. Risk of breast cancer in carriers of BRCA gene mutations. N Engl J Med 1997;337:788-789. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9289641.

22. Metcalfe KA, Poll A, Royer R, et al. Screening for founder mutations in BRCA1 and BRCA2 in unselected Jewish women. J Clin Oncol 2010;28:387-391. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20008623.

23. Bergman A, Einbeigi Z, Olofsson U, et al. The western Swedish BRCA1 founder mutation 3171ins5; a 3.7 cM conserved haplotype of today is a reminiscence of a 1500-year-old mutation. Eur J Hum Genet 2001;9:787-793. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11781691.

24. Csokay B, Udvarhelyi N, Sulyok Z, et al. High frequency of germline BRCA2 mutations among Hungarian male breast cancer patients without family history. Cancer Res 1999;59:995-998. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10070953.

25. Ji J, Hemminki K. Familial risk for histology-specific bone cancers: an updated study in Sweden. Eur J Cancer 2006;42:2343-2349. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16859907.

26. Mikaelsdottir EK, Valgeirsdottir S, Eyfjord JE, Rafnar T. The Icelandic founder mutation BRCA2 999del5: analysis of expression. Breast Cancer Res 2004;6:R284-290. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15217494.

27. Petrij-Bosch A, Peelen T, van Vliet M, et al. BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. Nat Genet 1997;17:341-345. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9354803.

28. Tonin PN, Mes-Masson AM, Futreal PA, et al. Founder BRCA1 and BRCA2 mutations in French Canadian breast and ovarian cancer families. Am J Hum Genet 1998;63:1341-1351. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9792861.

29. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998;62:676-689. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9497246.

30. Yun MH, Hiom K. Understanding the functions of BRCA1 in the DNA-damage response. Biochem Soc Trans 2009;37:597-604. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19442256.

31. Cipak L, Watanabe N, Bessho T. The role of BRCA2 in replication-coupled DNA interstrand cross-link repair in vitro. Nat Struct Mol Biol 2006;13:729-733. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16845393.

32. Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994;265:2088-2090. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8091231.

33. Abeliovich D, Kaduri L, Lerer I, et al. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients

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among Ashkenazi women. Am J Hum Genet 1997;60:505-514. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9042909.

34. Levy-Lahad E, Catane R, Eisenberg S, et al. Founder BRCA1 and BRCA2 mutations in Ashkenazi Jews in Israel: frequency and differential penetrance in ovarian cancer and in breast-ovarian cancer families. Am J Hum Genet 1997;60:1059-1067. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9150153.

35. Petrucelli N, Daly MB, Bars Culver JO, Feldman GL. BRCA1 and BRCA2 hereditary breast/ovarian cancer. GeneReviews; 2011. Available at: Available at: http://www.ncbi.nlm.nih.gov/books/NBK1247/.

36. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer 2000;83:1301-1308. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11044354.

37. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117-1130. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12677558.

38. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 2007;25:1329-1333. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17416853.

39. Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343:692-695. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7907678.

40. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643-646. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14576434.

41. Mavaddat N, Peock S, Frost D, et al. Cancer Risks for BRCA1 and BRCA2 Mutation Carriers: Results From Prospective Analysis of EMBRACE. J Natl Cancer Inst 2013. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23628597.

42. Risch HA, McLaughlin JR, Cole DE, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl Cancer Inst 2006;98:1694-1706. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17148771.

43. Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 Mutation. JAMA 2006;296:185-192. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16835424.

44. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001;68:700-710. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11179017.

45. Atchley DP, Albarracin CT, Lopez A, et al. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J Clin Oncol 2008;26:4282-4288. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18779615.

46. Eerola H, Heikkila P, Tamminen A, et al. Relationship of patients' age to histopathological features of breast tumours in BRCA1 and BRCA2 and mutation-negative breast cancer families. Breast Cancer Res 2005;7:R465-469. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15987451.

47. Lakhani SR, Reis-Filho JS, Fulford L, et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res 2005;11:5175-5180. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16033833.

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48. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002;20:2310-2318. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11981002.

49. Lee E, McKean-Cowdin R, Ma H, et al. Characteristics of Triple-Negative Breast Cancer in Patients With a BRCA1 Mutation: Results From a Population-Based Study of Young Women. J Clin Oncol 2011. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22010008.

50. Young SR, Pilarski RT, Donenberg T, et al. The prevalence of BRCA1 mutations among young women with triple-negative breast cancer. BMC Cancer 2009;9:86. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19298662.

51. Evans DG, Howell A, Ward D, et al. Prevalence of BRCA1 and BRCA2 mutations in triple negative breast cancer. J Med Genet 2011;48:520-522. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21653198.

52. Fostira F, Tsitlaidou M, Papadimitriou C, et al. Prevalence of BRCA1 mutations among 403 women with triple-negative breast cancer: implications for genetic screening selection criteria: a Hellenic Cooperative Oncology Group Study. Breast Cancer Res Treat 2012;134:353-362. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22434525.

53. Gonzalez-Angulo AM, Timms KM, Liu S, et al. Incidence and Outcome of BRCA Mutations in Unselected Patients with Triple Receptor-Negative Breast Cancer. Clin Cancer Res 2011;17:1082-1089. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21233401.

54. Rummel S, Varner E, Shriver CD, Ellsworth RE. Evaluation of BRCA1 mutations in an unselected patient population with triple-negative breast cancer. Breast Cancer Res Treat 2013;137:119-125. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23192404.

55. Saura C, Saanchez-Olle G, Bosch N, et al. High prevalence of BRCA1/2 germline mutations in female breast cancer patients with triple-negative phenotype (TNBC) and family history [abstract]. J Clin Oncol 2010;28(Supple 15):Abstract 1534. Available at: http://meeting.ascopubs.org/cgi/content/abstract/28/15_suppl/1534.

56. Tun NM, Villani GM, Ong K. Risk of having BRCA mutations in women with triple-negative breast cancer: A systematic review and meta-analysis [abstract]. J Clin Oncol 2011;29:Abstract 160. Available at: http://meeting.ascopubs.org/cgi/content/abstract/29/27_suppl/160.

57. Lee LJ, Alexander B, Schnitt SJ, et al. Clinical outcome of triple negative breast cancer in BRCA1 mutation carriers and noncarriers. Cancer 2011. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21264845.

58. Comen E, Davids M, Kirchhoff T, et al. Relative contributions of BRCA1 and BRCA2 mutations to "triple-negative" breast cancer in Ashkenazi Women. Breast Cancer Res Treat 2011;129:185-190. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21394499.

59. Meyer P, Landgraf K, Hogel B, et al. BRCA2 mutations and triple-negative breast cancer. PLoS One 2012;7:e38361. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22666503.

60. Cancer risks in BRCA2 mutation carriers. The Breast Cancer Linkage Consortium. J Natl Cancer Inst 1999;91:1310-1316. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10433620.

61. Liede A, Karlan BY, Narod SA. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. J Clin Oncol 2004;22:735-742. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14966099.

62. Agalliu I, Gern R, Leanza S, Burk RD. Associations of high-grade prostate cancer with BRCA1 and BRCA2 founder mutations. Clin Cancer Res 2009;15:1112-1120. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19188187.

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63. Gallagher DJ, Gaudet MM, Pal P, et al. Germline BRCA mutations denote a clinicopathologic subset of prostate cancer. Clin Cancer Res 2010;16:2115-2121. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20215531.

64. Kirchhoff T, Kauff ND, Mitra N, et al. BRCA mutations and risk of prostate cancer in Ashkenazi Jews. Clin Cancer Res 2004;10:2918-2921. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15131025.

65. Leongamornlert D, Mahmud N, Tymrakiewicz M, et al. Germline BRCA1 mutations increase prostate cancer risk. Br J Cancer 2012;106:1697-1701. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22516946.

66. Tulinius H, Olafsdottir GH, Sigvaldason H, et al. The effect of a single BRCA2 mutation on cancer in Iceland. J Med Genet 2002;39:457-462. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12114473.

67. van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, et al. Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. J Med Genet 2005;42:711-719. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16141007.

68. Moran A, O'Hara C, Khan S, et al. Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. Fam Cancer 2012;11:235-242. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22187320.

69. Castro E, Goh C, Olmos D, et al. Germline BRCA Mutations Are Associated With Higher Risk of Nodal Involvement, Distant Metastasis, and Poor Survival Outcomes in Prostate Cancer. J Clin Oncol 2013;31:1748-1757. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23569316.

70. Mitra A, Fisher C, Foster CS, et al. Prostate cancer in male BRCA1 and BRCA2 mutation carriers has a more aggressive phenotype. Br J Cancer 2008;98:502-507. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18182994.

71. Narod SA, Neuhausen S, Vichodez G, et al. Rapid progression of prostate cancer in men with a BRCA2 mutation. Br J Cancer 2008;99:371-374. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18577985.

72. Thorne H, Willems AJ, Niedermayr E, et al. Decreased prostate cancer-specific survival of men with BRCA2 mutations from multiple breast cancer families. Cancer Prev Res (Phila) 2011;4:1002-1010. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21733824.

73. Tryggvadottir L, Vidarsdottir L, Thorgeirsson T, et al. Prostate cancer progression and survival in BRCA2 mutation carriers. J Natl Cancer Inst 2007;99:929-935. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17565157.

74. Ferrone CR, Levine DA, Tang LH, et al. BRCA germline mutations in Jewish patients with pancreatic adenocarcinoma. J Clin Oncol 2009;27:433-438. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19064968.

75. Hahn SA, Greenhalf B, Ellis I, et al. BRCA2 germline mutations in familial pancreatic carcinoma. J Natl Cancer Inst 2003;95:214-221. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12569143.

76. Kim DH, Crawford B, Ziegler J, Beattie MS. Prevalence and characteristics of pancreatic cancer in families with BRCA1 and BRCA2 mutations. Fam Cancer 2009;8:153-158. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18855126.

77. Lynch HT, Deters CA, Snyder CL, et al. BRCA1 and pancreatic cancer: pedigree findings and their causal relationships. Cancer Genet Cytogenet 2005;158:119-125. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15796958.

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78. Murphy KM, Brune KA, Griffin C, et al. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. Cancer Res 2002;62:3789-3793. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12097290.

79. Ozcelik H, Schmocker B, Di Nicola N, et al. Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. Nat Genet 1997;16:17-18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9140390.

80. Lorenzo Bermejo J, Hemminki K. Risk of cancer at sites other than the breast in Swedish families eligible for BRCA1 or BRCA2 mutation testing. Ann Oncol 2004;15:1834-1841. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15550590.

81. Beiner ME, Finch A, Rosen B, et al. The risk of endometrial cancer in women with BRCA1 and BRCA2 mutations. A prospective study. Gynecol Oncol 2007;104:7-10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16962648.

82. Jazaeri AA, Lu K, Schmandt R, et al. Molecular determinants of tumor differentiation in papillary serous ovarian carcinoma. Mol Carcinog 2003;36:53-59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12557260.

83. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005;104:2807-2816. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16284991.

84. Gayther SA, Russell P, Harrington P, et al. The contribution of germline BRCA1 and BRCA2 mutations to familial ovarian cancer: no evidence for other ovarian cancer-susceptibility genes. Am J Hum Genet 1999;65:1021-1029. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10486320.

85. Sekine M, Nagata H, Tsuji S, et al. Localization of a novel susceptibility gene for familial ovarian cancer to chromosome 3p22-p25. Hum Mol Genet 2001;10:1421-1429. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11440995.

86. Lynch HT, Casey MJ, Snyder CL, et al. Hereditary ovarian carcinoma: heterogeneity, molecular genetics, pathology, and management. Mol Oncol 2009;3:97-137. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19383374.

87. Kauff ND, Mitra N, Robson ME, et al. Risk of ovarian cancer in BRCA1 and BRCA2 mutation-negative hereditary breast cancer families. J Natl Cancer Inst 2005;97:1382-1384. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16174860.

88. Alsop K, Fereday S, Meldrum C, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol 2012;30:2654-2663. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22711857.

89. Bolton KL, Chenevix-Trench G, Goh C, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 2012;307:382-390. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22274685.

90. Cass I, Baldwin RL, Varkey T, et al. Improved survival in women with BRCA-associated ovarian carcinoma. Cancer 2003;97:2187-2195. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12712470.

91. Chetrit A, Hirsh-Yechezkel G, Ben-David Y, et al. Effect of BRCA1/2 mutations on long-term survival of patients with invasive ovarian cancer: the national Israeli study of ovarian cancer. J Clin Oncol 2008;26:20-25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18165636.

92. Tan DS, Rothermundt C, Thomas K, et al. "BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with

Discussion updated in progress

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BRCA1 and BRCA2 mutations. J Clin Oncol 2008;26:5530-5536. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18955455.

93. Yang D, Khan S, Sun Y, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA 2011;306:1557-1565. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21990299.

94. Berchuck A, Heron KA, Carney ME, et al. Frequency of germline and somatic BRCA1 mutations in ovarian cancer. Clin Cancer Res 1998;4:2433-2437. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9796975.

95. Bjorge T, Lie AK, Hovig E, et al. BRCA1 mutations in ovarian cancer and borderline tumours in Norway: a nested case-control study. Br J Cancer 2004;91:1829-1834. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15477862.

96. Lakhani SR, Manek S, Penault-Llorca F, et al. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. Clin Cancer Res 2004;10:2473-2481. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15073127.

97. Press JZ, De Luca A, Boyd N, et al. Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer 2008;8:17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18208621.

98. Callahan MJ, Crum CP, Medeiros F, et al. Primary fallopian tube malignancies in BRCA-positive women undergoing surgery for ovarian cancer risk reduction. J Clin Oncol 2007;25:3985-3990. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17761984.

99. Finch A, Shaw P, Rosen B, et al. Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. Gynecol Oncol 2006;100:58-64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16137750.

100. Powell CB, Chen LM, McLennan J, et al. Risk-reducing salpingo-oophorectomy (RRSO) in BRCA mutation carriers: experience with a consecutive series of 111 patients using a standardized surgical-pathological protocol. Int J Gynecol Cancer 2011;21:846-851. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21670699.

101. Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005;23:127-132. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15625367.

102. Shaw PA, Rouzbahman M, Pizer ES, et al. Candidate serous cancer precursors in fallopian tube epithelium of BRCA1/2 mutation carriers. Mod Pathol 2009;22:1133-1138. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19543244.

103. Medeiros F, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006;30:230-236. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16434898.

104. Kindelberger DW, Lee Y, Miron A, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. Am J Surg Pathol 2007;31:161-169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17255760.

105. Basham VM, Lipscombe JM, Ward JM, et al. BRCA1 and BRCA2 mutations in a population-based study of male breast cancer. Breast Cancer Res 2002;4:R2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11879560.

106. Couch FJ, Farid LM, DeShano ML, et al. BRCA2 germline mutations in male breast cancer cases and breast cancer families. Nat Genet 1996;13:123-125. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8673091.

107. Ding YC, Steele L, Kuan CJ, et al. Mutations in BRCA2 and PALB2 in male breast cancer cases from the United States. Breast Cancer Res

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Treat 2011;126:771-778. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20927582.

108. Friedman LS, Gayther SA, Kurosaki T, et al. Mutation analysis of BRCA1 and BRCA2 in a male breast cancer population. Am J Hum Genet 1997;60:313-319. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9012404.

109. Evans DG, Susnerwala I, Dawson J, et al. Risk of breast cancer in male BRCA2 carriers. J Med Genet 2010;47:710-711. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20587410.

110. Tai YC, Domchek S, Parmigiani G, Chen S. Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2007;99:1811-1814. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18042939.

111. What are the key statistics about breast cancer in men? 2009. Available at: Available at: http://www.cancer.org/docroot/CRI/content/CRI_2_4_1X_What_are_the_key_statistics_for_male_breast_cancer_28.asp?sitearea=. Accessed March 1, 2010.

112. Sidransky D, Tokino T, Helzlsouer K, et al. Inherited p53 gene mutations in breast cancer. Cancer Res 1992;52:2984-2986. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1581912.

113. Gonzalez KD, Noltner KA, Buzin CH, et al. Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. J Clin Oncol 2009;27:1250-1256. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19204208.

114. Lane DP. Cancer. p53, guardian of the genome. Nature 1992;358:15-16. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1614522.

115. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323-331. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9039259.

116. Garber JE, Goldstein AM, Kantor AF, et al. Follow-up study of twenty-four families with Li-Fraumeni syndrome. Cancer Res 1991;51:6094-6097. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1933872.

117. Nichols KE, Malkin D, Garber JE, et al. Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomarkers Prev 2001;10:83-87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11219776.

118. Siddiqui R, Onel K, Facio F, et al. The TP53 mutational spectrum and frequency of CHEK2*1100delC in Li-Fraumeni-like kindreds. Fam Cancer 2005;4:177-181. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15951970.

119. Birch JM, Hartley AL, Tricker KJ, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. Cancer Res 1994;54:1298-1304. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8118819.

120. Krutilkova V, Trkova M, Fleitz J, et al. Identification of five new families strengthens the link between childhood choroid plexus carcinoma and germline TP53 mutations. Eur J Cancer 2005;41:1597-1603. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15925506.

121. Li FP, Fraumeni JF, Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med 1969;71:747-752. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5360287.

122. Li FP, Fraumeni JF, Jr., Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988;48:5358-5362. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3409256.

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123. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-1238. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1978757.

124. Varley JM, Evans DG, Birch JM. Li-Fraumeni syndrome--a molecular and clinical review. Br J Cancer 1997;76:1-14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9218725.

125. Melhem-Bertrandt A, Bojadzieva J, Ready KJ, et al. Early onset HER2-positive breast cancer is associated with germline TP53 mutations. Cancer 2011;118:908-913. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21761402.

126. Wilson JR, Bateman AC, Hanson H, et al. A novel HER2-positive breast cancer phenotype arising from germline TP53 mutations. J Med Genet 2010;47:771-774. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20805372.

127. Hisada M, Garber JE, Fung CY, et al. Multiple primary cancers in families with Li-Fraumeni syndrome. J Natl Cancer Inst 1998;90:606-611. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9554443.

128. Lustbader ED, Williams WR, Bondy ML, et al. Segregation analysis of cancer in families of childhood soft-tissue-sarcoma patients. Am J Hum Genet 1992;51:344-356. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1642235.

129. Birch JM, Blair V, Kelsey AM, et al. Cancer phenotype correlates with constitutional TP53 genotype in families with the Li-Fraumeni syndrome. Oncogene 1998;17:1061-1068. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9764816.

130. Chompret A. The Li-Fraumeni syndrome. Biochimie 2002;84:75-82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11900879.

131. Chompret A, Abel A, Stoppa-Lyonnet D, et al. Sensitivity and predictive value of criteria for p53 germline mutation screening. J Med Genet 2001;38:43-47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11332399.

132. Eeles RA. Germline mutations in the TP53 gene. Cancer Surv 1995;25:101-124. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8718514.

133. Lalloo F, Varley J, Ellis D, et al. Prediction of pathogenic mutations in patients with early-onset breast cancer by family history. Lancet 2003;361:1101-1102. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12672316.

134. Bougeard G, Sesboue R, Baert-Desurmont S, et al. Molecular basis of the Li-Fraumeni syndrome: an update from the French LFS families. J Med Genet 2008;45:535-538. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18511570.

135. Tinat J, Bougeard G, Baert-Desurmont S, et al. 2009 version of the Chompret criteria for Li Fraumeni syndrome. J Clin Oncol 2009;27:e108-109; author reply e110. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19652052.

136. Ginsburg OM, Akbari MR, Aziz Z, et al. The prevalence of germline TP53 mutations in women diagnosed with breast cancer before age 30. Fam Cancer 2009;8:563-567. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19714488.

137. Lalloo F, Varley J, Moran A, et al. BRCA1, BRCA2 and TP53 mutations in very early-onset breast cancer with associated risks to relatives. Eur J Cancer 2006;42:1143-1150. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16644204.

138. Lee DS, Yoon SY, Looi LM, et al. Comparable frequency of BRCA1, BRCA2 and TP53 germline mutations in a multi-ethnic Asian cohort suggests TP53 screening should be offered together with BRCA1/2 screening to early-onset breast cancer patients. Breast Cancer Res 2012;14:R66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22507745.

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Clinical Trials: NCCN believes that the best management for any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

To find clinical trials online at NCCN Member Institutions, click here: nccn.org/clinical_trials/physician.html.

NCCN Categories of Evidence and Consensus: All recommendations are category 2A unless otherwise specified.

See NCCN Categories of Evidence and Consensus.

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Updates in the 1.2014 version of the NCCN Guidelines for Breast Cancer Risk Reduction from the 1.2013 version include:

BRISK-1
- Under Familial/genetic factors:
- Known genetic predisposition for breast cancer (BRCA1/2, p53, PTEN, or other gene mutation) is new to the page,
  - Footnote "~~b~~" ~~See BRISK-1 for women with a known genetic predisposition for breast cancer (BRCA1/2, p53, PTEN, or other gene mutation)~~ has been deleted.
- Modified Criteria for further genetic risk evaluation to include, "for women who do not have a personal history of invasive breast cancer or DCIS"
- Under Family history:
  - 2nd sub sub-bullet was reorganized and modified:
    - ≥2 primary~~ies~~ breast *cancers* in a single individual
  - 3rd sub sub-bullet was reorganized and modified:
    - ≥2 individuals with breast ~~primaries~~ *cancers* on the same side of family (maternal or paternal)
  - 4th sub sub-bullet was reorganized and modified:
    - ≥1 ovarian/*fallopian tube/primary peritoneal* cancer from the same side of family (maternal or paternal)
  - 7th sub sub-bullet was modified:
    - From a population at increased risk for *genetic predisposition*
- Corresponding footnote "g" was modified: For populations at increased risk, requirements for inclusion may be modified (eg, women of Ashkenazi Jewish descent with *a personal history of* breast, ovarian, or pancreatic cancer at any age).
- 2nd sub-bullet under Family history has been modified: *Personal history of* ovarian/fallopian tube/primary peritoneal cancer
- The branch off the 2nd column–
  - Yes: *"Referral to cancer genetics professional recommended"* is new to the page as is corresponding footnote "h"
  - No: reader is directed to ***BRISK-2***

BRISK-2
- ~~Woman meets one or more of the familial risk criteria~~ has been deleted.
- ~~Referral to cancer genetics professional recommended~~ has been deleted.
- *Known genetic predisposition* is new to the page
- *Woman meets one or more of the familial risk criteria (See BRISK-1)* is a new footnote corresponding to "Pedigree suggestive of genetic predisposition." (Also for BRISK-4)
- Footnote "k" has been modified to include: *"See life expectancy calculator (www.eprognosis.com)."* (Also for BRISK-3, 4, and 5)

BRISK-3
- Under Elements of Risk:
- ~~Demographics~~ has been replaced with *Elements that increase risk*
- 1st bullet "Increasing age," is new to the page
  - ~~Utility of risk reduction agents for breast cancer risk reduction in women <35 years of age is unknown~~ has been deleted.
- 2nd bullet, "Ethnicity/race" has a new corresponding footnote: *"For example, there is an increased incidence of specific BRCA1/2 mutations in women of Ashkenazi Jewish decent."*
- 3rd bullet, "Lifestyle factors" is new to the page.
  - Increased body mass index (BMI)
  - Alcohol consumption
  - Current or prior estrogen and progesterone hormone therapy
    - ~~Risk factor in caucasian women~~ has been deleted.
    - ~~BMI and breast cancer risk in African American women is complex~~
    - ~~Obesity is a risk factor in postmenopausal women~~ has been deleted.
- 4th bullet, 2nd sub-bullet, *"Nulliparity"* is new to the page.
- New to the page: *Elements that decrease risk*, and includes the following:
  - Prior oophorectomy
  - Prior risk-reduction therapy
  - Exercise

BRISK-5
- 6th column, bottom branch, changed Exemestane to *Aromatase inhibitors* with a corresponding footnote, *"Exemestane and anastrozole are not currently FDA approved for breast cancer risk reduction. There are currently no data comparing the benefits and risks of exemestane and anastrozole to those of tamoxifen or raloxifene."* (Also for BRISK-A and BRISK-B)

BRISK-6 Footnotes:
- *"s" has been modified: Risk-reduction mastectomy should generally be considered only in women with a genetic mutation conferring a high risk for breast cancer (BRCA1/2, PTEN, TP53, CDH1, STK11), compelling family history, or possibly with LCIS or prior thoracic radiation therapy at <30 yrs of age. The value of risk-reducing mastectomy in women with deleterious mutations in other genes associated with a 2-fold or greater risk for breast cancer (based on large epidemiologic studies) in the absence of a compelling family history of breast cancer is unknown.* ~~Risk-reduction mastectomy should generally be considered only in women with BRCA1/2, or other strongly predisposing gene mutation, compelling~~

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BRISK-6 Footnotes (continued)

~~family history, or possibly with LCIS or prior thoracic radiation therapy at <30 y of age. Women considering risk reduction masteetomy should receive multidisciplinary counseling including consultation with genetics if not already done. Psychological consultation may also be of value.~~

- ~~"aa" Other aromatase inhibitors have shown prevention of contralateral breast cancer and there are ongoing clinical trials.~~ has been deleted.
- "y" Axillary node assessment *has limited indication at the time of* ~~is not part of the~~ risk reduction ~~procedure~~ *surgery.*

BRISK-7

- Footnote "ff": "Some selective serotonin reuptake inhibitors (SSRIs) decrease the formation of endoxifen, the active metabolite of tamoxifen. However, citalopram and venlafaxine appear to have minimal impact on tamoxifen metabolism. The clinical impact of these observations is not known." The footnote was deleted from this page and moved to BRISK-B, corresponding to Tamoxifen.

BRISK-A

- ~~Exemestane~~ has been replaced with *aromatase inibitors.*
- Under Surgery, 3rd sub-bullet, *Consider nipple-sparing mastectomy for risk reduction* is new to the page.
- Under Healthy lifestyle
  - 1st sub-bullet modified: Consider breast cancer risks associated with ~~hormone~~ *combined estrogen/progesterone* therapy
  - The reference that corresponds to "Limit alcohol consumption to less than 1 drink per day" has been deleted. ~~Mahoney MG, Bevers T, Linos E, Willett WC. Opportunities and strategies for breast cancer prevention through risk reduction. CA Cancer J Clin 2008;58(6):347-71.~~

BRISK-B

- Under Tamoxifen
  - 3rd sub-bullet modified: ~~Limited data are currently available regarding~~ The efficacy of tamoxifen risk reduction in women who are carriers of BRCA1/2 mutations or who have had prior thoracic radiation *is less well studied than in other risk groups. Available data suggest a benefit.*
- 3rd column: ~~Exemestane~~ has been changed to *Aromatase inhibitors (Exemestane and Anastrozole)*

BRISK-B (continued)

- The following is new to the guidelines: 2nd sub-bullet, Data regarding anastrozole are from a single large randomized study limited to postmenopausal women 40-70 years of age with the following risk compared with the general population:
  - Aged 40-44 years - 4 times higher
  - Aged 45-60 years - ≥2 times higher
  - Aged 60-70 years - ≥1.5 times higher

  Women who did not meet these criteria but had a Tyrer-Cuzick model 10-year breast cancer risk >5% were also included.

- Exemestane: 25 mg per day was found to reduce the relative incidence of invasive breast cancer by 65 % from 0.55% to 0.19% with a median follow-up of 3 years. ~~There are ongoing trials evaluating prolonged aromatase inibitor therapy in postmenopausal healthy women at risk for breast cancer.~~

- Anastrozole: 1 mg per day was found to reduce the relative incidence of breast cancer by 53% with a median follow-up of 5 years.

- There are no data regarding the use of ~~exemestane~~ *aromatase inhibitors* in women who are carriers of BRCA1/2 mutations or who have had prior thoracic radiation.

- For high-risk postmenopausal women, data regarding the risk/benefit ratio for ~~exemestane~~ *aromatase inhibitor* therapy are influenced by age and comorbid conditions such as osteoporosis (category 1). There are insufficient data on ethnicity and race.

- Use of ~~exemestane~~ *aromatase inhibitors* for breast cancer risk reduction in premenopausal women is inappropriate unless part of a clinical trial.

- Reference 3 has been deleted: ~~Fisher B, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90(18):1371-88.~~

MS-1

- The Discussion section has been updated to reflect the changes to the algorithm.

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Breast Cancer Risk Reduction

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FAMILIAL RISK ASSESSMENT[a]

Familial/genetic factors

- **Known genetic predisposition for breast cancer (BRCA1/2, p53, PTEN, or other gene mutation)** → See BRISK-2

- **Criteria for further genetic risk evaluation for women who do not have a personal history of invasive breast cancer or ductal carcinoma in situ (DCIS):[b]**
  - **Family history**
    - **Member of a family with a known mutation in a breast cancer susceptibility gene**
    - **≥2 primary breast cancers[c] in a single individual**
    - **≥2 individuals with breast cancers on the same side of family (maternal or paternal)**
    - **≥1 ovarian/fallopian tube/primary peritoneal cancer from the same side of family (maternal or paternal)**
    - **First- or second-degree relative with breast cancer ≤45 y**
    - **≥1 family member on same side of family with a combination of breast cancer and ≥1 of the following (especially if early onset): pancreatic cancer, aggressive prostate cancer (Gleason score ≥7), sarcoma, adrenocortical carcinoma, brain tumors, endometrial cancer, leukemia/lymphoma, thyroid cancer, dermatologic manifestations,[d] and/or macrocephaly, hamartomatous polyps of GI tract,[e] or diffuse gastric cancer[f]**
    - **From a population at increased risk for genetic predisposition[g]**
    - **Male breast cancer**
  - **Personal history of ovarian/fallopian tube/primary peritoneal cancer**

→ **Woman meets one or more of the familial/genetic risk criteria**
- **Yes** → **Referral to cancer genetics professional recommended[h]** See BRISK-2
- **No** → See BRISK-2

[a] See NCCN Guidelines for Genetic/Familial High-Risk Assessment.

[b] The criteria for further genetic risk assessment and genetic testing are not identical. For the purposes of these guidelines, invasive and ductal carcinoma in situ breast cancers should be included. The maternal and paternal sides of the family should be considered independently for familial patterns of cancer.

[c] Two breast primaries including bilateral disease or cases where there are two or more clearly separate ipsilateral primary tumors.

[d] For dermatologic manifestations, see NCCN Guidelines for Genetic/Familial High-Risk Assessment - Cowden Syndrome (See COWD-1).

[e] For hamartomatous colon polyps in conjunction with breast cancer and hyperpigmented macules of the lips and oral mucosa, STK11 testing should be considered. See NCCN Guidelines for Colorectal Cancer Screening: Peutz-Jeghers Syndrome.

[f] For lobular breast cancer with a family history of diffuse gastric cancer, *CDH1* gene testing should be considered.

[g] For populations at increased risk, requirements for inclusion may be modified (eg, women of Ashkenazi Jewish descent with a personal history of breast, ovarian, or pancreatic cancer at any age).

[h] A genetic counselor, medical geneticist, oncologist, surgeon, oncology nurse, or other health professional with expertise and experience in cancer genetics should be involved early in counseling patients who potentially meet criteria for an inherited syndrome. Genetic counseling is highly recommended when genetic testing is offered and after results are disclosed.

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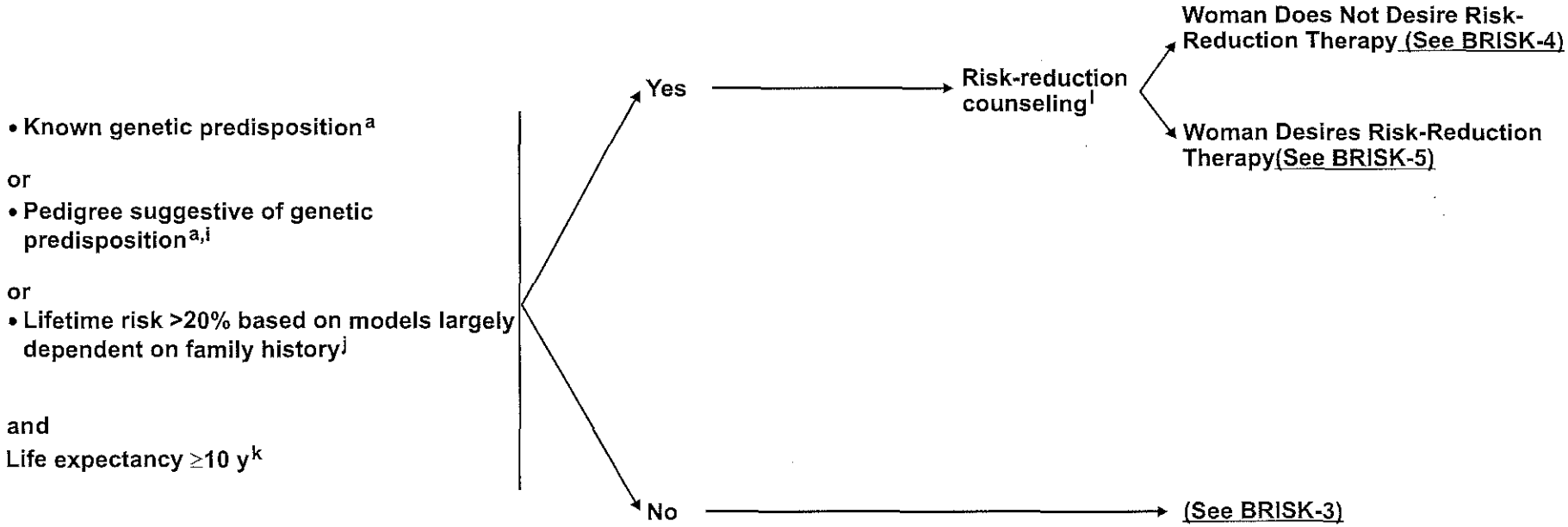
ADDITIONAL RISK ASSESSMENT



[a] See NCCN Guidelines for Genetic/Familial High-Risk Assessment.
[i] Woman meets one or more of the familial risk criteria (See BRISK-1).
[j] Risk models that are largely dependent on family history (eg, Claus, BRCAPRO, BOADICEA, Tyrer-Cuzick).
[k] See life expectancy calculator (www.eprognosis.com). For a reference point, the life expectancy of the average 78-y-old woman in the United States is 10.2 years. (See NCCN Guidelines for Senior Adult Oncology).
[l] See Components of Risk/Benefit Assessment and Counseling (BRISK-A).

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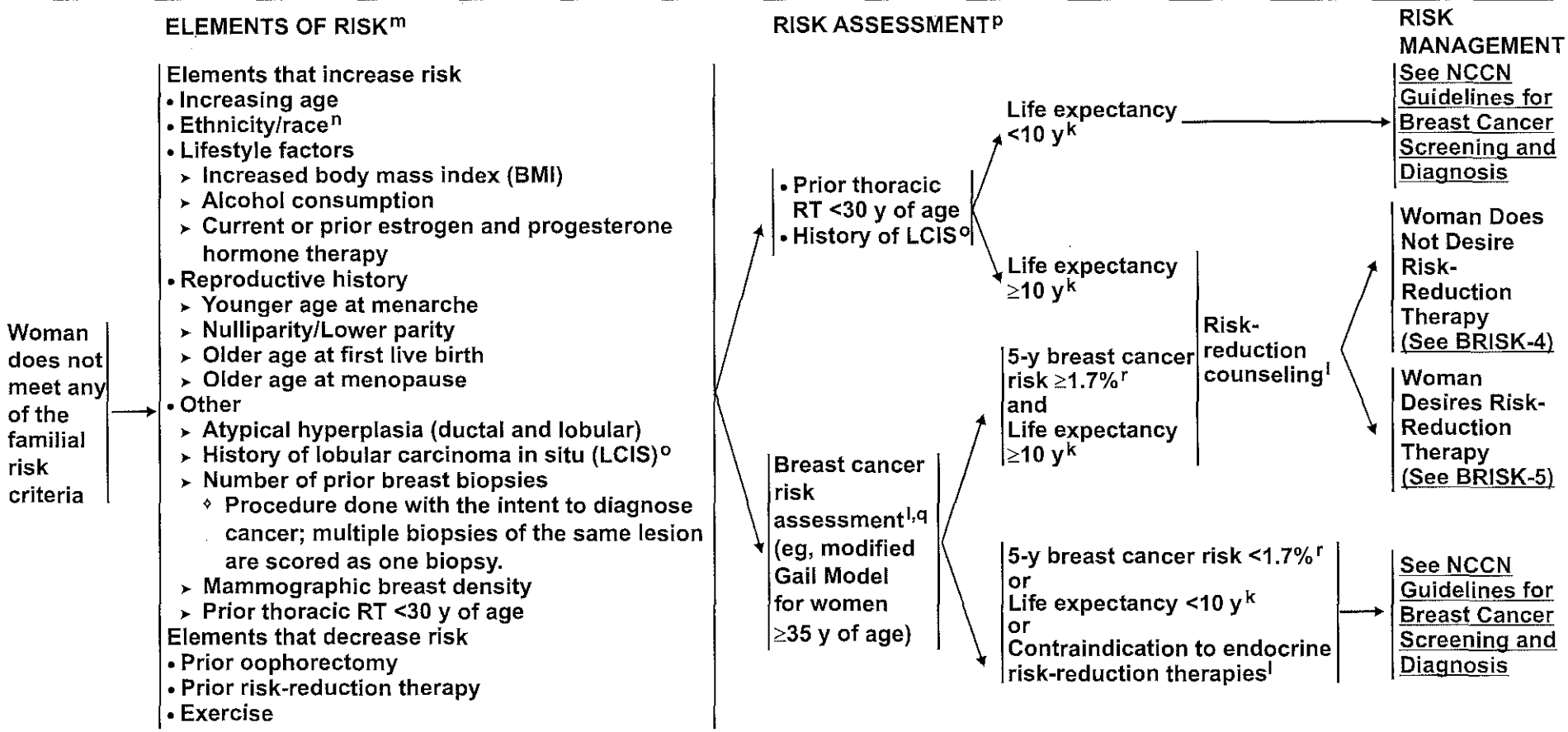

[k] See life expectancy calculator (www.eprognosis.com). For a reference point,the life expectancy of the average 78-y-old woman in the United States is 10.2 years. (See NCCN Guidelines for Senior Adult Oncology).

[l] See Components of Risk/Benefit Assessment and Counseling (BRISK-A).

[m] The management for women with DCIS is available in the NCCN Guidelines for Breast Cancer.

[n] For example, there is an increased incidence of specific BRCA1/2 mutations in women of Ashkenazi Jewish decent.

[o] The management for women with LCIS is also available in the NCCN Guidelines for Breast Cancer.

[p] The clinical utility and role of random periareolar fine needle aspiration, nipple aspiration, or ductal lavage are still being evaluated and should only be used in the context of a clinical trial.

[q] The modified Gail Model (NCI Breast Cancer Risk Assessment Tool) is a computer-based version and may be obtained through the NCI website (http://www.cancer.gov/bcrisktool/Default.aspx). There are circumstances in which the Gail Model underestimates risk for development of breast cancer, for instance, BRCA1/2 carriers and those with a strong family history of breast cancer or family history of ovarian cancer in the maternal or paternal family lineage or non-white women. The Claus, BRCAPRO, Tyrer-Cuzick, and BOADICEA models may be particularly helpful in determining risk for breast cancer in women with a strong family history of breast, ovarian, or other cancers. See Discussion, MS-2.

[r] The definition of risk as defined by the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (NSABP BCPT).

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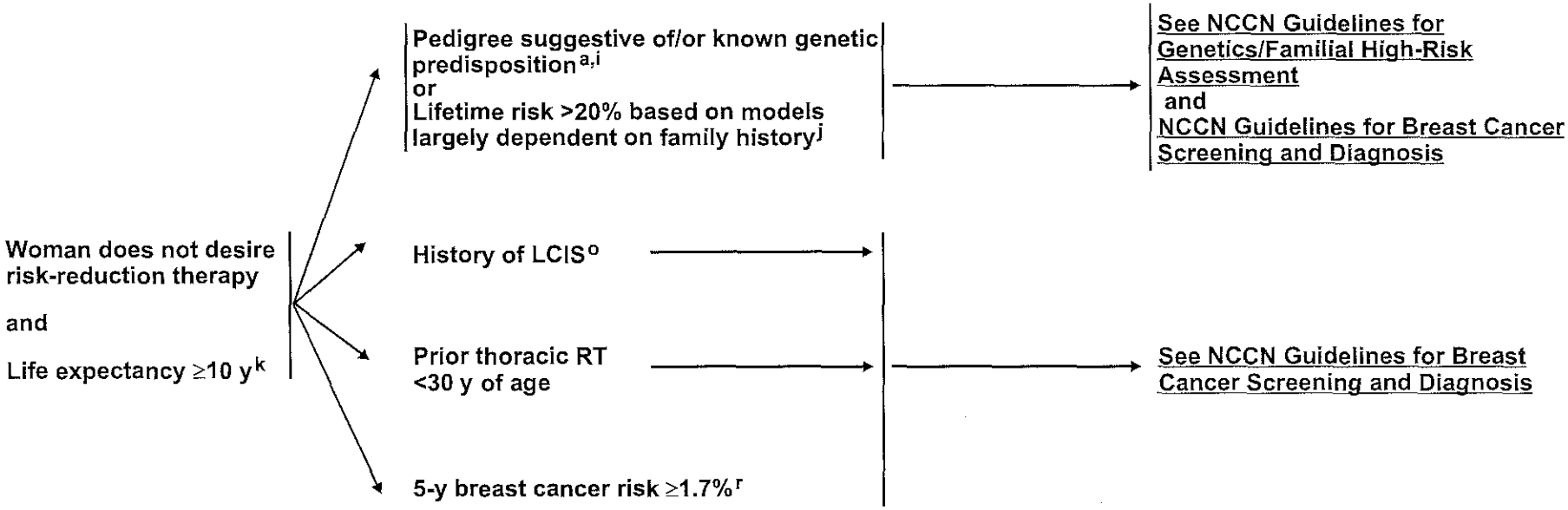
RISK-REDUCTION THERAPY NOT DESIRED | **RISK ASSESSMENT** | **SCREENING/FOLLOW-UP**



[a] See NCCN Guidelines for Genetic/Familial High-Risk Assessment.
[i] Woman meets one or more of the familial risk criteria (See BRISK-1).
[j] Risk models that are largely dependent on family history (eg, Claus, BRCAPRO, BOADICEA, Tyrer-Cuzick).
[k] See life expectancy calculator (www.eprognosis.com). For a reference point, the life expectancy of the average 78-y-old woman in the United States is 10.2 years. (See NCCN Guidelines for Senior Adult Oncology).
[o] The management for women with LCIS is also available in the NCCN Guidelines for Breast Cancer.
[r] The definition of risk as defined by the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (NSABP BCPT).

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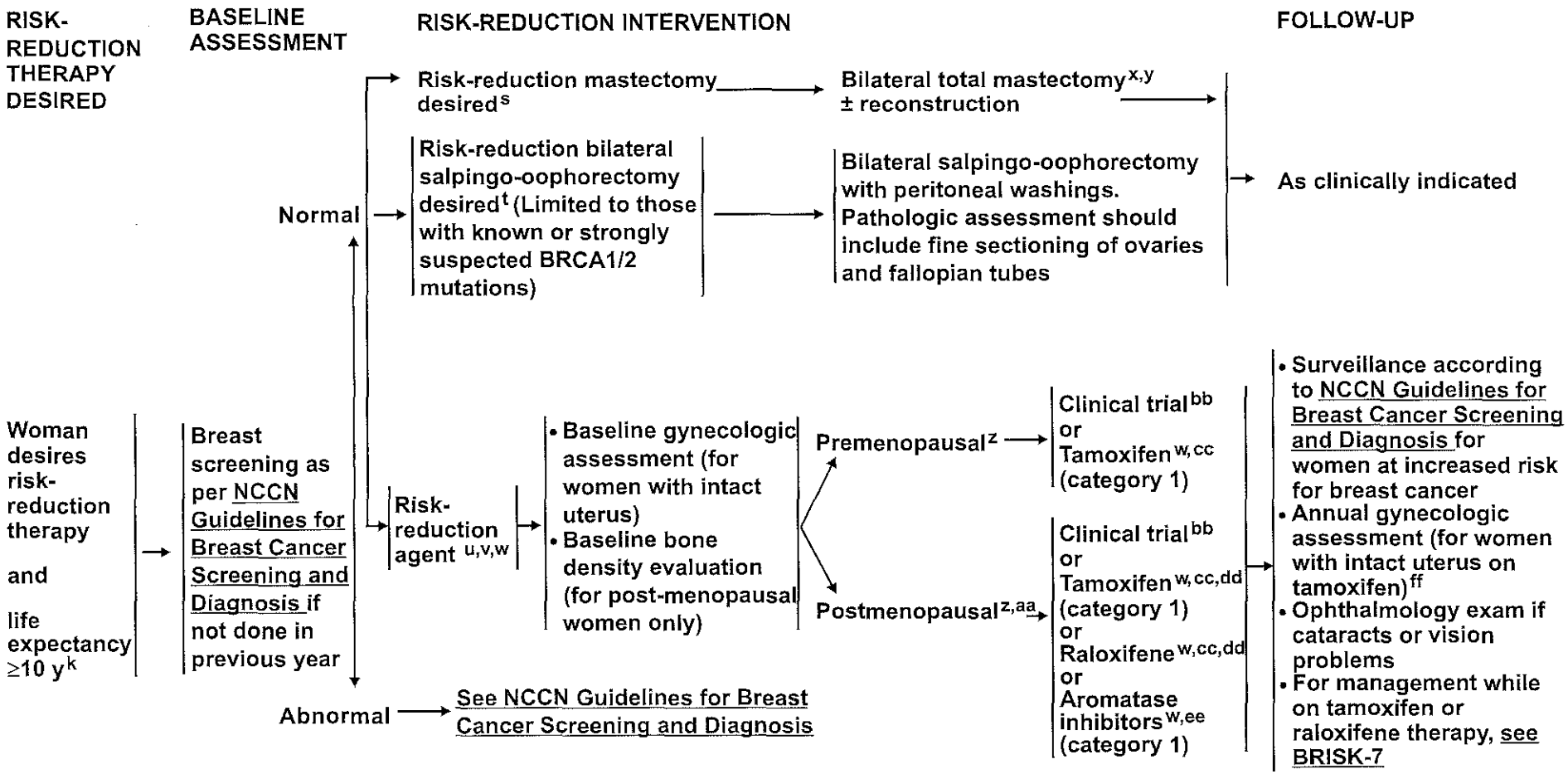

See footnotes on BRISK-6

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FOOTNOTES FOR RISK-REDUCTION THERAPY

[k] See life expectancy calculator (www.eprognosis.com). For a reference point, the life expectancy of the average 78-y-old woman in the United States is 10.2 years. (See NCCN Guidelines for Senior Adult Oncology).

[s] Risk-reduction mastectomy should generally be considered only in women with a genetic mutation conferring a high risk for breast cancer (ie, BRCA1/2, PTEN, TP53, CDH1, STK11), compelling family history, or possibly with LCIS or prior thoracic radiation therapy at <30 yrs of age. The value of risk-reducing mastectomy in women with deleterious mutations in other genes associated with a 2-fold or greater risk for breast cancer (based on large epidemiologic studies) in the absence of a compelling family history of breast cancer is unknown.

[t] The additional benefit of concurrent hysterectomy is not clear at this time.

[u] There are no data regarding the use of risk-reduction agents in women with prior thoracic radiation therapy.

[v] CYP2D6 genotype testing is not recommended in women considering tamoxifen.

[w] See Breast Cancer Risk-Reduction Agents (BRISK-B).

[x] Discuss risks and benefits of nipple-areolar sparing surgery.

[y] Axillary node assessment has limited indication at the time of risk-reduction surgery.

[z] Clinical trials in breast cancer have utilized a variety of definitions of menopause. Menopause is generally the permanent cessation of menses, and as the term is utilized in breast cancer management includes a profound and permanent decrease in ovarian estrogen synthesis. Reasonable criteria for determining menopause include any of the following: Prior bilateral oophorectomy; age ≥60 y; age <60 y; and amenorrhea for 12 or more months in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression and follicle-stimulating hormone (FSH) and estradiol in the postmenopausal range. If taking tamoxifen or toremifene and age <60 y, FSH and plasma estradiol level in postmenopausal ranges.

[aa] Bone density may play a role in choice of therapy.

[bb] Women in clinical trial should have baseline exam, follow-up, and monitoring as per protocol.

[cc] Utility of tamoxifen or raloxifene for breast cancer risk reduction in women <35 years of age is unknown. Raloxifene is only for post-menopausal women >35 y. While raloxifene in long-term follow-up appears to be less efficacious in risk reduction than tamoxifen, consideration of toxicity may still lead to the choice of raloxifene over tamoxifen in women with an intact uterus.

[dd] When counseling postmenopausal women regarding the risk/benefit of tamoxifen and raloxifene, refer to tables in Freedman AN, et al. Benefit/risk assessment for breast cancer chemoprevention with raloxifene or tamoxifen for women age 50 years or older. J Clin Oncol 2011;29(17):2327-2333.

[ee] Exemestane and anastrozole are not currently FDA approved for breast cancer risk reduction. There are currently no data comparing the benefits and risks of exemestane and anastrozole to those of tamoxifen or raloxifene.

[ff] Routine endometrial ultrasound and biopsy are not recommended for women in the absence of other symptoms.

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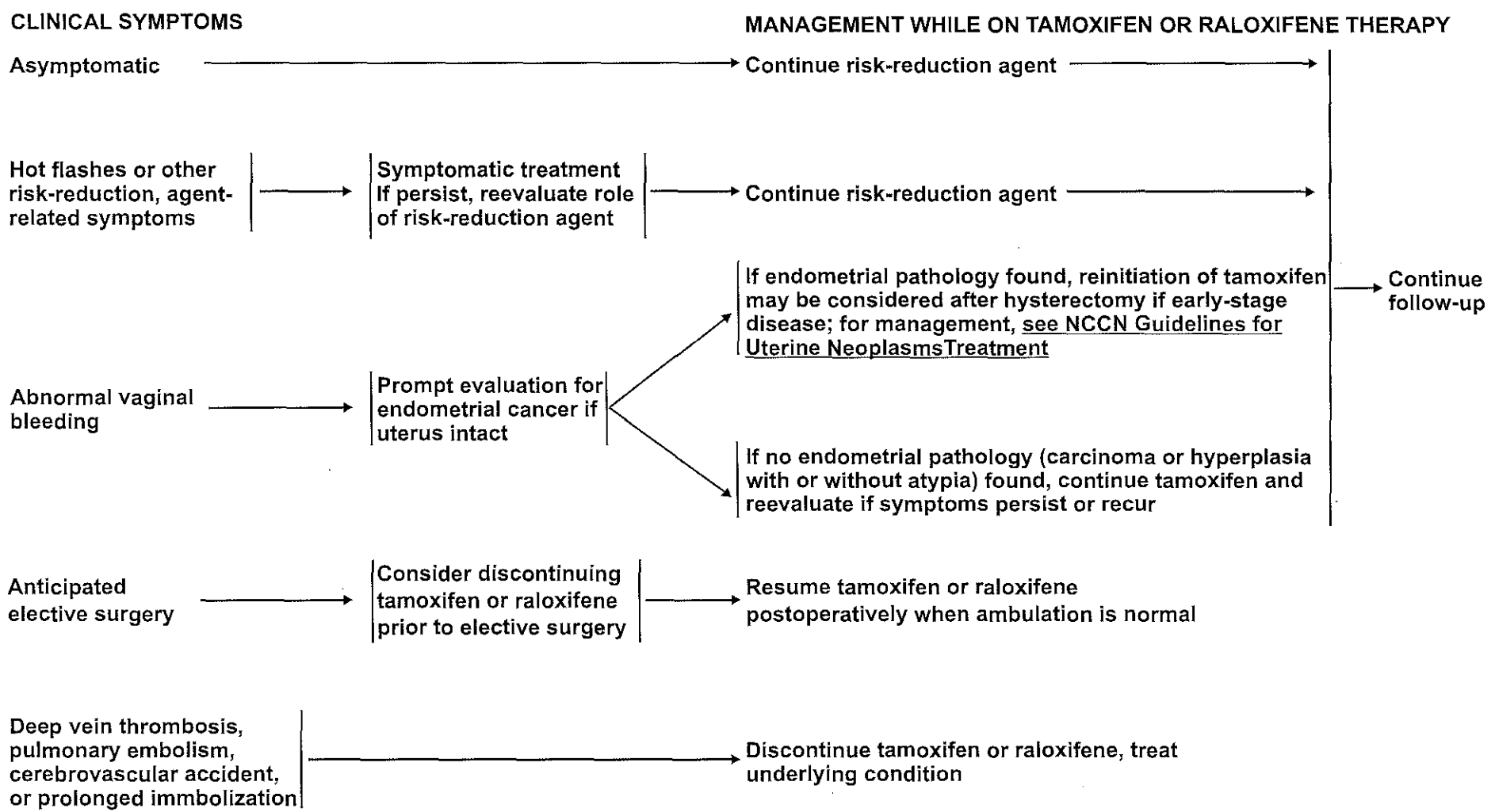

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COMPONENTS OF RISK/BENEFIT ASSESSMENT AND COUNSELING

Options for risk reduction should be discussed in a shared decision-making environment. For breast cancer risk reduction, elements of this discussion include:

- **If a woman is at high risk secondary to a strong family history or very early onset of breast or ovarian cancer, genetic counseling should be offered. See NCCN Guidelines for Genetic/Familial High-Risk Assessment.**

- **Risk-reduction agents - See the Discussion section.**
  - **Discussion of relative and absolute risk reduction with tamoxifen, raloxifene, or aromatase inhibitors[1].**
  - **Contraindications to tamoxifen or raloxifene: history of deep vein thrombosis, pulmonary embolus, thrombotic stroke, transient ischemic attack, or known inherited clotting trait.**
  - **Contraindications to tamoxifen, raloxifene, and aromatase inhibitors[1]: current pregnancy or pregnancy potential without effective nonhormonal method of contraception.**
  - **Common and serious adverse effects of tamoxifen, raloxifene, or aromatase inhibitors[1] with emphasis on age-dependent risks.**

- **Surgery**
  - **Discussion of risk-reduction mastectomy in high-risk women. Risk-reduction mastectomy should generally be considered only in women with BRCA1/2, or other strongly predisposing gene mutation, compelling family history, or possibly women with LCIS or prior thoracic RT <30 y of age. Evaluation should include consultation with surgery and reconstructive surgery. Psychological consultation may also be considered.**
  - **Discussion regarding the risk for breast or ovarian cancer and the option of risk-reduction bilateral salpingo-oophorectomy.**
  - **Consider nipple-sparing mastectomy for risk reduction.**

- **Option of participation in clinical research for screening, risk assessment, or other risk-reduction intervention.**

- **Healthy lifestyle**
  - **Consider breast cancer risks associated with combined estrogen/progesterone therapy**
  - **Limit alcohol consumption to less than 1 drink per day**
  - **Exercise[2]**
  - **Weight control**

[1] Exemestane and anastrozole are not currently FDA approved for breast cancer risk reduction. There are currently no data comparing the benefits and risks of exemestane and anastrozole to those of tamoxifen or raloxifene.

[2] See American Cancer Society Guidelines.

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BREAST CANCER RISK-REDUCTION AGENTS

- Tamoxifen[1,2,3]
  - Data regarding tamoxifen risk reduction are limited to pre- and postmenopausal women 35 y of age or older with a Gail Model 5-year breast cancer risk of ≥1.7% or a history of LCIS.
  - Tamoxifen: 20 mg per day for 5 years was shown to reduce risk of breast cancer by 49%. Among women with a history of atypical hyperplasia, this dose and duration of tamoxifen was associated with an 86% reduction in breast cancer risk.
  - The efficacy of tamoxifen risk reduction in women who are carriers of BRCA1/2 mutations or who have had prior thoracic radiation is less well studied than in other risk groups. Available data suggest a benefit.
  - For high-risk premenopausal women, data regarding the risk/benefit ratio for tamoxifen appear relatively favorable (category 1).
  - For high-risk postmenopausal women, data regarding the risk/benefit ratio for tamoxifen are influenced by age, presence of uterus, or comorbid conditions (category 1). There are insufficient data on ethnicity and race.

- Raloxifene[1,2]
  - Data regarding raloxifene risk reduction are limited to postmenopausal women 35 y of age or older with a Gail model 5-year breast cancer risk ≥1.7% or a history of LCIS.
  - Raloxifene: 60 mg per day was found to be equivalent to tamoxifen for breast cancer risk reduction in the initial comparison. While raloxifene in long-term follow-up appears to be less efficacious in risk reduction than tamoxifen, consideration of toxicity may still lead to the choice of raloxifene over tamoxifen in women with an intact uterus.
  - There are no data regarding the use of raloxifene in women who are carriers of BRCA1/2 mutations or who have had prior thoracic radiation.
  - For high-risk postmenopausal women, data regarding the risk/benefit ratio for raloxifene are influenced by age or comorbid conditions (category 1). There are insufficient data on ethnicity and race.
  - Use of raloxifene for breast cancer risk reduction in premenopausal women is inappropriate unless part of a clinical trial.

- Aromatase inhibitors (Exemestane and Anastrozole)[4]
  - Data regarding exemestane are from a single large randomized study limited to postmenopausal women 35 years of age or older with a Gail model 5-year breast cancer risk ≥1.7% or a history of LCIS.
  - Data regarding anastrozole are from a single large randomized study limited to postmenopausal women 40-70 years of age with the following risk compared with the general population:
    - Aged 40-44 years - 4 times higher
    - Aged 45-60 years - ≥2 times higher
    - Aged 60-70 years - ≥1.5 times higher

    Women who did not meet these criteria but had a Tyrer-Cuzick model 10-year breast cancer risk >5% were also included.
  - Exemestane: 25 mg per day was found to reduce the relative incidence of invasive breast cancer by 65% from 0.55% to 0.19% with a median follow-up of 3 years.
  - Anastrozole: 1 mg per day was found to reduce the relative incidence of breast cancer by 53% with a median follow-up of 5 years.
- There are no data regarding the use of aromatase inhibitors in women who are carriers of BRCA1/2 mutations or who have had prior thoracic radiation.
- For high-risk postmenopausal women, data regarding the risk/benefit ratio for aromatase inhibitor therapy are influenced by age and comorbid conditions such as osteoporosis (category 1). There are insufficient data on ethnicity and race.
- Use of aromatase inhibitors for breast cancer risk reduction in premenopausal women is inappropriate unless part of a clinical trial.

[1]There are limited data regarding >5 years of tamoxifen or raloxifene use in breast cancer prevention. Moreover, there may be safety concerns related to use of tamoxifen for greater than 5 years. Based on the recent update of the STAR trial data, continuing raloxifene beyond 5 years (there are no high-level experience or clinical trial data evaluating these agents for risk reduction beyond 5 years) may be an approach to maintain the risk-reduction activity of the agent.

[2]When counseling postmenopausal women regarding the risk/benefit of tamoxifen and raloxifene, refer to tables in Freedman AN, et al. Benefit/risk assessment for breast cancer chemoprevention with raloxifene or tamoxifen for women age 50 years or older. J Clin Oncol 2011;29(17):2327-2333.

[3]Some selective serotonin reuptake inhibitors (SSRIs) decrease the formation of endoxifen, the active metabolite of tamoxifen. However, citalopram and venlafaxine appear to have minimal impact on tamoxifen metabolism. The clinical impact of these observations is not known.

[4]Exemestane and anastrozole are not currently FDA approved for breast cancer risk reduction. There are currently no data comparing the benefits and risks of exemestane and anastrozole to those of tamoxifen or raloxifene.

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Discussion

NCCN Categories of Evidence and Consensus

Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All recommendations are category 2A unless otherwise noted.

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Overview

Breast cancer is the most commonly diagnosed cancer in American women, with an estimated 235,030 cases of invasive breast cancer and an estimated death of 40,430 women with breast cancer in 2014.[1] This highlights the need for effective breast cancer screening and risk-reduction strategies.

For a woman who does not have a personal history of breast cancer, the risk factors for the development of breast cancer can be grouped into categories including familial/genetic factors; factors related to demographics; reproductive history lifestyle factors; and other factors such as number of breast biopsies, atypical hyperplasia or lobular carcinoma in situ [LCIS], breast density, or prior thoracic irradiation before age 30 [eg, to treat Hodgkin's disease).

Estimating breast cancer risk for the individual woman is difficult, and most breast cancers are not attributable to risk factors other than female gender and increased age. The development of effective strategies for the reduction of breast cancer incidence has also been difficult, because few of the existing risk factors are modifiable and some of the potentially modifiable risk factors have social implications extending beyond concerns for breast cancer (eg, age at first live birth). Nevertheless, effective breast cancer risk-reduction strategies such as use of risk-reduction agents, and risk-reduction surgery, have been identified. However, women and their physicians who are considering interventions to reduce risk for breast cancer must balance the demonstrated benefits with the potential morbidities of the interventions. Surgical risk-reduction strategies (eg, risk-reduction bilateral mastectomy) may have psychosocial and/or physical consequences for the woman, and risk-reduction agents, used for non-surgical risk reduction, are associated with certain adverse effects.[2-4] To assist women who are at increased risk of developing breast cancer and their physicians in the application of individualized strategies to reduce breast cancer risk, NCCN has developed these guidelines for breast cancer risk reduction.

Risk Assessment

Estimation of breast cancer risk for an individual woman who does not have a personal history of invasive breast cancer or ductal carcinoma in situ (DCIS) begins with an initial assessment of familial/genetic factors associated with increased breast cancer risk for the purpose of determining whether more extensive genetic risk assessment and counseling should be undertaken.

Familial/Genetic Factors

The first step in this primary assessment is a broad and flexible evaluation of the personal and family history of the individual, primarily with respect to breast and/or ovarian cancer.[5,6]

Genetic predispositions conferring a high risk for breast cancer include Hereditary Breast and Ovarian Cancer (*BRCA1/2*),[7,8] Li-Fraumeni syndrome (*TP53*),[9] Peutz-Jeghers syndrome (*STK11*),[10] Cowden syndrome (*PTEN*),[11,12] and Hereditary Diffuse Gastric Cancer (*CDH1*).[13]

If the individual has a known genetic predisposition for breast cancer such as mutations in *BRCA1/2, TP53, PTEN*, or other gene mutation associated with breast cancer risk, that individual must be counselled for risk reduction.

If the familial/genetic factors are not known, a thorough evaluation must be performed. The magnitude of the risk increases with the number of affected relatives in the family, the closeness of the relationship, and

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age at which the affected relative was diagnosed.[14-16] The younger the age at diagnosis of the first- or second-degree relative, the more likely it is that a genetic component is present. The maternal *and* paternal sides of the family should be considered independently for familial patterns of cancer (see NCCN Guidelines for Genetic/Familial Risk Assessment: Breast and Ovarian).

Hereditary cancers are often characterized by gene mutations associated with a high probability of cancer development (ie, a high penetrance genotype), vertical transmission through either mother or father, and an association with other types of tumors.[17,18] They often have an early age of onset and exhibit an autosomal-dominant inheritance pattern (ie, they occur when the individual has a germline mutation in only one copy of a gene).

Familial cancers share some but not all features of hereditary cancers. For example, although familial breast cancers occur in a given family more frequently than in the general population, they generally do not exhibit the inheritance patterns or onset age consistent with hereditary cancers. Familial cancers may be associated with chance clustering of sporadic cancer cases within families, genetic variation in lower penetrance genes, a shared environment, or combinations of these factors.[19-22]

If an individual or a close family member of that individual meets one or more of the criteria listed in the NCCN Guidelines for Breast Cancer Risk Reduction under "Familial Risk Assessment" (and also NCCN Guidelines for Genetic/Familial Risk Assessment: Breast and Ovarian), that individual may be at increased risk for familial/hereditary breast cancer, and referral for formal genetic assessment/counseling is recommended.

A cancer genetic professional should be involved in determining whether the individual has a lifetime risk for breast cancer greater than 20% based on models dependent on family history (eg, Claus,[23] Tyrer-Cuzick,[24] and others[25-27]). BRCAPRO[28] and Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA)[29] are more commonly used to estimate the risk based on *BRCA* mutations. Strong genetic association between breast and ovarian cancer has been demonstrated in some families by linkage analyses. The Claus tables may be useful in providing breast cancer risk estimates for white women without a known cancer-associated gene mutation who have one or two first- or second-degree female relatives with breast cancer[23] and ovarian cancer.[30] Based on this risk assessment, women with a *BRCA1/2*, *TP53*, or *PTEN* gene mutation, or a pedigree strongly suggestive of genetic predisposition to breast cancer, may be identified. The NCCN Guidelines for Genetic/Familial Risk Assessment: Breast and Ovarian also describe management strategies for women with a known or suspected *BRCA1/2, TP53, or PTEN* mutation or a pedigree strongly suggestive of genetic predisposition to breast cancer.

Other Elements of Risk

For women not considered to be at risk for familial/hereditary breast cancer, an evaluation of other elements of risk that contribute to increased breast cancer risk is recommended. These include ethnicity/race. There is an increased incidence of BRCA1/2 mutation reported in women of Ashkenazi Jewish decent.[31] It also includes increasing age and reproductive history. Strong risk factors linked to reproductive history include nulliparity, current use of menopausal hormone therapy (HT), and prolonged interval between menarche and age at first birth.[32-36]

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Body mass index (BMI) is an independent risk factor for breast cancer, especially in Caucasian women. Several studies have established the association between high BMI and adult weight gain to increased risk for breast cancer in postmenopausal women. This increase in risk has been attributed to increase in circulating endogenous estrogen levels from fat tissue.[37-39] In addition, the association between BMI and risk for postmenopausal breast cancer is stronger for hormone-positive tumors.[40-43]

Lifestyle factors such as current or prior HT,[36] alcohol consumption,[44,45] and smoking[46] also contribute to the risk of developing breast cancer. Other factors to consider are number of breast biopsies, prior thoracic irradiation before age 30 (eg, to treat Hodgkin's disease), atypical hyperplasia or LCIS, and changes in breast density.[47] Breast density is not included in any of the commonly used risk assessment models/tools.[26] However, dense breast tissue as measured by mammography is increasingly recognized as an important risk factor for breast cancer.[48,49] For example, a report of a large case-cohort study of women 35 years and older with no history of breast cancer who underwent mammographic screening, first at baseline and then at an average of 6 years later, suggested that longitudinal changes in breast density are associated with changes in breast cancer risk.[49]

Women ≥35 years of age *without* a *BRCA1/2*, *TP53*, or *PTEN* mutation; a strong family history of breast cancer; a history of thoracic radiation before age 30; or a history of LCIS should have their risk for breast cancer estimated according to the modified Gail model.[50-52] The modified Gail model is a computer-based, multivariate, logistic regression model that uses age, race, age at menarche, age at first live birth or nulliparity, number of first-degree relatives with breast cancer, number of previous breast biopsies, and histology of the breast biopsies to produce actuarial estimates of future breast cancer risk.[50,51,53,54] The criteria used to determine risk by the modified Gail model are described in Table 1. The Gail model, as modified by the National Surgical Adjuvant Breast and Bowel Project (NSABP) investigators, is available on the National Cancer Institute website (http://www.cancer.gov/bcrisktool/Default.aspx).

The risk threshold required for a woman to consider the use of risk-reduction strategies must depend on an evaluation of the efficacy, morbidity, and expense of the proposed intervention. As a reasonable discriminating threshold, the NCCN Breast Cancer Risk Reduction Panel has adopted the 1.7% or greater 5-year actuarial breast cancer risk as defined by the modified Gail model, which was used to identify women eligible for the NSABP, Breast Cancer Prevention Trial (BCPT),[55,56] and the Study of Tamoxifen and Raloxifene (STAR) trial.[57,58]

The Gail model was updated using combined data from the Women's Contraceptive and Reproductive Experiences (CARE) study and the SEER database, as well as causes of death from the National Center of Health Statistics, to provide a more accurate determination of risk for African-American women.[59] The model was updated using data from the Asian American Breast Cancer Study (AABCS) and the SEER database to provide a more accurate risk assessment for Asian and Pacific Islander women in the United States.[60] Application of the Gail model to recent immigrants from Japan or China may overestimate the risk for breast cancer.[60]

As previously mentioned, the Gail model is not an appropriate breast cancer risk assessment tool for women who received prior thoracic radiation to treat Hodgkin's disease (eg, mantle radiation) or those with LCIS. In the Late Effects Study Group trial, the overall risk for breast cancer associated with prior thoracic irradiation at a young age was

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found to be 56.7–fold (55.5-fold for female patients) greater than the risk for breast cancer in the general population.[61] In that study, the relative risk according to follow-up interval was: 0 at 5 to 9 years; 71.3 at 10 to 14 years; 90.8 at 15 to 19 years; 50.9 at 20 to 24 years; 41.2 at 25 to 29 years; and 24.5 at >29 years.[61] Results from a case-control study of women treated at a young age (30 years or younger) for Hodgkin lymphoma with thoracic radiation indicated that the estimated, cumulative, absolute risk for breast cancer at 55 years of age was 29.0% (95% confidence interval [CI], 20.2%–40.1%) for a woman treated at 25 years of age with 40 Gy of radiation and no alkylating agents.[62] Women with a history of treatment with thoracic radiation for Hodgkin's disease are at a high risk for breast cancer on the basis of radiation exposure alone.[61-66] Women with a history of LCIS are also at substantially increased risk for invasive breast cancer in both the affected and contralateral breast.[67,68]

Women with a life expectancy ≥10 years and no diagnosis/history of breast cancer who are considered to be at increased risk for breast cancer based on any of the above-mentioned assessments should receive counseling, that is tailored to the individual, to decrease breast cancer risk (eg, risk-reduction surgery in *BRCA1/2* mutation carriers; therapy with risk-reduction agents in those without a contraindication to these agents) (see section below on *Components of Risk-Reduction Counseling*, page MS-20), and should undergo breast screening as detailed in the NCCN Guidelines for Breast Cancer Screening and Diagnosis.

If life expectancy is <10 years, there is probably minimal if any benefit to risk-reduction therapy or screening (see NCCN Guidelines for Breast Cancer Screening and Diagnosis and NCCN Guidelines for Breast Cancer).

Women with a diagnosis of DCIS should be managed according to recommendations outlined in the NCCN Guidelines for Breast Cancer.

Risk-Reduction Interventions

Lifestyle Modifications

Evidence from immigration studies indicate that in addition to family history and genetics, environmental factors play a significant role. Life style modifications such as diet, body weight, exercise, and alcohol consumption are some of the modifiable components of breast cancer risk. While there is no clear evidence that specific dietary components can effectively reduce breast cancer risk, weight gain and obesity in adulthood are risk factors for the development of postmenopausal breast cancer.[39,69,70] Alcohol consumption, even at moderate levels, increases breast cancer risk.[45,70-73] Patients should be encouraged to maintain a healthy lifestyle and to remain up-to-date with recommendations for screening and surveillance (see section on *Counseling regarding lifestyle modifications* on MS-24).

Risk-Reduction Surgery

Bilateral Total Mastectomy

The lifetime risk for breast cancer in *BRCA1/2* mutation carriers has been estimated to be 56% to 84%.[74-76] Retrospective analyses with median follow-up periods of 13 to 14 years have indicated that bilateral risk–reducing mastectomy (RRM) decreased the risk of developing breast cancer by at least 90% in moderate- and high-risk women and in known *BRCA1/2* mutation carriers.[77,78] An analysis of results from the study by Hartmann et al,[77] determined that to prevent one case of breast cancer in women with high risk, the number of needed to be treated with RRM was equal to 6.[79] Results from smaller prospective studies with shorter follow-up periods have provided support for concluding that RRM provides a high degree of protection against

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breast cancer in women with a *BRCA1/2* mutation.[80,81] The NCCN Breast Cancer Risk Reduction Panel supports the use of RRM for carefully selected women at high risk for breast cancer who desire this intervention (eg, women with a *BRCA1/2*, *TP53*, *PTEN*, *CDH1*, or *STK11* mutation or, possibly, women with a history of LCIS). Although the consensus of the NCCN Breast Cancer Risk Reduction Panel is that consideration of RRM is an option for a woman with LCIS without additional risk factors, it is not a recommended approach for most of these women. There are no data regarding RRM in women with prior mantle radiation exposure. The value of RRM in women with deleterious mutations in other genes associated with a high risk for breast cancer (based on large epidemiologic studies) in the absence of a compelling family history of breast cancer is unknown.

Women considering RRM should first have appropriate multidisciplinary consultations and a clinical breast examination and bilateral mammogram if not performed within the past 6 months. If results are normal, women who choose RRM may undergo the procedure with or without immediate breast reconstruction. Bilateral mastectomy performed for risk reduction should involve removal of all breast tissue (ie, a total mastectomy). Axillary node assessment has limited utility at the time of RRM. Women undergoing RRM do not require an axillary lymph node dissection unless breast cancer is identified on pathologic evaluation of the mastectomy specimen.[82]

Some patients may be at risk for an occult primary tumor, such as patients with abnormal imaging findings on either mammogram or breast MRI, who do not undergo biopsy, and patients with familial history who have not had a breast MRI prior to surgery. In such patients, a sentinel lymph node biopsy may be performed to stage the axilla for an occult cancer during the RRM, and a secondary axillary lymph node dissection could be avoided if an occult invasive cancer is discovered. This procedure has not been found to increase the risk for lymphedema.[83]

Following RRM, women who carry a *BRCA1/2* mutation should be monitored according to the NCCN Guidelines for Genetic/Familial Risk Assessment: Breast and Ovarian. Women found to have invasive breast cancer or DCIS at the time of RRM should be treated according to the NCCN Guidelines for Breast Cancer. All other women should be followed up with routine health maintenance following RRM. Most health maintenance recommendations are not related to the breast. For monitoring breast health, women should continue with annual exams of the chest/reconstructed breast as there is still a small risk of developing breast cancer. Mammograms are not recommended in this situation.

Bilateral Salpingo-oophorectomy

Women with a *BRCA1/2* mutation are at increased risk for both breast and ovarian cancers (including fallopian tube cancer). Although the risk for ovarian cancer is lower than the risk for breast cancer in a *BRCA1/2* mutation carrier (eg, estimated lifetime risks of 36%–46% and 10%–27% in *BRCA1* and *BRCA2* mutation carriers, respectively[75,84-87]), the absence of reliable methods of early detection and the poor prognosis associated with advanced ovarian cancer have lent support for the performance of bilateral risk-reducing salpingo-oophorectomy (RRSO) after completion of childbearing in these women. In the studies of Rebbeck et al, the mean age at diagnosis of ovarian cancer was 50.8 years for *BRCA1/2* carriers.[88]

The effectiveness of RRSO in reducing the risk for ovarian cancer in carriers of a *BRCA1/2* mutation has been demonstrated in a number of studies. For example, results of a meta-analysis involving 10 studies of *BRCA1/2* mutation carriers showed an approximately 80% reduction in

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the risk for ovarian or fallopian cancer following RRSO.[89] However, a 1% to 4.3% residual risk for a primary peritoneal carcinoma has been reported in some studies.[88-93]

RRSO is also reported to reduce the risk for breast cancer in carriers of a *BRCA1/2* mutation by approximately 50%.[88,89,93,94] In the case-control international study, a 56% (odds ratio = 0.44; 95% CI, 0.29–0.66) and a 46% (odds ratio = 0.57; 95% CI, 0.28–1.15) breast cancer risk reduction was reported following RRSO in carriers of a *BRCA1/2* mutation, respectively.[94] Hazard ratios of 0.47 (95% CI, 0.29–0.77)[88] and 0.30 (95% CI, 0.11–0.84)[92] were reported in two other studies comparing breast cancer risk in women with a *BRCA1/2* mutation who had undergone RRSO with carriers of these mutations who opted for surveillance only. These studies are further supported by a meta-analysis that found similar reductions in breast cancer risk of approximately 50% for *BRCA1/2* mutation carriers following RRSO.[89] The results of a prospective cohort study suggest that RRSO may be associated with a greater reduction in breast cancer risk for *BRCA1* mutation carriers compared with *BRCA2* mutation carriers.[95]

Reductions in breast cancer risk for carriers of a *BRCA1/2* mutation after RRSO may be associated with decreased hormonal exposure following surgical removal of the ovaries. Greater reductions in breast cancer risk were observed in women with a *BRCA1* mutation who had an RRSO at age 40 years or younger (odds ratio = 0.36, 95% CI, 0.20–0.64) relative to *BRCA1* carriers aged 41–50 years who had this procedure (odds ratio = 0.50, 95% CI, 0.27–0.92).[94] Nonsignificant risk reduction of developing breast cancer was found for women aged 51 years or older, although only a small number of women were included in this group.[94] However, results from Rebbeck et al also suggest that RRSO after age 50 is not associated with a substantial decrease in breast cancer risk.[93]

Although data are limited regarding an optimal age for RRSO, the Monte Carlo simulation model provides estimates of the survival impact of breast and ovarian risk-reduction strategies (eg, mammographic/MRI breast screening; risk-reduction surgery) in women who are carriers of *BRCA1/2* mutations according to the type of *BRCA* mutation present, the specific risk-reduction intervention(s), and the age of the women at the time of the intervention(s).[96] Survival estimates generated from this model can facilitate shared decision-making regarding choice of a risk-reduction approach (see Table 2).

A prospective multicenter study reported the benefit of risk-reducing procedures for women with strong genetic predispositions for breast cancer.[97] The study involved 2482 women diagnosed with *BRCA1/2* gene mutations, almost half of whom chose either RRSO or RRM. During the 3 years of follow up, no cases of breast cancer occurred in the women who opted for RRM. In the same time period, 7% of the women who adopted other approaches received a breast cancer diagnosis. In *BRCA2*-mutation carriers, no cases of ovarian cancer occurred after salpingo-oophorectomy over a 6-year follow-up period, whereas 3% of those who did not undergo the same surgery were diagnosed with ovarian cancer. None of the women who underwent RRM developed breast cancer. RRSO was associated with a reduction in overall mortality (hazard ratio [HR] = 0.40), breast cancer–specific mortality (HR = 0.44), and ovarian cancer–specific mortality (HR = 0.21). Among women who underwent RRSO, only 1.1% developed ovarian cancer.

The NCCN Breast Cancer Risk Reduction Panel recommends limiting RRSO to women with a known or strongly suspected *BRCA1/2* mutation. Peritoneal washings should be performed at surgery, and pathologic assessment should include fine sectioning of the ovaries and fallopian tubes.[98] The additional benefit of concurrent hysterectomy is

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not clear at this time. Women who undergo RRSO should continue with routine health maintenance and breast screening as per the NCCN Guidelines for Breast Cancer unless a woman has had RRM.

Risk-Reduction Agents

Risk-reduction agents (ie, tamoxifen, raloxifene, anastrozole, exemestane) are recommended for women ≥35 years of age only, as the utility of these agents in women younger than 35 years is unknown.

Tamoxifen for Risk Reduction

The benefits of tamoxifen, a selective estrogen receptor (ER) modulator (SERM), in the treatment of breast cancer in the adjuvant and metastatic settings are well documented. Retrospective analysis of randomized, controlled, clinical trials comparing tamoxifen to no tamoxifen in the adjuvant treatment of women with breast cancer has shown a reduction in the incidence of contralateral second primary breast cancer.[99-102] The meta analyses by Early Breast Cancer Trialists' Collaborative Group confirmed that the risk for contralateral primary breast cancer is substantially reduced (ie, a statistically significant annual recurrence rate ratio = 0.59) by 5 years of tamoxifen therapy in women with first breast cancers that are ER-positive or have an unknown ER status.[103]

NSABP Breast Cancer Prevention Trial

The effectiveness of tamoxifen in the setting of breast cancer treatment gave rise to the NSABP BCPT study, also known as the P-1 study. It was a randomized clinical trial of healthy women aged 60 years or older, aged 35 to 59 years with a 1.7% or greater cumulative 5-year risk for developing breast cancer, or with a history of LCIS.[55] Both premenopausal and postmenopausal women were enrolled in the trial and randomized in a double-blinded fashion to treatment with tamoxifen, 20 mg daily for 5 years, or placebo. Invasive breast cancer incidence was the primary study endpoint; high-priority secondary endpoints included the occurrence of thromboembolic disease, cardiovascular disease, bone fracture, endometrial cancer, noninvasive breast cancer, and breast cancer mortality. The trial was unblinded and initial findings were reported in 1998. A subsequent report on this trial has been published, which takes into account 7 years of follow-up data subsequent to the point where the study was unblinded. However, nearly one-third of the placebo participants began taking a SERM when the study was unblinded, which decreased the proportion of women in the placebo group relative to the tamoxifen group, potentially confounding the long-term results.[56]

The results of the P-1 study showed that treatment with tamoxifen decreased the short-term risk for breast cancer by 49% in healthy women aged 35 years or older who had an increased risk for the disease.[55] Risk-reduction benefits were demonstrated across all age groups, in pre-menopausal and post-menopausal women. The difference in average annual rates for invasive breast cancer was 3.30 cases per 1,000 women (ie, 6.76 cases per 1,000 women in the placebo group and 3.43 cases per 1,000 women in the group taking tamoxifen). The absolute risk reduction was 21.4 cases per 1,000 women over 5 years.[55] In terms of numbers needed to treat, this corresponds to treatment of 47 women with tamoxifen to prevent 1 case of invasive breast cancer. Updated results indicate that breast cancer risk was reduced by 43% in this population after 7 years of follow-up.[56] The reduction in invasive breast cancer risk in participants with atypical hyperplasia was particularly striking (risk ratio [RR], 0.14; 95% CI, 0.03–0.47) in the initial study analysis, and a RR of 0.25 (95% CI, 0.10–0.52) was found after 7 years of follow-up. An additional benefit of tamoxifen was a decrease in bone fractures (RR, 0.81; 95% CI, 0.63–1.05). However, as was anticipated from the experience in studies of

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women taking tamoxifen following a breast cancer diagnosis, major toxicities included hot flashes, invasive endometrial cancer in postmenopausal women, and cataracts. A significant increase in the incidence of pulmonary embolism was also observed in women ≥50 years of age taking tamoxifen. The average annual rates of pulmonary embolism per 1000 women were 1.00 versus 0.31 (RR= 3.19; 95% CI, 1.12–11.15).[55]

No differences were observed in overall rates of mortality by treatment group with a follow-up period of up to 7 years. The initial study analysis revealed that average annual mortality from all causes in the tamoxifen group was 2.17 per 1,000 women compared with 2.71 per 1,000 women treated with placebo, for a RR of 0.81 (95% CI, 0.56–1.16).[55]Annual mortality after 7 years of follow-up was 2.80 per 1,000 women compared with 3.08 per 1,000 women in the tamoxifen and placebo groups, respectively, for a RR of 1.10 (95% CI, 0.85–1.43).[56]

An evaluation of the subset of patients with a *BRCA1/2* mutation in the P-1 study revealed that breast cancer risk was reduced by 62% in study patients with a *BRCA2* mutation receiving tamoxifen relative to placebo (RR = 0.38; 95% CI, 0.06–1.56). However, tamoxifen use was not associated with a reduction in breast cancer risk in patients with a *BRCA1* mutation.[104] These findings may be related to the greater likelihood for development of ER-positive tumors in *BRCA2* mutation carriers relative to *BRCA1* mutation carriers. However, this analysis was limited by the very small number of patients with a *BRCA1/2* mutation.

Based on the BCPT [P-1] study results, in October 1998 the U.S. Food and Drug Administration (FDA) approved tamoxifen for breast cancer risk reduction for women at increased risk for breast cancer.

European Studies of Tamoxifen

Three European studies comparing tamoxifen with placebo for breast cancer risk reduction have also been reported. The Royal Marsden Hospital study was a pilot trial of tamoxifen versus placebo in women ages 30 to 70 years who were at increased breast cancer risk based largely on their family history.[105,106] Women in the trial were allowed to continue or to initiate postmenopausal HT. With 2471 participants available for interim analysis, no difference in the frequency of breast cancer was observed between the 2 study groups. Moreover, the toxicity experienced by the 2 groups did not show statistically significant differences.[106] An analysis of updated findings from the Royal Marsden Hospital study did demonstrate a nonsignificant breast cancer risk-reduction benefit with tamoxifen use (ie, 62 cases of breast cancer in 1238 women receiving tamoxifen vs. 75 cases of breast cancer in 1233 women in the placebo arm).[105]

Most recently, an analysis of blinded results from the Royal Marsden Hospital trial at 20-year follow-up showed no difference in breast cancer incidence between the groups randomly assigned to tamoxifen or placebo (HR = 0.78; 95% CI, 0.58– 1.04; P = .10).[107] However, the incidence of ER-positive breast cancer was significantly lower in the tamoxifen arm vs. placebo arm of the trial (HR = 0.61; 95% CI, 0.43–0.86; P = .005). Importantly, the difference between the 2 arms became significant only in the posttreatment period (ie, after 8 years of treatment).

The Italian Tamoxifen Prevention Study randomized 5408 women ages 35 to 70 years without breast cancer, who had undergone a previous hysterectomy, to receive tamoxifen or placebo for 5 years.[108] Women in the trial were allowed to receive HT. No significant difference in breast cancer occurrence in the overall study population was identified at median follow-up periods of 46, 81.2, and 109.2 months.[108-110]

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Thromboembolic events, predominantly superficial thrombophlebitis, were increased in women treated with tamoxifen. A subset of women in the Italian Tamoxifen Prevention Study who had used HT and were classified as at increased breast cancer risk based on reproductive and hormonal characteristics were found to have a significantly reduced risk for breast cancer with tamoxifen therapy.[110,111] However, only approximately 13% of the patients in the trial were at high risk for breast cancer.

It is unclear why no overall breast cancer risk reduction was observed in the Italian Tamoxifen Prevention Study. Possible reasons include concurrent use of HT, and different study populations (ie, populations at lower risk for breast cancer).[112]

The first International Breast Cancer Intervention Study (IBIS-I) randomized 7152 women aged 35 to 70 years at increased risk for breast cancer to receive either tamoxifen or placebo for 5 years.[113] Tamoxifen provided a breast cancer (invasive or ductal carcinoma in situ) risk reduction of 32% (95% CI, 8–50; $P = .013$). Thromboembolic events increased with tamoxifen (odds ratio = 2.5; 95% CI, 1.5–4.4; $P = .001$), and endometrial cancer showed a nonsignificant increase ($P = .20$). An excess of deaths from all causes was seen in the tamoxifen-treated women ($P = .028$).

In an updated analysis of the blinded IBIS-I trial at a median follow-up of 96 months, the relative risk for breast cancer for the tamoxifen arm compared with placebo was 0.73 (95% CI, 0.58–0.91; $P = .004$).[114] Although no difference in the risk for ER-negative–invasive tumors was observed between the 2 groups, those in the tamoxifen arm were found to have a 34% lower risk for ER-positive invasive breast cancer. Slightly higher risk reduction with tamoxifen was observed for premenopausal patients. Importantly, the increased risk for venous thromboembolism (VTE) observed with tamoxifen during the treatment period was no longer significant in the posttreatment period. Gynecologic and vasomotor side effects associated with active tamoxifen treatment were not observed during the posttreatment follow-up. These results provide randomized evidence that the benefits of tamoxifen continue following cessation of treatment while many of the side effects diminish or disappear.

The use of tamoxifen as a breast cancer risk-reduction agent has most recently been evaluated in the STAR Trial[57,58] (see section on *The STAR Trial* below, MS-11).

Raloxifene for Risk Reduction

Raloxifene is a second-generation SERM that is chemically different from tamoxifen and appears to have similar anti-estrogenic effects with considerably less endometrial stimulation. The efficacy of raloxifene as a breast cancer risk-reduction agent has been evaluated in several clinical studies. In 2007, the FDA expanded the indications for raloxifene to include reduction in risk for invasive breast cancer in postmenopausal women with osteoporosis, and reduction in risk for invasive breast cancer in postmenopausal women at high risk for invasive breast cancer.

The MORE Trial

The Multiple Outcomes of Raloxifene Evaluation (MORE) trial was designed to determine whether 3 years of raloxifene treatment reduced the risk of fracture in postmenopausal women with osteoporosis.[115] A total of 7705 postmenopausal women 31 to 80 years of age were randomized to receive placebo, 60 mg/day of raloxifene, or 120 mg/day of raloxifene for 3 years. At study entry, participants were required to have osteoporosis (defined as a bone density at least 2.5 standard deviations below the mean for young women) or a history of

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osteoporotic fracture. The study showed a reduction in the vertebral fracture risk and an increase in bone mineral density (BMD) in the femoral neck and spine for the women treated with raloxifene, compared with those who received placebo.

After a median follow-up of 40 months in the MORE trial, breast cancer was reported in 40 patients: 27 cases in 2576 women receiving placebo and 13 cases in 5129 women receiving raloxifene.[116] The relative risk of developing invasive breast cancer on raloxifene, compared with placebo, was 0.24 (95% CI, 0.13–0.44). Raloxifene markedly decreased the risk for ER-positive cancers (relative risk = 0.10; 95% CI, 0.04–0.24) but did not appear to influence the risk of developing an ER-negative cancer (relative risk = 0.88; 95% CI, 0.26–3.0). Although breast cancer incidence was a secondary endpoint in the MORE trial, it is important to note that breast cancer risk was not a prospectively determined characteristic for the women enrolled and stratified into treatment arms in this study.[112] Furthermore, the patients enrolled in the MORE trial were, on average, at lower risk for breast cancer and older than the patients enrolled in the NSABP BCPT [P-1] study.

Side effects associated with the raloxifene use included hot flashes, influenza-like syndromes, endometrial cavity fluid, peripheral edema, and leg cramps. In addition, there was an increased incidence of deep venous thromboses (DVT) (0.7% for women receiving 60 mg/day raloxifene vs. 0.2% for placebo) and pulmonary emboli (0.3% for women receiving 120 mg/day raloxifene vs. 0.1% for placebo) associated with raloxifene treatment. However, there was no increase in the risk for endometrial cancer associated with raloxifene.

The CORE Trial

The early findings related to breast cancer risk in the MORE trial led to the continuation of this trial under the name Continuing Outcomes Relevant to Evista (CORE) trial. Because breast cancer incidence was a secondary endpoint in the MORE trial, CORE was designed to assess the effect of 4 additional years of raloxifene on the incidence of invasive breast cancer in postmenopausal women with osteoporosis. A secondary endpoint was the incidence of invasive ER-positive breast cancer. Data from the CORE trial were reported in 2004.[117]

During the CORE trial, the 4-year incidence of invasive breast cancer was reduced by 59% (HR = 0.41; 95% CI, 0.24–0.71) in the raloxifene group compared with the placebo group. Raloxifene, compared to placebo, reduced the incidence of invasive ER-positive breast cancer by 66% (HR = 0.34; 95% CI, 0.18–0.66) but had no effect on invasive ER-negative breast cancers.[117] Over the 8 years of both trials (MORE + CORE), the incidence of invasive breast cancer was reduced by 66% (HR = 0.34; 95% CI, 0.22–0.50) in the raloxifene group compared with the placebo group. Compared to placebo, 8 years of raloxifene reduced the incidence of invasive ER-positive breast cancer by 76% (HR = 0.24; 95% CI, 0.15–0.40). Interestingly, the incidence of noninvasive breast cancer was not significantly different for patients in the raloxifene and placebo arms (HR = 1.78; 95% CI, 0.37–8.61).[117]

The adverse events in the CORE trial were similar to those seen in the MORE trial. There was a nonsignificant increase in the risk for thromboembolism (relative risk = 2.17; 95% CI, 0.83–5.70) in the raloxifene group of the CORE trial compared to the placebo group. There was no statistically significant difference in endometrial events (bleeding, hyperplasia, and cancer) between the raloxifene and placebo groups during the 4 years of CORE or the 8 years of MORE and CORE. During the 8 years of the MORE and CORE trials, raloxifene increased the risk for hot flushes and leg cramps compared with placebo; these risks were observed during the MORE trial but not during the additional 4 years of therapy in CORE. While it is possible

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that hot flushes and leg cramps are early events that do not persist with continued therapy, it is also possible that an increased risk of these adverse events was not observed in the CORE trial as a result of selection bias (ie, women who experienced these symptoms in the MORE trial may have chosen not to continue in the CORE trial).

The results from the CORE trial are not entirely straightforward because of the complex design of the trial. Of the 7705 patients randomized in the MORE trial, only 4011 chose to continue, blinded to therapy, in CORE; this drop off likely introduces bias in favor of the treatment group. In the CORE trial, the researchers did not randomize the patients again (1286 in the placebo arm, 2725 in the raloxifene arm), maintaining the double blinding of the original trial.

The RUTH Trial

In the Raloxifene Use for The Heart (RUTH) trial, postmenopausal women with an increased risk for coronary heart disease were randomly assigned to raloxifene or placebo arms.[118,119] Invasive breast cancer incidence was another primary endpoint of the trial, although only approximately 40% of the study participants had an increased risk for breast cancer according to the Gail model. Median exposure to study drug was 5.1 years and median duration of follow-up was 5.6 years.[119] Raloxifene did not reduce risk of cardiovascular events, but there was a 44% decrease in the incidence of invasive breast cancer in the raloxifene arm (HR=0.56; 95% CI, 0.38–0.83], with a 55% lower incidence of ER-positive breast cancer (HR=0.45; 95% CI, 0.28–0.72). No reduction in the risk for noninvasive breast cancer was found for patients receiving raloxifene, in agreement with the initial results of the STAR trial, although only 7% of breast cancers in the RUTH trial were noninvasive.

The STAR Trial

Despite issues of trial design, the results from the CORE trial and the previous MORE study provided support for concluding that raloxifene may be an effective breast cancer risk-reduction agent. However, neither of these studies was designed to directly evaluate the efficacy of raloxifene versus tamoxifen in this regard. This issue was addressed in the NSABP STAR trial (P-2), which was initiated in 1999; initial results became available in 2006.[57]

In the STAR trial, 19,747 postmenopausal women 35 years or older at increased risk for invasive breast cancer as determined by the modified Gail model or with a personal history of LCIS were enrolled into one of two treatment arms (no placebo arm). The primary study endpoint was invasive breast cancer; secondary endpoints included quality of life, and incidences of noninvasive breast cancer, DVT, pulmonary embolism, endometrial cancer, stroke, cataracts, and death. At an average follow-up of approximately 4 years, no statistically significant differences between patients receiving 20 mg/day of tamoxifen or 60 mg/day of raloxifene were observed with respect to invasive breast cancer risk reduction (RR = 1 .02;95% CI, 0.82–1.28). Because there was no placebo arm, it was not possible to determine a raloxifene-versus-placebo RR for invasive breast cancer; however, tamoxifen was shown in the BCPT [P-1] study to reduce breast cancer risk by nearly 50%. In addition, raloxifene was shown to be as effective as tamoxifen in reducing the risk for invasive cancer in the subset of patients with a history of LCIS or atypical hyperplasia. However, raloxifene was not as effective as tamoxifen in reducing the risk for noninvasive breast cancer, although the observed difference was not statistically significant (RR = 1.40; 95% CI, 0.98–2.00).[55]

At a median follow-up of nearly 8 years (81 months) involving 19,490 women, raloxifene was shown to be about 76% as effective as

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tamoxifen in reducing the risk for invasive breast cancer (RR = 1.24; 95% CI, 1.05–1.47), suggesting that tamoxifen has greater long-term benefit with respect to lowering invasive breast cancer risk.[58] Raloxifene remained as effective as tamoxifen in reducing the risk for invasive cancer in women with LCIS (RR, 1.13; 95% CI, 0.76–1.69), but was less effective than tamoxifen for those with a history of atypical hyperplasia (RR, 1.48; 95% CI = 1.06–2.09). Interestingly, at long-term follow-up, the risk for noninvasive cancer in the raloxifene arm grew closer to that observed for the group receiving tamoxifen (RR = 1.22; 95% CI, 0.95–1.50). No significant differences in mortality were observed between the 2 groups. In the initial analysis of the STAR trial data, invasive endometrial cancer occurred less frequently in the group receiving raloxifene compared with the tamoxifen group, although the difference did not reach statistical significance. It is important to note, however, that the incidence of endometrial hyperplasia and hysterectomy were significantly lower in the raloxifene group compared to the tamoxifen group. However, at long-term follow-up, the risk for endometrial cancer was significantly lower in the raloxifene arm (RR, 0.55; 95% CI, 0.36–0.83).

The lower incidences of thromboembolic events (RR, 0.75; 95% CI, 0.60–0.93) and cataract development (RR, 0.80; 95% CI, 0.72–0.89) observed in the raloxifene group compared to the tamoxifen group when the STAR trial results were initially analyzed were maintained at long-term follow-up.[58] The incidences of stroke, ischemic heart disease, and bone fracture were similar in the two groups. In the initial report, overall quality of life was reported to be similar for patients in both groups, although patients receiving tamoxifen reported better sexual function.[120]

Aromatase Inhibitors for Risk Reduction

A number of clinical trials testing the use of aromatase inhibitors (AIs) in the adjuvant therapy of postmenopausal women with invasive breast cancer have been reported. The first of these studies, the Arimidex, Tamoxifen, Alone or in Combination trial (ATAC trial), randomized postmenopausal women with invasive breast cancer to anastrozole versus tamoxifen versus anastrozole plus tamoxifen in a double-blinded fashion.[121] The occurrence of contralateral second primary breast cancers was a study endpoint. At 47 months median follow-up, a nonsignificant reduction in contralateral breast cancers was observed in women treated with anastrozole alone compared with tamoxifen (odds ratio = 0.62; 95% CI, 0.38–1.02; P = .062) and a significant reduction in contralateral breast cancers was seen in the subset of women with hormone receptor-positive first cancers (odds ratio= 0.56; 95% CI, 0.32–0.98; P = .04).[122] Similar reductions in the risk for contralateral breast cancer have been observed with sequential tamoxifen followed by exemestane compared with tamoxifen alone and with sequential tamoxifen followed by letrozole compared with tamoxifen followed by placebo.[123,124]

In the Breast International Group (BIG) 1-98 trial postmenopausal women with early-stage breast cancer were randomized to receive 5 years of treatment with one of the following therapeutic regimens: letrozole; sequential letrozole followed by tamoxifen; tamoxifen; or sequential tamoxifen followed by letrozole. Risk for breast cancer recurrence was lower in women in the letrozole arm relative to the tamoxifen arm.[125]

The results of the MAP.3 trial show promising use of exemestane in the breast cancer prevention setting. MAP.3 is a randomized, double-blind, placebo-controlled, multicenter, multinational trial in which 4560 women were randomly assigned to either exemestane (2285 patients) or

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placebo (2275 patients).[3] The study authors reported that about 5% of patients in each group had discontinued the protocol treatment. The major reasons for early discontinuation of the protocol treatments were toxic effects (15.4% in the exemestane group vs. 10.8% in the placebo group, $P < .001$) and patient refusal (6.9% vs. 6.0%, $P = .22$). After a median follow-up of 3 years, compared to the placebo exemestane was found to reduce the relative incidence of invasive breast cancers by 65%, from 0.55% to 0.19% (HR, 0.35 with exemestane; 95% CI, 0.18–0.70).[3]

The IBIS-II study included 3864 postmenopausal women at high risk for breast cancer, defined by family history of breast cancer or prior diagnosis of DCIS, LCIS, or atypical ductal hyperplasia.[4] (HR, 0.47; 95% CI, 0.32–0.68). The advantage of anastrozole was greater prevention of high-grade tumors (HR, 0.35; 95% CI, 0.16–0.74) compared with intermediate- or low-grade tumors. The follow-up period in this trial was longer than that for the MAP.3 trial. The cumulative incidence after 7 years was predicted to rise 2.8% in the anastrozole group compared with 5.6% in the placebo group.[4]

NCCN Breast Cancer Risk Reduction Panel Recommendations for Risk-Reduction Agents

Based on data from the BCPT[55] and STAR[57] trials, Freedman et al have developed tables of benefit/risk indices for women aged 50 years and older to compare raloxifene versus no treatment (placebo) and tamoxifen versus no treatment.[2] The risk and benefit of treatment with either tamoxifen or raloxifene depends on age, race, breast cancer risk, and history of hysterectomy. There are separate tables in the report listing the level of 5-year invasive breast cancer risk by age group for non-Hispanic white women with and without a uterus, black women with and without a uterus, and Hispanic women with and without a uterus. The NCCN Breast Cancer Risk Reduction Panel recommends using these tables [2] while counseling postmenopausal women regarding use of raloxifene and tamoxifen for breast cancer risk reduction.

Tamoxifen Recommendations

The NCCN Breast Cancer Risk Reduction Panel recommends tamoxifen (20 mg/day) as an option to reduce breast cancer risk in healthy pre- and postmenopausal women ≥35 years of age, whose life expectancy is ≥10 years, and who have a ≥1.7% 5-year risk for breast cancer as determined by the modified Gail model, or who have had LCIS (category 1). The consensus of the NCCN Breast Cancer Risk Reduction Panel is that the risk/benefit ratio for tamoxifen use in premenopausal women at increased risk for breast cancer is relatively favorable (category 1), and that the risk/benefit ratio for tamoxifen use in postmenopausal women is influenced by age, presence of uterus, or other comorbid conditions (category 1). Early studies suggest that lower doses of tamoxifen over shorter treatment periods may reduce breast cancer risk in postmenopausal women, but these findings need to be validated in phase III clinical trials.[126] Only limited data are currently available regarding the efficacy of tamoxifen risk reduction in *BRCA1/2* mutation carriers and women who have received prior thoracic radiation. The efficacy of tamoxifen risk reduction in women with *BRCA1/2* mutations is less well studied than in other risk groups. However, available data suggest a benefit.[104]

The utility of tamoxifen as a breast cancer risk-reduction agent in women <35 years of age is not known. There are insufficient data on the influence of ethnicity and race on the efficacy and safety of tamoxifen as a risk-reduction agent.

There is evidence that certain drugs (eg, serotonin reuptake inhibitors [SSRIs]) interfere with the enzymatic conversion of tamoxifen to endoxifen by inhibiting a particular isoform of cytochrome P-450 2D6

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enzyme (CYP2D6) involved in the metabolism of tamoxifen.[127] The consensus of the NCCN Breast Cancer Risk Reduction Panel is that alternative medications that have minimal or no impact on plasma levels of endoxifen should be substituted when possible.[127] Citalopram and venlafaxine do not disrupt tamoxifen metabolism.

It has also been reported that certain CYP2D6 genotypes are markers of poor tamoxifen metabolism.[128,129] Nevertheless, the consensus of the NCCN Breast Cancer Risk Reduction Panel is that further validation of this biomarker is needed before it can be used to select patients for tamoxifen therapy.

Raloxifene Recommendations

The NCCN experts serving on the Breast Cancer Risk Reduction Panel feel strongly that tamoxifen is a superior choice of risk-reduction agent for most postmenopausal women desiring non-surgical risk-reduction therapy. This is based on the updated STAR trial results that showed diminished benefits of raloxifene compared to tamoxifen after cessation of therapy.[58] However, consideration of toxicity may still lead to the choice of raloxifene over tamoxifen in some women.

If raloxifene is chosen, the NCCN Breast Cancer Risk Reduction Panel recommends use of 60 mg/day. Data regarding use of raloxifene to reduce breast cancer risk is limited to healthy postmenopausal women ≥35 years who have a ≥1.7% 5-year risk for breast cancer as determined by the modified Gail model, or who have a history of LCIS. The consensus of the NCCN Breast Cancer Risk Reduction Panel is that the risk/benefit ratio for raloxifene use in postmenopausal women at increased risk for breast cancer is influenced by age and comorbid conditions (category 1). Since there are no currently available data regarding the efficacy of raloxifene risk reduction in *BRCA1/2* mutation carriers and women who have received prior thoracic radiation, use of raloxifene in these populations is designated as a category 2A recommendation by the NCCN Breast Cancer Risk Reduction Panel. Use of raloxifene to reduce breast cancer risk in premenopausal women is inappropriate unless part of a clinical trial. The utility of raloxifene as a breast cancer risk-reduction agent in women <35 years of age is not known. There are insufficient data on the influence of ethnicity and race on the efficacy and safety of raloxifene as a risk-reduction agent.

AI Recommendations (Anastrozole and Exemestane)

The NCCN experts serving on the Breast Cancer Risk Reduction Panel have included exemestane and anastrozole as choices of risk-reduction agent for most postmenopausal women desiring non-surgical risk-reduction therapy (category 1). This is based on the results of the MAP.3 trial[3] and the IBIS-II trial.[4] The NCCN Breast Cancer Risk Reduction Panel recommends use of 25 mg/day of exemestane or 1 mg per day of anastrozole. Data regarding use of AI (exemestane and anastrozole) to reduce breast cancer risk are limited to postmenopausal women 35 years of age or older with a Gail model 5-year risk score >1.66% or a history of LCIS. The consensus of the NCCN Breast Cancer Risk Reduction Panel is that the risk/benefit ratio for use of an AI in postmenopausal women at increased risk for breast cancer is influenced by age, bone density, and comorbid conditions. Use of an AI to reduce breast cancer risk in premenopausal women is inappropriate unless part of a clinical trial. The utility of an AI as a breast cancer risk-reduction agent in women <35 years of age is not known. There are insufficient data on the influence of ethnicity and race on the efficacy and safety of raloxifene as a risk-reduction agent.

Exemestane and anastrazole are not currently FDA approved for breast cancer risk reduction. Currently, there are no data comparing the benefits and risks of AI to those of tamoxifen or raloxifene.

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Monitoring Patients on Risk Reduction Agents

Follow-up of women treated with risk-reduction agents for breast cancer risk reduction should focus on the early detection of breast cancer and the management of adverse symptoms or complications. Appropriate monitoring for breast cancer and the evaluation of breast abnormalities should be performed according to the guidelines described for high-risk women in the NCCN Guidelines for Breast Cancer Screening and Diagnosis. The population of women eligible for risk-reduction therapy with tamoxifen, raloxifene, anastrozole, or exemestane is at sufficiently increased risk for breast cancer to warrant, at a minimum, yearly bilateral mammography, a clinical breast examination every 6–12 months, and encouragement of breast awareness.

Endometrial Cancer

Results from the BCPT [P-1] study indicated that women ≥50 years of age treated with tamoxifen have an increased risk of developing invasive endometrial cancer. For women ≥50 years the risk of developing endometrial cancer while on tamoxifen compared to placebo was high (RR, 4.01, 95% CI 1.70–10.90).[55,56] An increased risk for endometrial cancer was *not* observed in women ≤49 years of age treated with tamoxifen in this study (RR, 1.21, 95% CI, 0.41–3.60).[55,56] Although the only death from endometrial cancer in the NSABP BCPT [P-1] occurred in a placebo-treated subject,[55,56] analyses of the NSABP data have revealed a small number of uterine sarcomas among the number of patients with an intact uterus taking tamoxifen. Uterine sarcoma is a rare form of uterine malignancy reported to occur in 2% to 4% of all patients with uterine cancer.[130] Compared with other uterine cancers, uterine sarcomas present at a more advanced stage and thus may carry a worse prognosis in terms of disease-free and overall survival.[131,132]

Updated results from the NSABP studies have indicated that incidence of both endometrial adenocarcinoma and uterine sarcoma is increased in women taking tamoxifen when compared to the placebo arm.[133] Several other studies have also supported an association between tamoxifen therapy and an increased risk of developing uterine sarcoma.[131,132,134,135] A "black box" FDA warning has been included on the package insert of tamoxifen to highlight the endometrial cancer risk (both epithelial endometrial cancer and uterine sarcoma) of tamoxifen.[136]

Use of raloxifene has not been associated with an increased incidence of endometrial cancer in the MORE trial.[116] Long-term results from the STAR trial showed the incidence of invasive endometrial cancer to be significantly lower in the group receiving raloxifene compared with the tamoxifen group (RR, 0.55, 09% CI 0.36–0.83).[58] For women with an intact uterus, a baseline gynecologic assessment is recommended prior to administration of the risk-reduction agent, and follow-up gynecologic assessments should be performed at each visit.[137] The vast majority of women with tamoxifen-associated endometrial cancer present with vaginal spotting as an early symptom of cancer. Therefore, prompt evaluation of vaginal spotting in the postmenopausal woman is essential.

At present, there is insufficient evidence to recommend the performance of uterine ultrasonography or endometrial biopsy for routine screening in asymptomatic women.[138-140] In women diagnosed with endometrial cancer while taking a risk-reduction agent, the drug should be discontinued until the endometrial cancer has been fully treated. The NCCN Breast Cancer Risk Reduction Panel believes that it is safe and reasonable to resume therapy with a risk-reduction agent after completion of treatment for early-stage endometrial cancer.

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Retinopathy and Cataract Formation

There have been reports of tamoxifen being associated with the occurrence of retinopathy, although most of this information has come from case studies.[141,142] Furthermore, these reports have not been confirmed in the randomized controlled trials of tamoxifen. A 1.14 relative risk of cataract formation (95% CI, 1.01–1.29), compared with placebo, has been reported in the BCPT [P-1] study, and individuals developing cataracts while on tamoxifen have a relative risk for cataract surgery of 1.57 (95% CI, 1.16–2.14), compared with placebo.[55] After 7 years of follow-up in the BCPT [P-1] study, relative risks of cataract formation and cataract surgery were similar to those initially reported.[56] In the MORE trial, raloxifene use was not associated with an increase in the incidence of cataracts compared with placebo (relative risk = 0.9; 95% CI, 0.8–1.1).[143] In the STAR trial, the incidence of cataract development and occurrence of cataract surgery was significantly higher in the group receiving tamoxifen compared with the group receiving raloxifene.[58,143] The rate of cataract development (RR = 0.80; 95% CI, 0.72– 0.89) and the rate of cataract surgery (RR = 0.79; 95% CI, 0.70–0.90) were about 20% less in the raloxifene group than in the tamoxifen group.[58,143] Thus, patients experiencing visual symptoms while undergoing treatment with tamoxifen should seek ophthalmologic evaluation.

Bone Mineral Density

Bone is an estrogen-responsive tissue, and tamoxifen can act as either an estrogen agonist or estrogen antagonist with respect to bone, depending on the menstrual status of a woman.[106,144-146] In premenopausal women, tamoxifen may oppose the more potent effects of estrogen on the bone and potentially increase the risk for osteoporosis, whereas tamoxifen in the presence of typically lower estrogen levels in postmenopausal women is associated with an increase in BMD.[55,56] However, the NCCN Breast Cancer Risk Reduction Panel does not recommend monitoring BMD in premenopausal patients on tamoxifen, since development of osteopenia/osteoporosis in this population is considered unlikely. Changes in BMD are of concern in women on AI therapy. Therefore, a baseline BMD scan is recommended before initiating therapy with an AI such as anastrozole or exemestane.

Raloxifene has been shown to increase BMD and to reduce incidence of vertebral bone fracture in postmenopausal women when compared with placebo.[115,118] Results from the STAR trial did not reveal any difference in the incidence of bone fracture in the groups of postmenopausal women on either raloxifene or tamoxifen.[57,58]

Thromboembolic Disease and Strokes

Tamoxifen and raloxifene have been associated with an increased risk of thromboembolic events (ie, DVT, pulmonary embolism) and stroke.[55,56-58,116,147] Increased incidences of VTE were observed in the tamoxifen arms of all the placebo-controlled, randomized, risk-reduction trials. Although not statistically significant, all of these trials with the exception of the Royal Marsden trial (which enrolled only younger women) also showed an increase in risk for stroke for women receiving tamoxifen. This risk was found to be significantly elevated in 2 meta analyses of randomized controlled trials evaluating tamoxifen for breast cancer risk reduction or treatment.[148,149] Comparison of the raloxifene and tamoxifen arms of the STAR trial did not show a difference with respect to incidence of stroke,[57,58] and the risk of fatal stroke was significantly higher for women in the RUTH trial with underlying heart disease receiving raloxifene.[119] However, evidence has shown that women with a Factor V Leiden or prothrombin G20210 → A mutation receiving tamoxifen therapy in the BCPT [P-1] study were not at increased risk of developing VTE compared to women without these mutations.[150] Although prospective screening of women for Factor V Leiden or

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prothrombin mutations or intermittent screening of women for thromboembolic disease is unlikely to be of value, women taking tamoxifen or raloxifene should be educated regarding the symptoms associated with DVT and pulmonary emboli. They should also be informed that prolonged immobilization may increase risk of VTE, and should be instructed to contact their physicians immediately if they develop symptoms of DVT or pulmonary emboli. Women with documented thromboembolic disease should receive appropriate treatment for the thromboembolic condition and should permanently discontinue tamoxifen or raloxifene therapy.

Managing Side Effects of Risk-Reduction Agents

Hot flashes are a common menopausal complaint. In the BCPT [P-1] study, hot flashes occurred in approximately 81% of women treated with tamoxifen and 69% of women treated with placebo.[55] In the STAR trial, women receiving tamoxifen reported a significantly increased incidence of vasomotor symptoms relative to women receiving raloxifene,[120] although raloxifene use has also been associated with an increase in hot flash severity and/or frequency when compared with placebo.[116] In women whose quality of life is diminished by hot flashes, an intervention to eliminate or minimize hot flashes should be undertaken. Estrogens and/or progestins have the potential to interact with SERMs and are not recommended by the NCCN Breast Cancer Risk Reduction Panel for the treatment of hot flashes for women on a risk-reduction agent outside of a clinical trial.

Gabapentin, a gamma-aminobutyric acid (GABA) analog used primarily for seizure control and management of neuropathic pain, has been reported to moderate both the severity and duration of hot flashes.[151-154] It has been hypothesized that the mode of action of gabapentin is via central temperature regulatory centers.[151,152] Results from a randomized, double-blind, placebo-controlled study involving the use of gabapentin to treat hot flashes in 420 women with breast cancer have been reported. The three treatment arms of the trial were as follows: 300 mg/day gabapentin; 900 mg/day gabapentin; and placebo. Study duration was 8 weeks, and most of the women in the study (68%–75% depending on treatment arm) were taking tamoxifen as adjuvant therapy. Women in the placebo group experienced reductions in severity of hot flashes of 21% and 15% at 4 and 8 weeks, respectively, whereas those in the treatment arms reported reductions of 33% and 31% with lower-dose gabapentin, and 49% and 46% with higher-dose gabapentin at 4 and 8 weeks, respectively. Only women receiving the higher dose of gabapentin had significantly fewer and less severe hot flashes. Side effects of somnolence or fatigue were reported in a small percentage of women taking gabapentin.[154]

Venlafaxine, a serotonin and norepinephrine inhibitor anti-depressant, has been shown to be effective in the management of hot flash symptoms in a group of breast cancer survivors, 70% of whom were taking tamoxifen. Significant declines were observed for both hot flash frequency and severity scores for all doses of venlafaxine (37.5 mg, 75 mg, and 150 mg) compared to placebo; incremental improvement was seen at 75 mg versus 37.5 mg (P = .03).[155] Participants receiving venlafaxine reported mouth dryness, reduced appetite, nausea, and constipation with increased prevalence at increased dosages. Based on these findings the authors suggested a starting dose of 37.5 mg with an increase, as necessary after one week, to 75 mg if a greater degree of symptom control is desired. However, this study followed subjects for only 4 weeks.

Another antidepressant, paroxetine, an SSRI, has also been studied for the relief of hot flash symptoms. A double-blind, placebo-controlled trial recruited 165 menopausal women who were randomized into 3 arms

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(placebo, paroxetine 12.5 mg daily, or paroxetine 25 mg daily). After 6 weeks, significant reductions in composite hot flash scores were noted for both dosages of paroxetine (12.5 mg, 62% reduction and 25 mg, 65% reduction); there were no significant differences between dose levels.[156] Adverse events, reported by 54% of subjects receiving placebo and 58% receiving paroxetine, generally included nausea, dizziness, and insomnia.

In a stratified, randomized, double-blind, cross-over, placebo-controlled study, 151 women reporting a history of hot flashes were randomized to one of 4 treatment arms (10 mg or 20 mg of paroxetine for 4 weeks followed by 4 weeks of placebo or 4 weeks of placebo followed by 4 weeks of 10 mg or 20 mg of paroxetine).[157] Hot flash frequency and composite score were reduced by 40.6% and 45.6%, respectively, for patients receiving 10 mg paroxetine compared to reductions of 13.7% and 13.7% in the placebo group. Likewise, reductions of 51.7% and 56.1% in hot flash frequency and score were found in women receiving 20 mg paroxetine compared with values of 26.6% and 28.8% in the placebo group. No significant differences in efficacy were observed with the lower and higher paroxetine doses. Rates of the most commonly reported side effects did not differ among the 4 arms, although nausea was significantly increased in women receiving 20 mg paroxetine relative to the other arms, and a greater percentage of patients receiving the higher dose of paroxetine discontinued treatment.

While these reports appear promising, further randomized studies of the use of these agents in women experiencing hot flash symptoms, especially those also taking tamoxifen, are needed to assess the long-term effectiveness and safety of these agents. In this context it should be noted that recent evidence has suggested that concomitant use of tamoxifen with certain SSRIs (eg, paroxetine and fluoxetine) may decrease plasma levels of endoxifen and 4-OH tamoxifen, active metabolites of tamoxifen, and may impact its efficacy.[127,158] These SSRIs may interfere with the enzymatic conversion of tamoxifen to its active metabolites by inhibiting a particular isoform of cytochrome P-450 enzyme (CYP2D6) involved in the metabolism of tamoxifen. Caution is advised about co-administration of these drugs with tamoxifen. Citalopram and venlafaxine appear to have only minimal effects on tamoxifen metabolism.

Of interest in this context are results of a retrospective evaluation of data from the Women's Healthy Eating and Living randomized trial, which suggest an inverse association between hot flashes and breast cancer recurrence for women with a history of breast cancer receiving tamoxifen. These results suggest that hot flashes in women receiving tamoxifen may be an indicator of the biologic availability and, thus, effectiveness of the drug. However, additional studies are needed to further elucidate whether hot flashes are predictive of benefit from tamoxifen.[159]

A recent report of 2 nonrandomized, parallel study cohorts of women with DCIS or those at high risk for breast cancer (eg, those with LCIS, atypical hyperplasia, or ≥1.7% 5-year breast cancer risk by the Gail model) comparing women receiving tamoxifen alone with women receiving tamoxifen concomitantly with HT (mean duration of HT at start of study approximately 10 years) did not show a difference in the rate of tamoxifen-induced hot flashes.[160] The NCCN Breast Cancer Risk Reduction Panel recommends against the use of HT for women taking tamoxifen or raloxifene outside of a clinical trial.

A variety of other substances for the control of hot flashes have been described.[161] Both the oral and transdermal formulations of clonidine reduce hot flashes in a dose-dependent manner.[162-164] Toxicities associated with clonidine include dry mouth, constipation, and

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drowsiness. Anecdotal evidence suggests that the use of a number of different herbal or food supplements may alleviate hot flashes. Vitamin E may decrease the frequency and severity of hot flashes, but results from a randomized clinical trial demonstrated that only a very modest improvement in hot flashes was associated with this agent compared with placebo.[165] Results from a double-blind, randomized, placebo-controlled, crossover trial of the use of black cohosh to treat hot flashes did not show significant differences between groups with respect to improvement in hot flash symptoms.[166] Some herbal or food supplements contain active estrogenic compounds, the activity and safety of which are unknown. Other strategies such as relaxation training, acupuncture, avoidance of caffeine and alcohol, and exercise for the management of hot flashes, while potentially beneficial, remain unsupported.[167]

It should be noted that the observed placebo effect in the treatment of hot flashes is considerable, typically falling in the range 25% or more,[151,153-157] suggesting that a considerable proportion of patients might be helped through a trial of therapy of limited duration. However, not all women who experience hot flashes require medical intervention, and the decision to intervene requires consideration of the efficacy and toxicity of the intervention. In addition, a study of women receiving tamoxifen for early-stage breast cancer showed a decrease in hot flashes over time.[168]

Components of Risk Reduction Counseling

Women should be monitored according to the NCCN Guidelines for Breast Cancer Screening and Diagnosis. Women with known or suspected *BRCA 1/2, TP53, PTEN*, or other gene mutations associated with breast cancer risk or those with close relatives with breast and/or ovarian cancer should also be followed according to the NCCN Guidelines for Genetic/Familial Risk Assessment: Breast and Ovarian whether or not they choose to undergo risk-reduction therapy. Women who have abnormal results from their clinical breast examination or bilateral mammogram should be managed according to the NCCN Guidelines for Breast Cancer Screening and Diagnosis or, if results indicate malignancy, the women should be treated according to the NCCN Guidelines for Breast Cancer. Although the NCCN Breast Cancer Risk Reduction Panel recommends that women with LCIS be managed according to the NCCN Breast Cancer Risk Reduction Guidelines, risk-reduction strategies for patients with LCIS are also described in the NCCN Guidelines for Breast Cancer.

All women who are appropriate candidates for breast cancer risk-reduction intervention should undergo counseling that provides a description of the available strategies, including a healthy lifestyle, to decrease breast cancer risk.[169] Options for breast cancer risk reduction should be discussed in a shared decision-making environment. The counseling should include a discussion and consideration of: 1) the individual's overall health status, including menopausal status, medical history, and medication history (eg, hysterectomy status, prior history of VTE, current use of hormones or SSRI, previous use of a SERM); 2) absolute and relative breast cancer risk reduction achieved with the risk-reduction intervention; 3) risks of risk-reduction therapy with an emphasis on age-dependent risks; 4) the contraindications to therapy with tamoxifen and raloxifene (eg, history of VTE, history of thrombotic stroke, history of transient ischemic attack, pregnancy or pregnancy potential without an effective nonhormonal method of contraception); and 5) the common and serious side effects of tamoxifen and raloxifene.

The 2009 ASCO Guidelines comparing the effectiveness of breast cancer risk-reduction agents provide some estimates of either the

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number needed to treat (NNT) to prevent breast cancer or the number needed to harm (NNH) by causing a specific side effect in a single patient receiving a specific risk-reduction agent.[170] Both NNT and NNH can be useful aids in communicating risks and benefits of tamoxifen and raloxifene in this setting (eg, using long-term data from the IBIS-1 trial, NNH with respect to VTE was determined to be 73 with tamoxifen, whereas this value was 150 for patients receiving raloxifene using data from the RUTH study).

Counseling Prior to Therapy with Risk Reduction Agents

Counseling sessions with women who are considering non-surgical breast cancer risk reduction should incorporate an explanation of data from the BCPT [P-1], STAR, MAP.3, and/or IBIS-II trial as appropriate.

Germline mutations in *PTEN* occur in 85% of patients with Cowden syndrome, an inherited condition associated with increased endometrial carcinoma risk. Therefore, increased risk for endometrial cancer in women with *PTEN* mutations should be discussed while considering a risk-reducing agent.

Counseling on Use of a SERM for Breast Cancer Risk Reduction

The BCPT [P-1] study showed that the toxicity profile of tamoxifen is much more favorable in younger women, and the benefits in relative risk reduction are similar across all age groups and risk groups.[55] The tamoxifen treatment risk/benefit ratio is especially favorable in women between the ages of 35 and 50 years. Unfortunately, individualized data regarding the risk/benefit ratio for tamoxifen are not generally available except for the broad age categories of ages 50 years and younger versus older than 50 years of age. Tamoxifen, unlike raloxifene, is a risk-reduction agent that can be used by premenopausal women. In addition, tamoxifen may be more effective than raloxifene in reducing the incidence of noninvasive breast cancer, although the difference is not statistically significant at long-term follow-up.[57,58] Further, tamoxifen was reported by patients in the STAR trial to be associated with better sexual function than raloxifene.[120] However, tamoxifen has been associated with an increased incidence of invasive endometrial cancer relative to placebo in women ≥50 years of age,[55,56] and an increased incidence of endometrial hyperplasia and invasive endometrial cancer relative to raloxifene,[57,58] possibly making it a less attractive choice in women with a uterus. Use of raloxifene to reduce breast cancer risk may be preferred by postmenopausal women with a uterus or those at risk for developing cataracts. All women receiving a breast cancer risk-reduction agent should be counseled with respect to signs and symptoms of possible side effects associated with use of these agents, and the recommended schedules for monitoring for the presence of certain adverse events. Contraindications to tamoxifen or raloxifene include history of VTE, thrombotic stroke, transient ischemic attack, current pregnancy or pregnancy potential without effective method of contraception, or known inherited clotting trait.

The optimal duration of SERM therapy for breast cancer risk reduction is not known. The BCPT [P-1] and STAR trials studied 5 years of risk-reduction therapy with either tamoxifen or raloxifene.[55,57] However, based on the updated STAR results, which showed that the benefits of raloxifene diminished after cessation of therapy,[58] continuing raloxifene beyond 5 years might be an approach to maintain the risk-reduction activity of the agent.

The use of tamoxifen for periods longer than 5 years has been evaluated in the *adjuvant treatment* setting. Results of two randomized trials on extended adjuvant tamoxifen treatment[171,172] have demonstrated that tamoxifen for up to 10 years is more effective than shorter durations at preventing cancer *recurrence* and improving breast cancer survival. The option of 10 years of adjuvant tamoxifen therapy is

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now recommended for both premenopausal women and postmenopausal women for preventing cancer recurrence in the NCCN Guidelines for Breast Cancer and the recently updated ASCO Guidelines.[173] There are limited data on tamoxifen use for more than 5 years in the risk-reduction setting. Until further information is available, a period of 5 years appears to be appropriate for tamoxifen therapy when the agent is used to reduce breast cancer risk.

After completing 5 years of tamoxifen therapy, women should continue to be monitored according to the NCCN Guidelines for Breast Cancer Screening and Diagnosis and should continue to undergo monitoring for late toxicity, especially for endometrial cancer and cataracts.

The prolonged effectiveness of tamoxifen as an agent to reduce breast cancer risk, particularly with respect to the development of ER-positive disease, is supported by results of several placebo-controlled, randomized trials at long-term follow-up.[56,107,114] The recent results from the STAR trial suggest that although a 5-year course of raloxifene retains considerable benefit with respect to the prevention of invasive breast cancer at a median follow-up of 81 months, the breast cancer preventive benefit of tamoxifen therapy for 5 years appears to be sustained for a longer period of time.[58]

The NCCN Breast Cancer Risk Reduction Panel recommends using the tables from the Freedman et al publication[2] while counseling postmenopausal women regarding use of raloxifene and tamoxifen for breast cancer risk reduction.

Counseling on Use of an AI for Breast Cancer Risk Reduction

Currently, there are no data comparing the benefits and risks of AIs (exemestane or anastrozole) to those of tamoxifen or raloxifene. Data regarding exemestane are from the single, large, randomized MAP.3 trial[3] limited to postmenopausal women 35 years of age or older with a Gail model 5-year breast cancer risk of 1.7% or a history of LCIS, which may be used while counseling patients. The data show that exemestane has a completely different toxicity profile than the SERMs. Compared to the placebo group in the MAP.3 trial, exemestane had no increased risk of serious side effects. The incidence of osteoporosis, cardiac events, and bone fractures were identical for women in the MAP.3 trial taking exemestane and for those taking the placebo. Women taking exemestane had a small, but not statistically significant increase in menopausal symptoms, such as hot flashes (18.3% vs. 11.9%) and arthritis (6.5% vs. 4.0%).[3]

Data regarding anastrozole are from a single, large, randomized trial, IBIS-II.[4] The trial included postmenopausal women 40 to 70 years of age with a higher risk of developing cancer compared with the general population. Women who did not meet these criteria but had a Tyrer-Cuzick model 10-year breast cancer risk >5% were also included.[4] Musculoskeletal and vasomotor events were reported in both arms of the trial and were found to be significantly higher in the anastrazole arm (P = .0001); fracture rates were similar in both arms.[4] The optimal duration of AI therapy is currently unknown. Changes in BMD are of concern in women receiving AI therapy. Therefore, a baseline BMD scan is recommended before initiating exemestane therapy. The role of calcium, vitamin D, and maintaining a healthy lifestyle in maintaining bone health must be emphasized in healthy postmenopausal women who are receiving exemestane.

Counseling Prior to Risk Reduction Surgery

For women at very high risk for breast cancer who are considering RRM, it is important that the potential psychosocial effects of RRM are addressed, although these effects have not been well studied.[174-176] Such surgery has the potential to negatively impact perceptions of body image, ease of forming new relationships, and the quality of existing

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relationships. Moreover, the procedure also eliminates the breast as a sexual organ. Multidisciplinary consultations are recommended prior to surgery, and should include a surgeon familiar with the natural history and therapy of benign and malignant breast disease[177] to enable the woman to become well informed regarding treatment alternatives, the risks and benefits of surgery, nipple-sparing mastectomy, and surgical breast reconstruction options. Immediate breast reconstruction is an option for many women following RRM, and early consultation with a reconstructive surgeon is recommended for those considering either immediate or delayed breast reconstruction.[178] Psychological consultations may also be considered.

Discussions regarding the risk for ovarian cancer and the option of RRSO for breast and ovarian cancer risk reduction should also be undertaken with women who are known carriers of a *BRCA1/2* mutation. Other topics that should be addressed with respect to RRSO include the increased risk for osteoporosis and cardiovascular disease associated with premature menopause, as well as the potential effects of possible cognitive changes, accelerated bone loss, and vasomotor symptoms on quality of life. Furthermore, the surgery itself may have some associated complications.

It has been reported that short-term HT in women undergoing RRSO did not negate the reduction in breast cancer risk associated with the surgery.[179] In addition, results of a recent case-control study of *BRCA1* mutation carriers showed no association between use of HT and increased breast cancer risk in postmenopausal *BRCA1* mutation carriers.[179] However, the consensus of the NCCN Breast Cancer Risk Reduction Panel is that caution should be used when considering HT use in mutation carriers following RRSO, given the limitations inherent in nonrandomized studies (see also section below on *Breast Cancer Risks Associated with Hormone Therapy*).[180,181] It is unlikely that a prospective randomized study on the use of RRSO for breast cancer risk reduction will be performed. Whether the resulting reduction in the risk for breast cancer from this procedure is preferable to a RRM is likely to remain a personal decision.[182] Table 2 provides estimates based on a Monte Carlo simulation model of the survival impact of breast and ovarian risk-reduction strategies. These data can be used as a tool to facilitate shared decision-making regarding choice of a risk-reduction approach, particularly with respect to issues related to risk-reduction surgery (see Table 2).

Counseling Regarding Lifestyle Modifications

There is evidence to indicate that certain lifestyle characteristics, such as obesity, increased alcohol consumption, and use of certain types of HT, are factors or markers for an elevated risk for breast cancer.[70] However, the association between a lifestyle modification and a change in breast cancer risk is not as clear. Nevertheless, a discussion of lifestyle characteristics associated with increased risk for breast cancer also provides "a teachable moment" for the promotion of overall health, and an opportunity to encourage women to make choices and changes compatible with a healthy lifestyle.

Breast Cancer Risks Associated with Hormone Therapy

The Women's Health Initiative (WHI) enrolled 161,809 postmenopausal women 50 to 79 years of age into a set of clinical trials from 1993 through 1998. Two of these trials were randomized controlled studies involving the use of HT (estrogen with/without progestin) in primary disease prevention: a trial involving 16,608 women with intact uteri at baseline randomized to receive estrogen plus progestin or placebo,[183] and a trial of 10,739 women with prior hysterectomy randomized to receive estrogen alone or placebo.[184] The former trial was terminated early due to evidence for breast cancer harm, along with a global index associated with overall harm. In that study, a 26% increased incidence

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of breast cancer was observed in the treatment group (HR =1.26; 95% CI, 1.00–1.59). An increased incidence of abnormal mammograms was also observed for women in the WHI who received estrogen plus progestin, and was attributed to an increase in breast density.[185] Of greater concern is that HT was associated with significant increase in rates of both breast cancer incidence and breast cancer–related mortality,[186] although the increased risk for breast cancer rapidly declined following cessation of HT.[187]

However, an increased risk for breast cancer was not observed in the trial of women who had undergone hysterectomies and were receiving unopposed estrogen. In fact, the rate of breast cancer was lower in the group receiving estrogen relative to the placebo group, although this difference was not considered to be statistically significant.[184] The lower incidence of breast cancer seen among women randomized to estrogen alone during the intervention period became statistically significant with extended follow-up for a mean of 10.7 years.[188] However, an increased incidence of abnormal mammograms was observed in the group of women receiving estrogen,[189] as well as a doubling of the risk for benign proliferative breast disease.[190] Analysis of the data from this randomized controlled WHI trial showed use of estrogen alone to significantly increase mammographic breast density compared with women receiving placebo; this effect was observed for at least a 2-year period.[191] Contrary to the results from the WHI randomized controlled trials, results from several prospective, population-based, observational studies have shown use of estrogen-only HT to be associated with increased risks for breast cancer. These studies include the Black Women's Health Study where use of estrogen alone for a duration of 10 years or longer was associated with a nonsignificant increase in risk for invasive breast cancer (relative risk = 1.41; 95% CI, 0.95–2.10);[192] the Million Women Study of women 50 to 64 years of age, which showed an association between current use of estrogen-only HT and increased risk for breast cancer (relative risk = 1.30; 95% CI, 1.21–1.40; $P <$ .0001);[193] and the Nurses' Health Study, which demonstrated a significantly increased breast cancer risk after long-term use (20 years or longer) of estrogen alone (relative risk = 1.42; 95% CI, 1.13–1.77).[194]

It has been noted that there are important differences in the populations enrolled in the WHI randomized clinical trials relative to the women followed in the observational studies with respect to duration of exposure to HT and age at initiation of HT.[195] For example, many of the women in the WHI clinical trials did not start receiving HT until years after menopause, whereas those in the population-based studies were more likely to initiate HT at menopause and to have been exposed to such treatment for longer periods of time. One hypothesis put forward to explain the apparent contradictions in the summary of studies of HT described above is that short-term use of estrogen following a period of estrogen deprivation may decrease breast cancer risk by inducing apoptosis of occult breast cancer tumors, whereas long-term use of estrogen may initiate and promote the growth of new tumors, thereby increasing breast cancer risk.[196] However, further studies are needed to evaluate this hypothesis. Another possible explanation for the decrease in breast cancer risk observed in the first 2 years of the WHI randomized controlled trial of postmenopausal women receiving estrogen plus progestin may be related to HT effects on breast tissue and subsequent interference with the ability of mammography to detect new breast cancer tumors.[195]

The use of estrogen/progestin therapy and estrogen therapy alone has also been associated with increased risk for cardiovascular disease (eg, stroke) and decreased risk for bone fractures.[183,184] However, a more recent secondary analysis from the WHI randomized controlled trials showed a trend for more effective reduction in the risk for

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cardiovascular disease with initiation of HT closer to menopause compared with administration of HT to women who experienced a greater time gap between menopause and the start of such therapy.[197] Nevertheless, recent results from a large French cohort control study show a significantly increased risk for breast cancer in women receiving short-term (ie, 2 years or less) estrogen and progesterone shortly after menopause when compared with nonusers.[198]

The NCCN Breast Cancer Risk Reduction Panel recommends against the use of HT for women taking tamoxifen, raloxifene, anastrozole, or exemestane outside of a clinical trial.

Alcohol Consumption

Numerous studies have demonstrated that the intake of moderate amounts of alcohol (one to two drinks per day) is associated with an increased risk for breast cancer. [70,71,72] A 10% increase in breast cancer risk for every 10 grams of alcohol consumed each day was seen in analyses of two cohort studies.[45,73] A population-based study of 51,847 postmenopausal women provided evidence to support an association between increased alcohol consumption and an increased likelihood of development of ER-positive breast cancer.[199] However, the effect of a reduction in alcohol consumption on the incidence of breast cancer has not been well studied. Even one drink per day modestly elevates breast cancer risk.[70] The consensus of the NCCN Breast Cancer Risk Reduction Panel is that alcohol consumption should be limited to <1 drink per day.

Exercise

Increased levels of physical activity have been associated with a decreased risk for breast cancer.[70,200-203] For example, the effect of exercise on breast cancer risk was evaluated in a population-based study of 90,509 women between the ages of 40 and 65 years.[203] A relative risk of 0.62 (95% CI, 0.49–0.78) was observed for women who reported more than five hours of vigorous exercise per week compared to women who did not participate in recreational activities. These results are supported by another population-based, case-control study of 4538 case patients with newly diagnosed invasive breast cancer and control patients grouped according to race (eg, 1605 black and 2933 white patients). Both black and white women with annual lifetime exercise activity levels exceeding the median activity level for active control subjects were found to have a 20% lower risk for breast cancer when compared to inactive women (odds ratio = 0.82; 95% CI, 0.71–0.93).[200] In addition, a prospective assessment evaluating the association of physical activity among 45,631 women showed the greatest reduction in breast cancer risk for women who reported walking/hiking for ≥10 hours per week (relative risk = 0.57; 95% CI, 0.34–0.95).[201] A study of 320 postmenopausal sedentary women randomly assigned to 1 year of aerobic exercise or a control group showed modest but significant changes in serum levels of estradiol and sex hormone-binding globulin from baseline (ie, a decrease and an increase in these levels, respectively).[204] However, it has been suggested that other, as yet unidentified, mechanisms are more likely to be responsible for the association between increased activity level and decreased risk for breast cancer.[205]

Diet

Results from the WHI controlled intervention trial of 48,835 postmenopausal women designed to test the effect of a low-fat diet (eg, fat intake limited to 20% of total caloric intake per day, and increased consumption of fruits, vegetables, and grains) on risk for breast cancer did not show a statistically significant reduction in the incidence of invasive breast cancer in women who followed a low-fat diet over an average of 8.1 years (HR = 0.91, 95% CI, 0.83–1.01).[206] Limitations of

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this type of study include inherent difficulties in assuring compliance with dietary interventions, recall biases, the relatively short duration of the follow-up period, and the likelihood of insufficient differences between the 2 arms with respect to fat intake.[207] Furthermore, it is possible that the impact of certain diets on breast cancer risk may be dependent on the age of the study population.[207,208] For example, results of a number of population-based studies have suggested that the effect of diet composition on breast cancer risk may be much greater during adolescence and early adulthood.[208,209] Nevertheless, diets in which the main sources of dietary fat are non-hydrogenated and unsaturated have been shown to have cardiovascular benefits.[208,210]

Epidemiologic studies suggest that vitamin D (from dietary sources and the sun) may play a protective role with respect to decreasing risk of breast cancer development.[208,211] Furthermore, there is some evidence to suggest that such protection is greatest for women who had more prolonged skin exposure to sunlight and higher dietary intake of sources of vitamin D during adolescence.[212,213] Current studies are ongoing to evaluate the role of vitamin D on breast cancer risk.

Weight/BMI

There is a substantial amount of evidence indicating that overweight or obese women have a higher risk for postmenopausal breast cancer.[39,69,70]

Recent results from the Nurses' Health Study evaluating the effect of weight change on the incidence of invasive breast cancer in 87,143 postmenopausal women suggested that women experiencing a weight gain of 25.0 kg or more since age 18 have an increased risk for breast cancer when compared with women who have maintained their weight (relative risk = 1.45; 95% CI, 1.27–1.66).[39] Furthermore, women who had never used postmenopausal HT and lost 10.0 kg or more since menopause and kept the weight off had a significantly lower risk for breast cancer than women who had maintained their weight (relative risk = 0.43; 95% CI, 0.21–0.86). Interestingly, there is evidence that the risk for breast cancer is lower in premenopausal women who are overweight compared with women who are not overweight.[70]

Results from a case-control study of 1073 pairs of women with *BRCA1/2* mutations indicated that a weight loss of 10 or more pounds in women with the *BRCA1* mutation between the ages of 18 and 30 was associated with a decreased risk of developing breast cancer between the ages of 30 and 40 years. (odds ratio = 0.35; 95% CI, 0.18–0.67).[214]

Clinical Trials

Risk-reduction counseling should include a discussion of breast cancer risk-reduction interventions available in clinical trials.

Summary

Breast cancer risk assessment provides a means of identifying healthy women without a history of personal breast cancer, who are at increased risk for future development of this disease. All women should be counseled regarding healthy lifestyle recommendations to decrease breast cancer risk and to avoid lifestyles that would adversely impact their chance of developing the disease. However, many of the risk factors for breast cancer are not modifiable. The demonstration that use of tamoxifen, raloxifene, anastrozole, or exemestane substantially decreases the future risk for breast cancer provides an opportunity for a risk-reduction intervention. However, the risks and benefits associated with use of risk-reduction agents for an individual woman should be evaluated and discussed with the woman as part of a shared decision-making process. Women taking a risk-reduction agent must be closely monitored for potential side effects associated with use of these

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agents. In special circumstances, such as in women who are carriers of a *BRCA1/2* mutation, where the risk for breast cancer is very high, the performance of a bilateral mastectomy or bilateral salpingo-oophorectomy may be considered for breast cancer risk reduction. Women considering either surgery should undergo multidisciplinary consultations prior to surgery so as to become well informed about all treatment alternatives, the risks and benefits of risk-reduction surgery, and, in the case of bilateral mastectomy, the various reconstruction options available. The NCCN Guidelines for Breast Cancer Risk Reduction Panel strongly encourage women and health care providers to participate in clinical trials to test new strategies for decreasing the risk for breast cancer. Only through the accumulated experience gained from prospective and well-designed clinical trials will additional advances in breast cancer risk reduction be realized.

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Table 1

Criteria Used in Calculation of 5-year Risk for Breast Cancer According to the Modified Gail Model
(Available at www.breastcancerprevention.com)

Question	Response
Age	____
Age at menarche (first menstrual period)	____
Age at first live birth or nulliparity	____
Number of breast biopsies	____
Atypical hyperplasia	Y / N
Number of first-degree relatives with breast cancer	____
Race/Ethnicity	Caucasian, African American, Hispanic, Other

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Table 2

Survival Probability According to Breast/Ovarian Cancer Risk-Reduction Strategy at Age 70* for 25-Year-Old *BRCA1/2* Mutation Carrier

Variable	Survival probability (%) in *BRCA1* mutation carriers	Survival probability (%) in *BRCA2* mutation carriers
No intervention	53% [BCD=41%;OCD=36%]	71% [BCD=36%;OCD=20%]
RRSO only at age 40	68% [BCD=45%;OCD=12%]	77% [BCD=30%;OCD=4%]
RRSO only at age 50	61% [BCD=51%;OCD=20%]	75% [BCD=42%;OCD=6%]
RRM only at age 25	66% [BCD=5%;OCD=58%]	79% [BCD=4%;OCD=30%]
RRM only at age 40	64% [BCD=13%;OCD=53%]	78% [BCD=9%;OCD=28%]
Breast Screening only from ages 25-69	59% [BCD=26%;OCD=46%]	75% [BCD=21%;OCD=25%]
RRSO at age 40 and RRM at age 25	79% [BCD=6%;OCD=21%]	83% [BCD=3%;OCD=6%]
RRSO at age 40 and breast screening from ages 25-69	74% [BCD=30%;OCD=15%]	80% [BCD=18%;OCD=5%]
RRSO at age 40, RRM at age 40; and breast screening from ages 25-39	77% [BCD=18%;OCD=18%]	82% [BCD=9%;OCD=6%]

*Survival probability for 70-year-old woman from general population = 84%

[Probability of death as a result of breast cancer (BCD) or ovarian cancer (OCD); RRSO – risk-reducing bilateral salpingo-oophorectomy; RRM – risk-reducing bilateral mastectomy; Breast screening – annual mammography and MRI]

Data from: Kurian AW, Sigal BM, Plevritis SK. Survival analysis of cancer risk reduction strategies for *BRCA1/2* mutation carriers. J Clin Oncol. 2010;28:222-231.

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References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24399786.

2. Freedman AN, Yu B, Gail MH, et al. Benefit/risk assessment for breast cancer chemoprevention with raloxifene or tamoxifen for women age 50 years or older. J Clin Oncol 2011;29:2327-2333. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21537036.

3. Goss PE, Ingle JN, Ales-Martinez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 2011;364:2381-2391. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21639806.

4. Cuzick J, Sestak I, Forbes JF, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. Lancet 2014;383:1041-1048. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24333009.

5. Murff HJ, Byrne D, Syngal S. Cancer risk assessment: quality and impact of the family history interview. Am J Prev Med 2004;27:239-245. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15450637.

6. Murff HJ, Spigel DR, Syngal S. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. JAMA 2004;292:1480-1489. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15383520.

7. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378:789-792. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8524414.

8. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266:66-71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7545954.

9. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-1238. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1978757.

10. van Lier MG, Wagner A, Mathus-Vliegen EM, et al. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. Am J Gastroenterol 2010;105:1258-1264; author reply 1265. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20051941.

11. Tan MH, Mester JL, Ngeow J, et al. Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res 2012;18:400-407. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22252256.

12. Bubien V, Bonnet F, Brouste V, et al. High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. J Med Genet 2013;50:255-263. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23335809.

13. Berx G, Staes K, van Hengel J, et al. Cloning and characterization of the human invasion suppressor gene E-cadherin (CDH1). Genomics 1995;26:281-289. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7601454.

14. Colditz GA, Willett WC, Hunter DJ, et al. Family history, age, and risk of breast cancer. Prospective data from the Nurses' Health Study. JAMA 1993;270:338-343. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8123079.

15. Slattery ML, Kerber RA. A comprehensive evaluation of family history and breast cancer risk. The Utah Population Database. JAMA 1993;270:1563-1568. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8371466.

16. Colditz GA, Kaphingst KA, Hankinson SE, Rosner B. Family history and risk of breast cancer: nurses' health study. Breast Cancer Res

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Treat 2012. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22350789.

17. Lynch HT, Watson P, Conway TA, Lynch JF. Clinical/genetic features in hereditary breast cancer. Breast Cancer Res Treat 1990;15:63-71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2322650.

18. Pharoah PD, Day NE, Duffy S, et al. Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer 1997;71:800-809. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9180149.

19. Berliner JL, Fay AM. Risk assessment and genetic counseling for hereditary breast and ovarian cancer: recommendations of the National Society of Genetic Counselors. J Genet Couns 2007;16:241-260. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17508274.

20. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med 2008;359:2143-2153. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19005198.

21. Pharoah PD, Antoniou A, Bobrow M, et al. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33-36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11984562.

22. Trepanier A, Ahrens M, McKinnon W, et al. Genetic cancer risk assessment and counseling: recommendations of the national society of genetic counselors. J Genet Couns 2004;13:83-114. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15604628.

23. Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. Cancer 1994;73:643-651. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8299086.

24. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med 2004;23:1111-1130. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15057881.

25. Amir E, Evans DG, Shenton A, et al. Evaluation of breast cancer risk assessment packages in the family history evaluation and screening programme. J Med Genet 2003;40:807-814. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14627668.

26. Evans DG, Howell A. Breast cancer risk-assessment models. Breast Cancer Res 2007;9:213. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17888188.

27. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007;57:75-89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17392385.

28. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet 1998;62:145-158. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9443863.

29. Antoniou AC, Cunningham AP, Peto J, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. Br J Cancer 2008;98:1457-1466. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18349832.

30. Claus EB, Risch N, Thompson WD. The calculation of breast cancer risk for women with a first degree family history of ovarian cancer. Breast Cancer Res Treat 1993;28:115-120. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8173064.

31. Roa BB, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nat Genet 1996;14:185-187. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8841191.

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32. Li CI, Malone KE, Daling JR, et al. Timing of menarche and first full-term birth in relation to breast cancer risk. Am J Epidemiol 2008;167:230-239. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17965112.

33. Yang XR, Chang-Claude J, Goode EL, et al. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. J Natl Cancer Inst 2011;103:250-263. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21191117.

34. Islam T, Matsuo K, Ito H, et al. Reproductive and hormonal risk factors for luminal, HER2-overexpressing, and triple-negative breast cancer in Japanese women. Ann Oncol 2012;23:2435-2441. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22328736.

35. Li CI, Beaber EF, Tang MT, et al. Reproductive factors and risk of estrogen receptor positive, triple-negative, and HER2-neu overexpressing breast cancer among women 20-44 years of age. Breast Cancer Res Treat 2013;137:579-587. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23224237.

36. Chlebowski RT, Hendrix SL, Langer RD, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. JAMA 2003;289:3243-3253. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12824205.

37. Han D, Nie J, Bonner MR, et al. Lifetime adult weight gain, central adiposity, and the risk of pre- and postmenopausal breast cancer in the Western New York exposures and breast cancer study. Int J Cancer 2006;119:2931-2937. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17016824.

38. Kawai M, Minami Y, Kuriyama S, et al. Adiposity, adult weight change and breast cancer risk in postmenopausal Japanese women: the Miyagi Cohort Study. Br J Cancer 2010;103:1443-1447. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20842123.

39. Eliassen AH, Colditz GA, Rosner B, et al. Adult weight change and risk of postmenopausal breast cancer. JAMA 2006;296:193-201. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16835425.

40. Suzuki R, Iwasaki M, Inoue M, et al. Body weight at age 20 years, subsequent weight change and breast cancer risk defined by estrogen and progesterone receptor status--the Japan public health center-based prospective study. Int J Cancer 2011;129:1214-1224. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21064092.

41. Suzuki R, Rylander-Rudqvist T, Ye W, et al. Body weight and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status among Swedish women: A prospective cohort study. Int J Cancer 2006;119:1683-1689. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16646051.

42. Feigelson HS, Patel AV, Teras LR, et al. Adult weight gain and histopathologic characteristics of breast cancer among postmenopausal women. Cancer 2006;107:12-21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16718671.

43. Vrieling A, Buck K, Kaaks R, Chang-Claude J. Adult weight gain in relation to breast cancer risk by estrogen and progesterone receptor status: a meta-analysis. Breast Cancer Res Treat 2010;123:641-649. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20711809.

44. Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. JAMA 2001;286:2143-2151. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11694156.

45. Hamajima N, Hirose K, Tajima K, et al. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer 2002;87:1234-1245. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12439712.

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46. Rosenberg L, Boggs DA, Bethea TN, et al. A prospective study of smoking and breast cancer risk among African-American women. Cancer Causes Control 2013;24:2207-2215. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24085586.

47. Greendale GA, Reboussin BA, Slone S, et al. Postmenopausal hormone therapy and change in mammographic density. J Natl Cancer Inst 2003;95:30-37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12509398.

48. Chiu SY, Duffy S, Yen AM, et al. Effect of baseline breast density on breast cancer incidence, stage, mortality, and screening parameters: 25-year follow-up of a Swedish mammographic screening. Cancer Epidemiol Biomarkers Prev 2010;19:1219-1228. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20406961.

49. Vachon CM, Sellers TA, Scott CG, et al. Longitudinal breast density and risk of breast cancer [abstract]. Cancer Research 2011;70:Abstract 4828. Available at: http://cancerres.aacrjournals.org/cgi/content/meeting_abstract/70/8_MeetingAbstracts/4828.

50. Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. J Natl Cancer Inst 1999;91:1541-1548. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10491430.

51. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81:1879-1886. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2593165.

52. Spiegelman D, Colditz GA, Hunter D, Hertzmark E. Validation of the Gail et al. model for predicting individual breast cancer risk. J Natl Cancer Inst 1994;86:600-607. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8145275.

53. Gail MH, Costantino JP. Validating and improving models for projecting the absolute risk of breast cancer. J Natl Cancer Inst 2001;93:334-335. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11238688.

54. Rockhill B, Spiegelman D, Byrne C, et al. Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. J Natl Cancer Inst 2001;93:358-366. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11238697.

55. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371-1388. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9747868.

56. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst 2005;97:1652-1662. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16288118.

57. Vogel VG, Costantino JP, Wickerham DL, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA 2006;295:2727-2741. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16754727.

58. Vogel VG, Costantino JP, Wickerham DL, et al. Update of the National Surgical Adjuvant Breast and Bowel Project Study of Tamoxifen and Raloxifene (STAR) P-2 Trial: Preventing breast cancer. Cancer Prev Res (Phila) 2010;3:696-706. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20404000.

59. Gail MH, Costantino JP, Pee D, et al. Projecting individualized absolute invasive breast cancer risk in African American women. J Natl Cancer Inst 2007;99:1782-1792. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18042936.

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60. Matsuno RK, Costantino JP, Ziegler RG, et al. Projecting Individualized Absolute Invasive Breast Cancer Risk in Asian and Pacific Islander American Women. J Natl Cancer Inst 2011. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21562243.

61. Bhatia S, Yasui Y, Robison LL, et al. High risk of subsequent neoplasms continues with extended follow-up of childhood Hodgkin's disease: report from the Late Effects Study Group. J Clin Oncol 2003;21:4386-4394. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14645429.

62. Travis LB, Hill D, Dores GM, et al. Cumulative absolute breast cancer risk for young women treated for Hodgkin lymphoma. J Natl Cancer Inst 2005;97:1428-1437. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16204692.

63. Hancock SL, Tucker MA, Hoppe RT. Breast cancer after treatment of Hodgkin's disease. J Natl Cancer Inst 1993;85:25-31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8416252.

64. Hudson MM, Poquette CA, Lee J, et al. Increased mortality after successful treatment for Hodgkin's disease. J Clin Oncol 1998;16:3592-3600. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9817280.

65. van Leeuwen FE, Klokman WJ, Stovall M, et al. Roles of radiation dose, chemotherapy, and hormonal factors in breast cancer following Hodgkin's disease. J Natl Cancer Inst 2003;95:971-980. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12837833.

66. Yahalom J, Petrek JA, Biddinger PW, et al. Breast cancer in patients irradiated for Hodgkin's disease: a clinical and pathologic analysis of 45 events in 37 patients. J Clin Oncol 1992;10:1674-1681. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1403050.

67. Rosen PP, Kosloff C, Lieberman PH, et al. Lobular carcinoma in situ of the breast. Detailed analysis of 99 patients with average follow-up of 24 years. Am J Surg Pathol 1978;2:225-251. Available at: http://www.ncbi.nlm.nih.gov/pubmed/210682.

68. Haagensen C, Bodian C, Haagensen D, Jr. Breast carcinoma: Risk and detection. Philadelphia, PA: W.B. Saunders; 1981.

69. van den Brandt PA, Spiegelman D, Yaun SS, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000;152:514-527. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10997541.

70. Mahoney MC, Bevers T, Linos E, Willett WC. Opportunities and strategies for breast cancer prevention through risk reduction. CA Cancer J Clin 2008;58:347-371. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18981297.

71. Zhang SM, Lee IM, Manson JE, et al. Alcohol consumption and breast cancer risk in the Women's Health Study. Am J Epidemiol 2007;165:667-676. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17204515.

72. Terry MB, Zhang FF, Kabat G, et al. Lifetime alcohol intake and breast cancer risk. Ann Epidemiol 2006;16:230-240. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16230024.

73. Smith-Warner SA, Spiegelman D, Yaun SS, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 1998;279:535-540. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9480365.

74. Antoniou AC, Pharoah PD, Easton DF, Evans DG. BRCA1 and BRCA2 cancer risks. J Clin Oncol 2006;24:3312-3313; author reply 3313-3314. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16829658.

75. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998;62:676-689. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9497246.

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76. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336:1401-1408. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9145676.

77. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 1999;340:77-84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9887158.

78. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. J Natl Cancer Inst 2001;93:1633-1637. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11698567.

79. Hamm RM, Lawler F, Scheid D. Prophylactic mastectomy in women with a high risk of breast cancer. N Engl J Med 1999;340:1837-1838; author reply 1839. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10366319.

80. Meijers-Heijboer H, van Geel B, van Putten WL, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2001;345:159-164. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11463009.

81. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 2004;22:1055-1062. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14981104.

82. Boughey JC, Khakpour N, Meric-Bernstam F, et al. Selective use of sentinel lymph node surgery during prophylactic mastectomy. Cancer 2006;107:1440-1447. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16955504.

83. Miller CL, Specht MC, Skolny MN, et al. Sentinel lymph node biopsy at the time of mastectomy does not increase the risk of lymphedema: implications for prophylactic surgery. Breast Cancer Res Treat 2012;135:781-789. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22941538.

84. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer 2000;83:1301-1308. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11044354.

85. Antoniou A, Pharoah PDP, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117-1130. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12677558.

86. King M-C, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643-646. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14576434.

87. Satagopan JM, Boyd J, Kauff ND, et al. Ovarian cancer risk in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. Clin Cancer Res 2002;8:3776-3781. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12473589.

88. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002;346:1616-1622. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12023993.

89. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst 2009;101:80-87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19141781.

90. Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with

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a BRCA1 or BRCA2 Mutation. JAMA 2006;296:185-192. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16835424.

91. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002;346:1609-1615. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12023992.

92. Kemel Y, Kauff ND, Robson ME, et al. Four-year follow-up of outcomes following risk-reducing salpingo-oophorectomy in BRCA mutation carriers [abstract]. ASCO Meeting Abstracts 2005;23:Abstract 1013. Available at: http://meeting.ascopubs.org/cgi/content/abstract/23/16_suppl/1013.

93. Rebbeck TR, Levin AM, Eisen A, et al. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. J Natl Cancer Inst 1999;91:1475-1479. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10469748.

94. Eisen A, Lubinski J, Klijn J, et al. Breast cancer risk following bilateral oophorectomy in BRCA1 and BRCA2 mutation carriers: an international case-control study. J Clin Oncol 2005;23:7491-7496. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16234515.

95. Kauff ND, Domchek SM, Friebel TM, et al. Risk-reducing salpingo-oophorectomy for the prevention of BRCA1- and BRCA2-associated breast and gynecologic cancer: a multicenter, prospective study. J Clin Oncol 2008;26:1331-1337. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18268356.

96. Kurian AW, Sigal BM, Plevritis SK. Survival analysis of cancer risk reduction strategies for BRCA1/2 mutation carriers. J Clin Oncol 2010;28:222-231. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19996031.

97. Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA 2010;304:967-975. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20810374.

98. Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005;23:127-132. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15625367.

99. Adjuvant tamoxifen in the management of operable breast cancer: the Scottish Trial. Report from the Breast Cancer Trials Committee, Scottish Cancer Trials Office (MRC), Edinburgh. Lancet 1987;2:171-175. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2885637.

100. Cyclophosphamide and tamoxifen as adjuvant therapies in the management of breast cancer. CRC Adjuvant Breast Trial Working Party. Br J Cancer 1988;57:604-607. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2900646.

101. Fisher B, Costantino J, Redmond C, et al. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. N Engl J Med 1989;320:479-484. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2644532.

102. Rutqvist LE, Cedermark B, Glas U, et al. Contralateral primary tumors in breast cancer patients in a randomized trial of adjuvant tamoxifen therapy. J Natl Cancer Inst 1991;83:1299-1306. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1886157.

103. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365:1687-1717. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15894097.

104. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: National Surgical Adjuvant Breast and Bowel Project (NSABP-

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P1) Breast Cancer Prevention Trial. JAMA 2001;286:2251-2256. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11710890.

105. Cuzick J, Powles T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. Lancet 2003;361:296-300. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12559863.

106. Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. Lancet 1998;352:98-101. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9672274.

107. Powles TJ, Ashley S, Tidy A, et al. Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. J Natl Cancer Inst 2007;99:283-290. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17312305.

108. Veronesi U, Maisonneuve P, Sacchini V, et al. Tamoxifen for breast cancer among hysterectomised women. Lancet 2002;359:1122-1124. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11943263.

109. Veronesi U, Maisonneuve P, Costa A, et al. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among hysterectomised women. Italian Tamoxifen Prevention Study. Lancet 1998;352:93-97. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9672273.

110. Veronesi U, Maisonneuve P, Rotmensz N, et al. Tamoxifen for the prevention of breast cancer: late results of the Italian Randomized Tamoxifen Prevention Trial among women with hysterectomy. J Natl Cancer Inst 2007;99:727-737. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17470740.

111. Veronesi U, Maisonneuve P, Rotmensz N, et al. Italian randomized trial among women with hysterectomy: tamoxifen and hormone-dependent breast cancer in high-risk women. J Natl Cancer Inst 2003;95:160-165. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12529349.

112. Bevers TB. Breast cancer chemoprevention: current clinical practice and future direction. Biomed Pharmacother 2001;55:559-564. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11769967.

113. Cuzick J, Forbes J, Edwards R, et al. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. Lancet 2002;360:817-824. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12243915.

114. Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer--96-month follow-up of the randomized IBIS-I trial. J Natl Cancer Inst 2007;99:272-282. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17312304.

115. Ettinger B, Black DM, Mitlak BH, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA 1999;282:637-645. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10517716.

116. Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. JAMA 1999;281:2189-2197. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10376571.

117. Martino S, Cauley JA, Barrett-Connor E, et al. Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. J Natl Cancer Inst 2004;96:1751-1761. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15572757.

118. Barrett-Connor E, Mosca L, Collins P, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med 2006;355:125-137. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16837676.

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119. Grady D, Cauley JA, Geiger MJ, et al. Reduced incidence of invasive breast cancer with raloxifene among women at increased coronary risk. J Natl Cancer Inst 2008;100:854-861. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18544744.

120. Land SR, Wickerham DL, Costantino JP, et al. Patient-reported symptoms and quality of life during treatment with tamoxifen or raloxifene for breast cancer prevention: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA 2006;295:2742-2751. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16754728.

121. Baum M, Budzar AU, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. Lancet 2002;359:2131-2139. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12090977.

122. Baum M, Buzdar A, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses. Cancer 2003;98:1802-1810. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14584060.

123. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med 2004;350:1081-1092. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15014181.

124. Goss PE, Ingle JN, Martino S, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 2003;349:1793-1802. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14551341.

125. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med 2005;353:2747-2757. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16382061.

126. Decensi A, Gandini S, Serrano D, et al. Randomized dose-ranging trial of tamoxifen at low doses in hormone replacement therapy users. J Clin Oncol 2007;25:4201-4209. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17709798.

127. Sideras K, Ingle JN, Ames MM, et al. Coprescription of tamoxifen and medications that inhibit CYP2D6. J Clin Oncol 2010;28:2768-2776. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20439629.

128. Higgins MJ, Stearns V. CYP2D6 polymorphisms and tamoxifen metabolism: clinical relevance. Curr Oncol Rep 2010;12:7-15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20425602.

129. Lash TL, Rosenberg CL. Evidence and practice regarding the role for CYP2D6 inhibition in decisions about tamoxifen therapy. J Clin Oncol 2010;28:1273-1275. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20124162.

130. Curtin J, Kavanagh J. Corpus: Mesenchymal tumors. In: Hoskins W, Perez C, Young R, eds. Principles and Practice of Gynecologic Oncology (ed 3rd). Philadelphia, PA; 2000:961-979.

131. Bergman L, Beelen ML, Gallee MP, et al. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of Liver and Endometrial cancer Risk following Tamoxifen. Lancet 2000;356:881-887. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11036892.

132. Curtis RE, Freedman DM, Sherman ME, Fraumeni JF, Jr. Risk of malignant mixed mullerian tumors after tamoxifen therapy for breast cancer. J Natl Cancer Inst 2004;96:70-74. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14709741.

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133. Wickerham DL, Fisher B, Wolmark N, et al. Association of tamoxifen and uterine sarcoma. J Clin Oncol 2002;20:2758-2760. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12039943.

134. Bouchardy C, Verkooijen HM, Fioretta G, et al. Increased risk of malignant mullerian tumor of the uterus among women with breast cancer treated by tamoxifen. J Clin Oncol 2002;20:4403. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12409344.

135. Rieck GC, Freites ON, Williams S. Is tamoxifen associated with high-risk endometrial carcinomas? A retrospective case series of 196 women with endometrial cancer. J Obstet Gynaecol 2005;25:39-41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16147692.

136. Wysowski DK, Honig SF, Beitz J. Uterine sarcoma associated with tamoxifen use. N Engl J Med 2002;346:1832-1833. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12050351.

137. ACOG committee opinion. No. 336: Tamoxifen and uterine cancer. Obstet Gynecol 2006;107:1475-1478. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16738185.

138. Barakat RR, Gilewski TA, Almadrones L, et al. Effect of adjuvant tamoxifen on the endometrium in women with breast cancer: a prospective study using office endometrial biopsy. J Clin Oncol 2000;18:3459-3463. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11032585.

139. Fung MF, Reid A, Faught W, et al. Prospective longitudinal study of ultrasound screening for endometrial abnormalities in women with breast cancer receiving tamoxifen. Gynecol Oncol 2003;91:154-159. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14529676.

140. Gerber B, Krause A, Muller H, et al. Effects of adjuvant tamoxifen on the endometrium in postmenopausal women with breast cancer: a prospective long-term study using transvaginal ultrasound. J Clin Oncol 2000;18:3464-3470. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11032586.

141. Gorin MB, Costantino JP, Kulacoglu DN, et al. Is tamoxifen a risk factor for retinal vaso-occlusive disease? Retina 2005;25:523-526. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15933605.

142. Nayfield SG, Gorin MB. Tamoxifen-associated eye disease. A review. J Clin Oncol 1996;14:1018-1026. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8622006.

143. Grady D, Ettinger B, Moscarelli E, et al. Safety and adverse effects associated with raloxifene: multiple outcomes of raloxifene evaluation. Obstet Gynecol 2004;104:837-844. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15458908.

144. Powles TJ, Hickish T, Kanis JA, et al. Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women. J Clin Oncol 1996;14:78-84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8558225.

145. Sverrisdottir A, Fornander T, Jacobsson H, et al. Bone mineral density among premenopausal women with early breast cancer in a randomized trial of adjuvant endocrine therapy. J Clin Oncol 2004;22:3694-3699. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15365065.

146. Vehmanen L, Elomaa I, Blomqvist C, Saarto T. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. J Clin Oncol 2006;24:675-680. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16446340.

147. Decensi A, Maisonneuve P, Rotmensz N, et al. Effect of tamoxifen on venous thromboembolic events in a breast cancer prevention trial. Circulation 2005;111:650-656. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15699284.

148. Braithwaite RS, Chlebowski RT, Lau J, et al. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J Gen Intern

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Med 2003;18:937-947. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14687281.

149. Bushnell CD, Goldstein LB. Risk of ischemic stroke with tamoxifen treatment for breast cancer: a meta-analysis. Neurology 2004;63:1230-1233. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15477543.

150. Abramson N, Costantino JP, Garber JE, et al. Effect of Factor V Leiden and prothrombin G20210-->A mutations on thromboembolic risk in the national surgical adjuvant breast and bowel project breast cancer prevention trial. J Natl Cancer Inst 2006;98:904-910. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16818854.

151. Guttuso T, Jr., Kurlan R, McDermott MP, Kieburtz K. Gabapentin's effects on hot flashes in postmenopausal women: a randomized controlled trial. Obstet Gynecol 2003;101:337-345. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12576259.

152. Guttuso TJ, Jr. Gabapentin's effects on hot flashes and hypothermia. Neurology 2000;54:2161-2163. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10851385.

153. Loprinzi L, Barton DL, Sloan JA, et al. Pilot evaluation of gabapentin for treating hot flashes. Mayo Clin Proc 2002;77:1159-1163. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12440550.

154. Pandya KJ, Morrow GR, Roscoe JA, et al. Gabapentin for hot flashes in 420 women with breast cancer: a randomised double-blind placebo-controlled trial. Lancet 2005;366:818-824. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16139656.

155. Loprinzi CL, Kugler JW, Sloan JA, et al. Venlafaxine in management of hot flashes in survivors of breast cancer: a randomised controlled trial. Lancet 2000;356:2059-2063. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11145492.

156. Stearns V, Beebe KL, Iyengar M, Dube E. Paroxetine controlled release in the treatment of menopausal hot flashes: a randomized controlled trial. JAMA 2003;289:2827-2834. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12783913.

157. Stearns V, Slack R, Greep N, et al. Paroxetine is an effective treatment for hot flashes: results from a prospective randomized clinical trial. J Clin Oncol 2005;23:6919-6930. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16192581.

158. Jin Y, Desta Z, Stearns V, et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 2005;97:30-39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15632378.

159. Mortimer JE, Flatt SW, Parker BA, et al. Tamoxifen, hot flashes and recurrence in breast cancer. Breast Cancer Res Treat 2008;108:421-426. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17541741.

160. Osborne CR, Duncan A, Sedlacek S, et al. The addition of hormone therapy to tamoxifen does not prevent hot flashes in women at high risk for developing breast cancer. Breast Cancer Res Treat 2009;116:521-527. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19139988.

161. Nelson HD, Vesco KK, Haney E, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA 2006;295:2057-2071. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16670414.

162. Goldberg RM, Loprinzi CL, O'Fallon JR, et al. Transdermal clonidine for ameliorating tamoxifen-induced hot flashes. J Clin Oncol 1994;12:155-158. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8270972.

163. Laufer LR, Erlik Y, Meldrum DR, Judd HL. Effect of clonidine on hot flashes in postmenopausal women. Obstet Gynecol 1982;60:583-586. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7145250.

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164. Nagamani M, Kelver ME, Smith ER. Treatment of menopausal hot flashes with transdermal administration of clonidine. Am J Obstet Gynecol 1987;156:561-565. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3826200.

165. Barton DL, Loprinzi CL, Quella SK, et al. Prospective evaluation of vitamin E for hot flashes in breast cancer survivors. J Clin Oncol 1998;16:495-500. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9469333.

166. Pockaj BA, Gallagher JG, Loprinzi CL, et al. Phase III double-blind, randomized, placebo-controlled crossover trial of black cohosh in the management of hot flashes: NCCTG Trial N01CC1. J Clin Oncol 2006;24:2836-2841. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16782922.

167. Carpenter JS. Hot flashes and their management in breast cancer. Semin Oncol Nurs 2000;16:214-225. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10967794.

168. Fallowfield LJ, Bliss JM, Porter LS, et al. Quality of life in the intergroup exemestane study: a randomized trial of exemestane versus continued tamoxifen after 2 to 3 years of tamoxifen in postmenopausal women with primary breast cancer. J Clin Oncol 2006;24:910-917. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16484701.

169. Mahoney MC. Breast cancer risk reduction and counseling: lifestyle, chemoprevention, and surgery. J Natl Compr Canc Netw 2007;5:702-710. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17927927.

170. Visvanathan K, Chlebowski RT, Hurley P, et al. American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. J Clin Oncol 2009;27:3235-3258. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19470930.

171. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 2013;381:805-816. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23219286.

172. Gray RG, Rea D, Handley K, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. ASCO Meeting Abstracts 2013;31:5. Available at: http://meeting.ascopubs.org/cgi/content/abstract/31/18_suppl/5.

173. Burstein HJ, Temin S, Anderson H, et al. Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Focused Update. J Clin Oncol 2014. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24868023.

174. Bresser PJC, Van Gool AR, Seynaeve C, et al. Who is prone to high levels of distress after prophylactic mastectomy and/or salpingo-ovariectomy? Ann Oncol 2007;18:1641-1645. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17660493.

175. Patenaude AF, Orozco S, Li X, et al. Support needs and acceptability of psychological and peer consultation: attitudes of 108 women who had undergone or were considering prophylactic mastectomy. Psychooncology 2008;17:831-843. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18636423.

176. van Dijk S, van Roosmalen MS, Otten W, Stalmeier PF. Decision making regarding prophylactic mastectomy: stability of preferences and the impact of anticipated feelings of regret. J Clin Oncol 2008;26:2358-2363. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18467728.

177. Giuliano AE, Boolbol S, Degnim A, et al. Society of Surgical Oncology: position statement on prophylactic mastectomy. Approved by the Society of Surgical Oncology Executive Council, March 2007. Ann

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Surg Oncol 2007;14:2425-2427. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17597344.

178. Morrow M, Mehrara B. Prophylactic mastectomy and the timing of breast reconstruction. Br J Surg 2009;96:1-2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19109821.

179. Rebbeck TR, Friebel T, Wagner T, et al. Effect of short-term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 2005;23:7804-7810. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16219936.

180. Chlebowski RT, Prentice RL. Menopausal hormone therapy in BRCA1 mutation carriers: uncertainty and caution. J Natl Cancer Inst 2008;100:1341-1343. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18812547.

181. Garber JE, Hartman A-R. Prophylactic oophorectomy and hormone replacement therapy: protection at what price? J Clin Oncol 2004;22:978-980. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14981100.

182. Grann VR, Jacobson JS, Thomason D, et al. Effect of prevention strategies on survival and quality-adjusted survival of women with BRCA1/2 mutations: an updated decision analysis. J Clin Oncol 2002;20:2520-2529. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12011131.

183. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321-333. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12117397.

184. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701-1712. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15082697.

185. McTiernan A, Martin CF, Peck JD, et al. Estrogen-plus-progestin use and mammographic density in postmenopausal women: Women's Health Initiative randomized trial. J Natl Cancer Inst 2005;97:1366-1376. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16174858.

186. Chlebowski RT, Anderson GL, Gass M, et al. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. JAMA 2010;304:1684-1692. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20959578.

187. Chlebowski RT, Kuller LH, Prentice RL, et al. Breast cancer after use of estrogen plus progestin in postmenopausal women. N Engl J Med 2009;360:573-587. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19196674.

188. LaCroix AZ, Chlebowski RT, Manson JE, et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. JAMA 2011;305:1305-1314. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21467283.

189. Stefanick ML, Anderson GL, Margolis KL, et al. Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. JAMA 2006;295:1647-1657. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16609086.

190. Rohan TE, Negassa A, Chlebowski RT, et al. Conjugated equine estrogen and risk of benign proliferative breast disease: a randomized controlled trial. J Natl Cancer Inst 2008;100:563-571. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18398105.

191. McTiernan A, Chlebowski RT, Martin C, et al. Conjugated equine estrogen influence on mammographic density in postmenopausal women in a substudy of the women's health initiative randomized trial. J

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Clin Oncol 2009;27:6135-6143. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19901118.

192. Rosenberg L, Palmer JR, Wise LA, Adams-Campbell LL. A prospective study of female hormone use and breast cancer among black women. Arch Intern Med 2006;166:760-765. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16606813.

193. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 2003;362:419-427. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12927427.

194. Chen WY, Manson JE, Hankinson SE, et al. Unopposed estrogen therapy and the risk of invasive breast cancer. Arch Intern Med 2006;166:1027-1032. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16682578.

195. Chlebowski RT. Menopausal hormone therapy, hormone receptor status, and lung cancer in women. Semin Oncol 2009;36:566-571. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19995648.

196. Santen RJ, Allred DC. The estrogen paradox. Nat Clin Pract Endocrinol Metab 2007;3:496-497. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17519914.

197. Rossouw JE, Prentice RL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 2007;297:1465-1477. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17405972.

198. Fournier A, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F. Estrogen-progestagen menopausal hormone therapy and breast cancer: does delay from menopause onset to treatment initiation influence risks? J Clin Oncol 2009;27:5138-5143. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19752341.

199. Suzuki R, Ye W, Rylander-Rudqvist T, et al. Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study. J Natl Cancer Inst 2005;97:1601-1608. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16264180.

200. Bernstein L, Patel AV, Ursin G, et al. Lifetime recreational exercise activity and breast cancer risk among black women and white women. J Natl Cancer Inst 2005;97:1671-1679. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16288120.

201. Howard RA, Leitzmann MF, Linet MS, Freedman DM. Physical activity and breast cancer risk among pre- and postmenopausal women in the U.S. Radiologic Technologists cohort. Cancer Causes Control 2009;20:323-333. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18941914.

202. Lahmann PH, Friedenreich C, Schuit AJ, et al. Physical activity and breast cancer risk: the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 2007;16:36-42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17179488.

203. Tehard B, Friedenreich CM, Oppert J-M, Clavel-Chapelon F. Effect of physical activity on women at increased risk of breast cancer: results from the E3N cohort study. Cancer Epidemiol Biomarkers Prev 2006;15:57-64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16434587.

204. Friedenreich CM, Woolcott CG, McTiernan A, et al. Alberta physical activity and breast cancer prevention trial: sex hormone changes in a year-long exercise intervention among postmenopausal women. J Clin Oncol 2010;28:1458-1466. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20159820.

205. Chlebowski RT, Chen Z, Cauley JA, et al. Oral bisphosphonate use and breast cancer incidence in postmenopausal women. J Clin Oncol 2010;28:3582-3590. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20567009.

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206. Prentice RL, Manson JE, Langer RD, et al. Benefits and risks of postmenopausal hormone therapy when it is initiated soon after menopause. Am J Epidemiol 2009;170:12-23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19468079.

207. Michels KB, Willett WC. The Women's Health Initiative Randomized Controlled Dietary Modification Trial: a post-mortem. Breast Cancer Res Treat 2009;114:1-6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18373274.

208. Linos E, Willett WC. Diet and breast cancer risk reduction. J Natl Compr Canc Netw 2007;5:711-718. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17927928.

209. Tretli S, Gaard M. Lifestyle changes during adolescence and risk of breast cancer: an ecologic study of the effect of World War II in Norway. Cancer Causes Control 1996;7:507-512. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8877047.

210. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288:2569-2578. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12444864.

211. Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1991-1997. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16103450.

212. Blackmore KM, Lesosky M, Barnett H, et al. Vitamin D from dietary intake and sunlight exposure and the risk of hormone-receptor-defined breast cancer. Am J Epidemiol 2008;168:915-924. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18756015.

213. Knight JA, Lesosky M, Barnett H, et al. Vitamin D and reduced risk of breast cancer: a population-based case-control study. Cancer Epidemiol Biomarkers Prev 2007;16:422-429. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17372236.

214. Kotsopoulos J, Olopado OI, Ghadirian P, et al. Changes in body weight and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 2005;7:R833-843. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16168130.

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CDT Code	Code description	Proposed Placement	Comments
D0171	re-evaluation – post-operative office visit	**Excluded**	Service should be bundled into the original treatment code
D0351	3D PHOTOGRAPHIC IMAGE-This procedure is for dental or maxillofacial diagnostic purposes. Not applicable for a CAD-CAM procedure	**Excluded**	Experimental
D1353	SEALANT REPAIR-PER TOOTH	**57** PREVENTIVE DENTAL SERVICES	D1351 Sealant is on line 57. Rule limits sealants to every 5 yrs unless they fail, then sealant can be redone. Monitor use to ensure not abused.
D6110	implant /abutment supported removable denture for edentulous arch – maxillary	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6111	implant /abutment supported removable denture for edentulous arch – mandibular	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6112	implant /abutment supported removable denture for partially edentulous arch – maxillary	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6113	implant /abutment supported removable denture for partially edentulous arch – mandibular	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6114	implant /abutment supported fixed denture for edentulous arch – maxillary	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6115	implant /abutment supported fixed denture for edentulous arch – mandibular	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6116	implant /abutment supported fixed denture for partially edentulous arch – maxillary	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6117	implant /abutment supported fixed denture for partially edentulous arch – mandibular	**627** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: IMPLANTS	
D6549	RESIN RETAINER--FOR RESIN BONDED FIXED PROSTHESIS	**609** DENTAL CONDITIONS (EG. MISSING TEETH) Treatment: COMPLEX PROSTHODONTICS	
D9931	Cleaning and inspection of a removable appliance. This procedure does not include any required adjustments	**457** DENTAL CONDITIONS (EG. MISSING TEETH, PROSTHESIS FAILURE) Treatment: REMOVABLE PROSTHODONTICS	Includes ultrasonic cleaning of dentures. DMAP to create rule to limit frequency to once per year and can not be billed with a prophylactic cleaning.
D9936	missed appointment	**Excluded**	Specifically excluded in OAR from being billed
D9987	Cancelled appointment	**Excluded**	Specifically excluded in OAR from being billed
D9219	evaluation for deep sedation or general anesthesia	**Exempt**	Anesthesia codes are all Exempt

Dentures Guideline and Rule Conflict

Question: What should be done about a conflict between the removable prosthodontics guideline and rule?

Question source: Katie Noah, Willamette Dental; DMAP

Issue:
Katie Noah noticed a discrepancy between DMAP rules and Guideline Note 62. Under the Dental Services Program Rulebook, 410-123-1260, the rule shows that under 21 is 4 missing posterior teeth but over 21 is 6 missing posterior teeth for the benefit.
http://www.dhs.state.or.us/policy/healthplan/guides/dental/123rb010112.pdf

However, Guideline note 62 states that **4** or more posterior missing teeth per arch are required, not **6**.

On 1/1/2012, DMAP changed the coverage rules so adults age 21+ could only receive coverage of a partial dentures if they are missing 6 or more posterior or one or more anterior teeth. This was a benefit reduction and made coverage different for adults versus children. The guideline did not change at the same time. The rationale for this was:
"Limits on some dental services for adult clients (age 21 and older) due to reduced payments to managed Dental Care Organizations (DCOs) to comply with budget limitations required by the 2011 Legislative Assembly in SB 5529. This will help assure access to services through sufficient provider networks will be maintained following this approximate 11% reduction in capitation rates."

Prioritized List Status:

Line:	**477**
Condition:	DENTAL CONDITIONS (EG. MISSING TEETH, PROSTHESIS FAILURE) (See Guideline Note 62)
Treatment:	REMOVABLE PROSTHODONTICS (E.G. FULL AND PARTIAL DENTURES, RELINES)
HCPCS:	D5110-D5212,D5520-D5761,D5820,D5821

GUIDELINE NOTE 62, REMOVEABLE PROSTHODONTICS
Line 477
Must have one or more anterior teeth missing or four or more posterior teeth missing per arch with resulting space equivalent to that loss demonstrating inability to masticate; third molars are not a consideration when counting missing teeth (D5211, D5212).

Expert input:
OHAP reviewed this guideline and felt that deletion of the guideline would provide the most clarity to the dental community.

Dentures, Issue #719

Dentures Guideline and Rule Conflict

HERC staff recommendation:

1) Delete guideline note 62 REMOVEABLE PROSTHODONTICS

Dentures, Issue #719