AGENDA

Cannabis Research Task Force

Friday, January 8, 2016
3:00 p.m. –6:00 p.m.
Portland State Office Building
800 NE Oregon St., Room 1A
Portland, OR 97232

Senate Bill 844, passed in 2015, created a task force to research the medical and public health properties of Cannabis. The task force consists of 15 members, appointed by the Governor. It is charged with studying and publishing a report on the development of a medical Cannabis industry that provides patients with medical products that meet individual patient needs. The report is due to the Legislative Assembly by February 1, 2016.

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1. Welcome and Introductions	3:00-3:15	Katrina Hedberg
2. Task Force deliverables	3:15-3:30	Mowgli Holmes
3. Summary of Background on Federal Laws and Institutional Review Boards	3:30-4:15	Karen Volmar
4. Break	4:15-4:30	
5. Panel Discussion on Legal Barriers to Cannabis Research	4:30-5:15	
6. Group discussion	5:15-5:30	Mowgli Holmes
7. Next Steps	5:30-5:45	Rosa Klein
8. Public Comment	5:45-6:00	Rosa Klein

Final Task Force Meeting:

Tuesday, Jan 26, 2015 4:00 PM- 7:00 PM

115 Hallie Ford Center Bldg.
Corvallis, Oregon

Oregon State Cannabis Research Task Force

Legal and Reglatory Landscape

By Mowgli Holmes

Oregon State Cannabis Research Task Force
(Background and questions for session 2)

Legal cannabis is an extremely unusual hybrid type of industry. It is an agricultural crop capable of producing dozens of different products, including fiber, oilseed, recreational products with a vast market size, and a diverse array of pharmaceutically active chemical compounds.

The regulatory challenge around it is complex, both because it is represents a disconnect between state and federal laws, and because it does not fit neatly into any of the existing regulatory models that might apply. cannabis is not like other agricultural crops, it is not like alcohol, and it is not like other pharmaceutical products.

An additional reason that why the regulatory challenges around cannabis are so complex is because we lack the basic body of research that underpins all of these other industries. Other agricultural and pharmaceutical industries today are highly modernized, because of years of research and development conducted both by public institutions and by companies. Most importantly, a huge federally-funded research infrastructure supports the basic science that agriculture and medicine are dependent on, as well as public health and safety research that places critical limits on industry.

Because of federal restrictions on cannabis research, this massive new industry is operating without any of this basic science foundation that other industries take for granted. We don't know the most basic information that we know for other plants, other medicines, or even other alternative therapies. (One informative example: a PubMed search for the term "medical marijuana" in article titles returns 443 publications, while searches for the terms "ginseng" in titles returns over 3000 publications.)

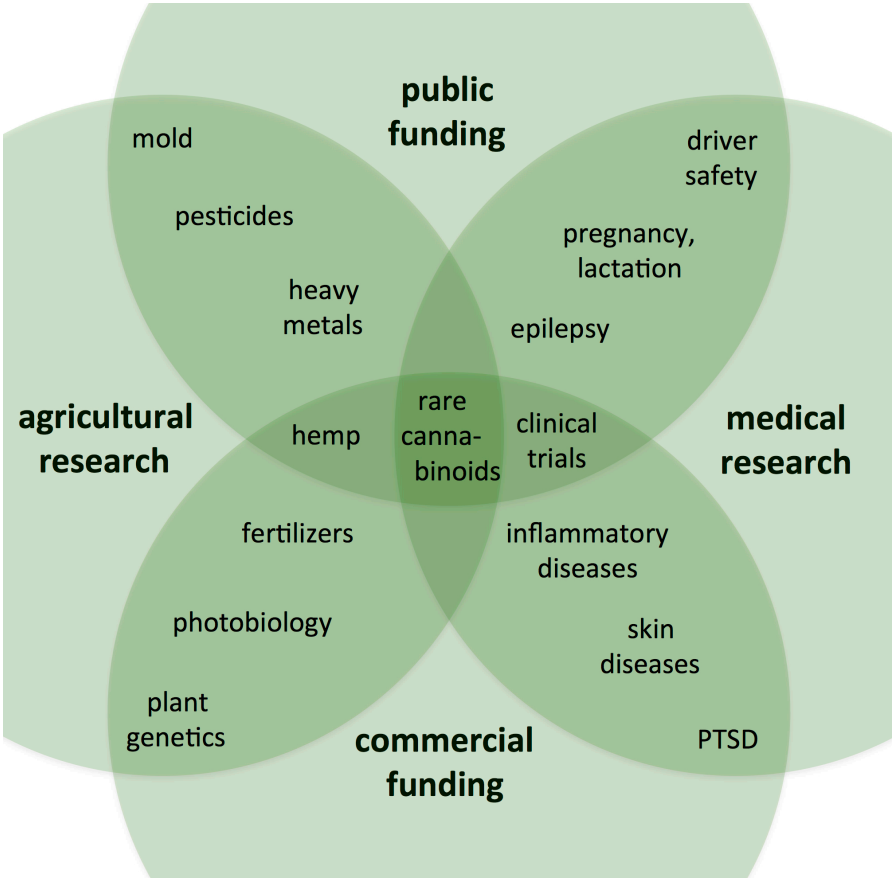
Federal restrictions are beginning to loosen somewhat, but it will still take a great deal of creativity to enable meaningful research on this subject. It was the clear will of the Oregon Legislature to create a strong and science-driven cannabis industry. This Task Force is in the position to make it possible for Oregon to lead the country in what will shortly be a huge wave of critical new cannabis research.

The diagram on the next page is an overview of the complex ways that different categories of research intersect with respect to cannabis. It is broken down into four overlapping categories: public, private, agricultural, and medical. There is broad understanding of the deficiencies of existing medical research on cannabis. It is less commonly understood how important, and how lacking, is agricultural research on cannabis. This new industry cannot survive without extensive investment in both types of research. Medical cannabis

products won't serve the industry or the needs of patients without high-quality medical and biochemical research data. Likewise, no industry will survive to produce these products without rigorous agricultural and public health research.

Other agricultural industries have access to both state and federal agriculture departments, and farmers have constant educational and R&D support from land-grant universities such as OSU. Oregon needs to either enable this support or find other ways to provide it. The clearest example of the need for this kind of industrial research is the pesticide issue. Other agricultural crops are very tightly regulated with respect to pesticide use, and they have access to vast educational and training resources about toxicity, application, and sustainable practices. The cannabis industry is right now highly dependent on pesticides, and without such research and education it will not be able to transition to safer practices.

This diagram is a very incomplete schematic, but it indicates what the Oregon Legislature understood when it passed SB 844 and HB 3400 this year—that there is a need for both public and commercial research to support this industry. HB 3400 created a commercial research license to enable companies to carry out such research. SB 844 created a Task Force in order to figure out how to accomplish the types of research that will most likely not be undertaken by private companies.



The goal of this task force should be to determine how to enable the research that the State of Oregon needs for its cannabis patients, users, and the industry itself, and that is currently not available. It should be the kind of research that is capable of encouraging doctors to change their prescribing habits and states to change their laws.

There are a limited number of settings in which any research at all can take place. I believe this is an exhaustive list of the current possibilities: Universities, commercial companies, non-profit entities, or state agencies. I'd like to argue that none of these are adequate. Universities will not do any work outside of the existing federal restrictions. Commercial companies will not invest adequately in public health research. Non-profits generally don't have access to adequate funding or skilled researchers. State agencies have limited resources, narrow charters, and face many political obstacles.

I'd also like to suggest that one reason the current wave of state-level legalization has not yet resulted in a burst of new cannabis research is that state governments have not been able to envision how to perform research outside of the federal system. Four states have now chosen to violate federal law in order to allow their citizens to use cannabis. As a result they have had to take on a host of regulatory issues that are normally handled by federal agencies. State agencies don't have the experience, resources, or—importantly—the access to research, that federal agencies have. These states have taken on the challenge of building regulatory structures normally handled at the federal level, even when doing so is at odds with federal law. But they have not been willing to take on the responsibility of generating the necessary research that is normally handled at the federal level—even though that research is precisely what allows federal agencies to effectively regulate agricultural and medical products.

So when Colorado and California decided to promote cannabis research, they did so entirely within the limits of the federal research system. They provided some funding, but they did not enable any types of research that are not already possible. Therefore, these programs have not expanded our body of knowledge about cannabis as much as they could have. Research within the existing federal guidelines is necessary, and is becoming more feasible. But it is extremely slow, extremely limited, and there are many types of research that are simply not even possible within those structures.

Oregon could take effective steps to enable all of these types of research. Certainly the state should provide funding that would allow federally-approved research to move more quickly. But more importantly, it could empower or create state institutions to engage in research that is not currently possible in any way, such as medical and agricultural research using Oregon-grown cannabis.

Questions for Session Two

Before the next meeting, it would be useful if each member of the Task Force could prepare responses to the following two questions:

1. What sort of new organizational or institutional structures could enable the kind of cannabis research that has until now not been possible?

Please feel free to engage in wild speculation.

2. What are some of the pressing research questions relating to cannabis that we do not currently have answers to? Why don't we have them, and what could be done to facilitate the work necessary to generate them?

Two very different examples of this type of question are on the following page.

Are cannabinoids a useful therapy for melanoma?

Many anecdotal reports exist. Several in-vitro and mouse studies have shown positive results, and theese types of studies are critical groundwork for medical questions. They can be done effectively in university settings, although clearly there are political and funding issues. Human studies have not been done. The assumption has been that they cannot be done without multiple federal approvals and IRB approval.

Potential solutions:

* More funding and institutional support for federally approved studies of all kinds.
* Observational studies have lower quality data, but could be done by coordinating data collection with medical institutions. Would such a study have to be based in a university setting?
* Could human subjects studies be done in a state-run institution outside of the federal university system if it contained its own IRB?
* Could human subjects studies be done in a state-run institution, without federal approvals, if the data generated was not intended to result in an FDA-approved drug?

Does cannabis concentrate heavy metals in its flowers and leaves?

Many plants take up and concentrate toxic metal ions, and this is a particular threat in agricultural regions where there was historical use of arsenic-based pesticides. There is currently very limited and contradictory information on the ability of cannabis to concentrate metals. Universities *will not* handle cannabis plants, and cannot generate this data.

Potential solutions:

* A surveillance study could be done that samples plants and soil from around the state and analyzes both for heavy metals. Any commercial or state entity could do this work, but would need structure and funding.
* A laboratory study with live plants on known soil environments could quantitatively analyze metal uptake in cannabis plants over time. Any commercial or state entity could do this work, but would need structure and funding.

State and Federal Cannabis Research Programs

Presented by: Karen Volmar JD MPH

January 8, 2016

International and Federal Laws

- International Convention on Narcotic Drugs
  - Requires federal agency control of access to narcotic drugs. Cannabis is explicitly included.
- Controlled Substances Act
  - Designates cannabis and hemp as Schedule I “high potential for abuse” and “no known medical benefit” and requires DEA to approve all research with Schedule I substances.
  - Requires registration of manufacturers, distributors of all scheduled substances
  - Separate researcher registration required for Schedule I
- Drug Free Schools and Communities Act
  - Requires universities to follow federal drug laws or risk loss of federal funding.

The Current Enforcement Environment

- Cole Memorandum
  - Articulates Department of Justice enforcement priorities. The department will not prosecute where states have effective regulatory schemes.

- 2016 Appropriations Bill
  - Prohibits the Department of Justice from using federal fund to prosecute any cannabis legal as part of a state medical marijuana program.

- These articulations of enforcement activities do not alter the underlying laws. Cannabis manufacturing, possession, distribution not otherwise authorized by federal law is still illegal unless approved by the DEA.

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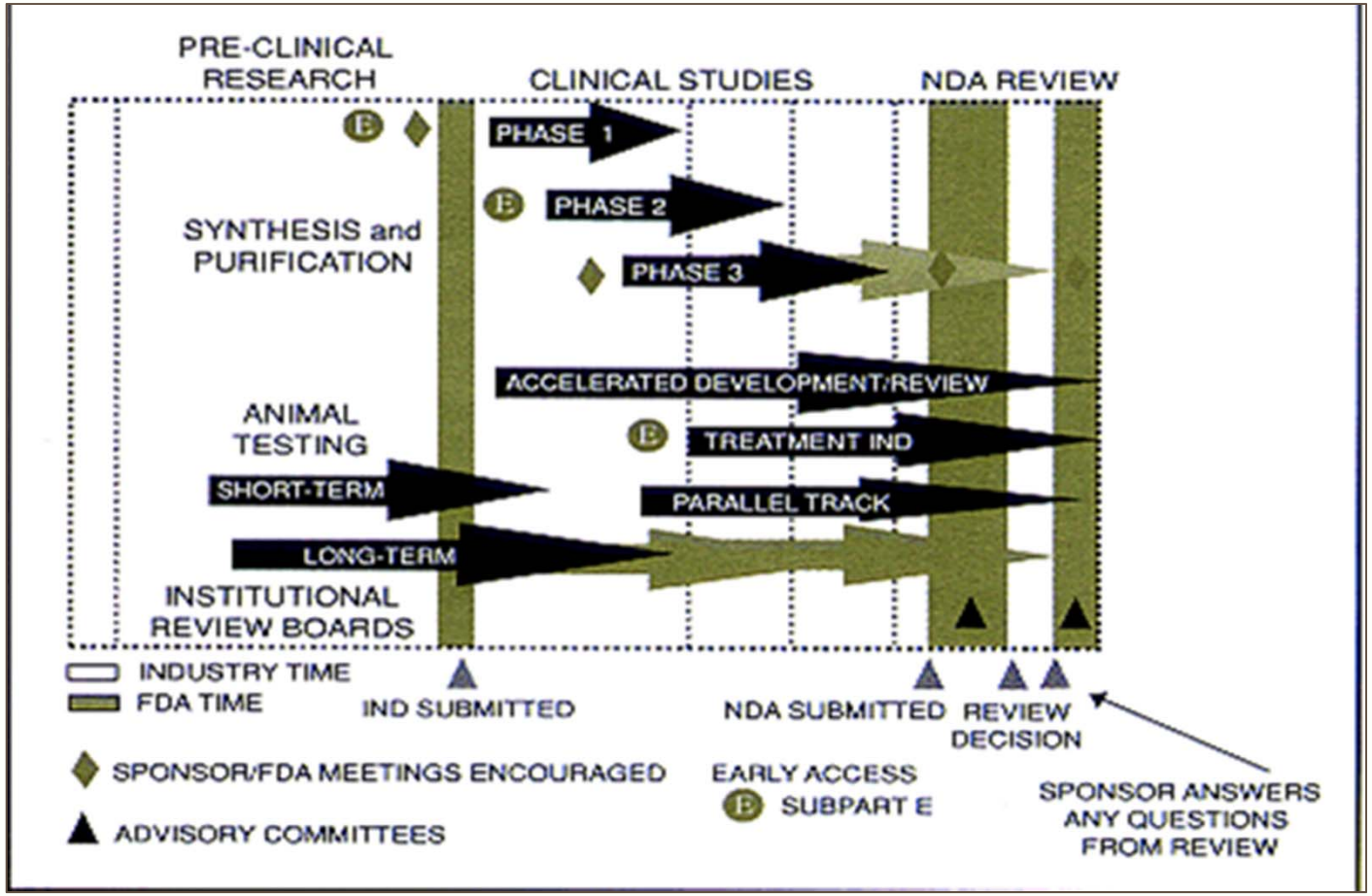
Drug Approval

- Food Drug and Cosmetics Act
  - Requires FDA review of new drugs, including safety, efficacy, and manufacturing quality.
- Protection of Human Subjects (The Common Rule)/FDA Institutional Review Board Rules
  - Requires that all federally funded or regulated research involving human subjects comply with federal human subjects protections.

FDA Drug Approval Process



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The FDA Approval Process

- The FDA distinguishes synthetic (or highly purified) drugs and botanical drugs.
- For botanical drugs the FDA "will rely on a combination of tests to ensure *identity, quality, strength, potency and consistency.*
- The amount of information necessary for an IND depends on
  - the novelty of the drug,
  - the extent to which it has been studied previously,
  - the drug product's known or suspected risks, and
  - the developmental phase of the drug

The Research Environment - Clinical Research

- Approval Process for cannabis research
  - Food and Drug Administration – Investigational New Drug Application
    - Institutional Review Boards. Local review of research protocols to insure compliance with federal human subjects research requirements. Required for FDA submission.
  - Drug Enforcement Agency – registration to handle, conduct research using Schedule I substances
  - National Institute on Drug Abuse – application for cannabis
    - Only federally authorized supply of research grade cannabis. Priority given to federally funded research (mostly relating to negative effects).

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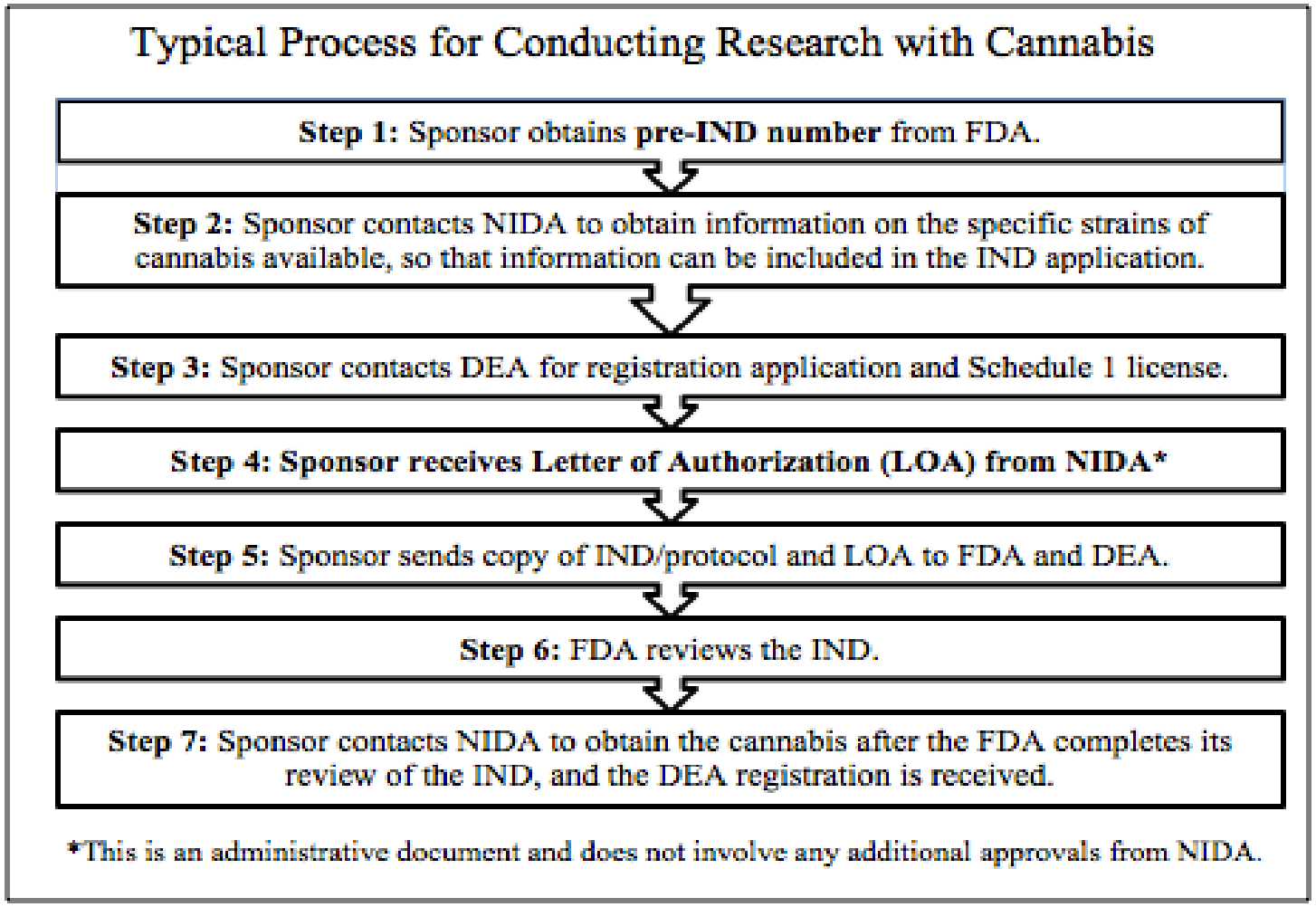

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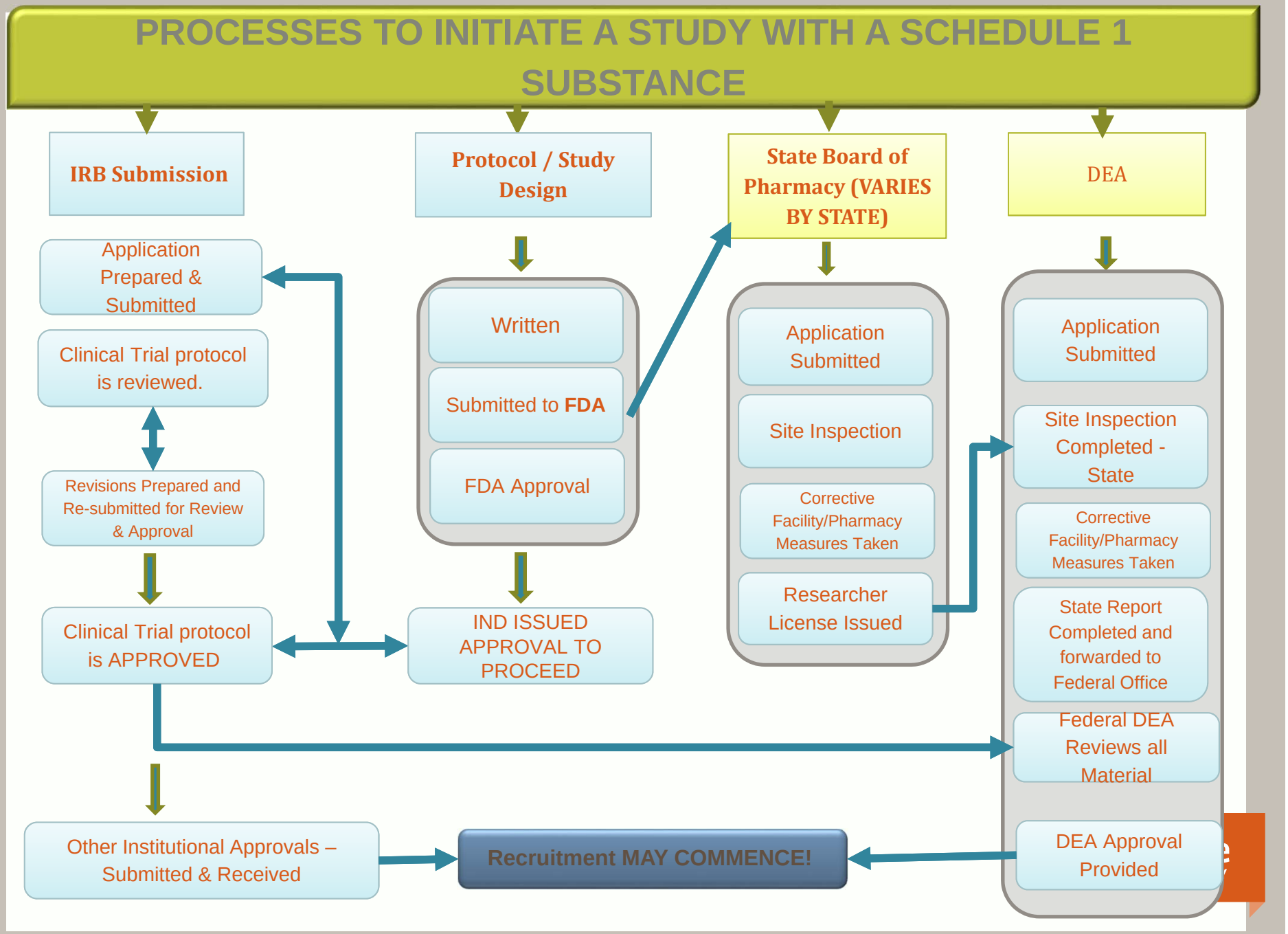
PROCESSES TO INITIATE A STUDY WITH A SCHEDULE 1 SUBSTANCE
IRB Submission
Protocol / Study Design
State Board of Pharmacy (VARIES BY STATE)
DEA
Application Prepared & Submitted
Clinical Trial protocol is reviewed.
Revisions Prepared and Re-submitted for Review & Approval
Clinical Trial protocol is APPROVED
Written
Submitted to FDA
FDA Approval
IND ISSUED APPROVAL TO PROCEED
Application Submitted
Site Inspection
Corrective Facility/Pharmacy Measures Taken
Researcher License Issued
Application Submitted
Site Inspection Completed - State
Corrective Facility/Pharmacy Measures Taken
State Report Completed and forwarded to Federal Office
Federal DEA Reviews all Material
DEA Approval Provided
Other Institutional Approvals – Submitted & Received
Recruitment MAY COMMENCE!

Cannabis Research Supply Through NIDA

Category	THC	CBD
Placebo marijuana (produced by solvent extraction)	THC (0%) /	CBD (0%)
Low THC varieties	Low THC (<1%)	Medium CBD (1-5%)
	Low THC (<1%)	High CBD (5-10%)
	Low THC (<1%)	Very High CBD (>10%)
Medium THC varieties	Medium THC (1-5%)	Low CBD (<1%)
	Medium THC (1-5%)	Medium CBD (1-5%)
	Medium THC (1-5%)	High CBD (5-10%)
	Medium THC (1-5%)	Very High CBD (>10%)
High THC varieties	High THC (5-10%)	Low CBD (<1%)
	High THC (5-10%)	High CBD (5-10%)
	High THC (5-10%)	Very High CBD (>10%)
Very high THC varieties	Very High THC (>10%)	Low CBD (<1%)

Institutional Review Boards

- National Research Act of 1974/Belmont Report
  - Outlined primary ethical principles involved in human subjects research
- IRBs established by federal regulation (both FDA and DHHS) to review, monitor, and approve research involving human subjects.
- IRBs primary purpose is to protect the rights and welfare of human participating as subjects in research by insuring
  - Risk to subjects are minimized
  - Risks to subjects are reasonable in relation to anticipated benefits
  - All participants provide informed consent
  - Additional requirements for vulnerable populations met

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IRBs continued

- Institutional review boards operate under “assurance” (registration) or FDA Bioresearch Monitoring Program.
- When reviewing research involving FDA regulated products, IRBs must comply with both FDA and HHS regulations.
- Generally, outside IRBs can be used to review studies, however, independent IRBs generally cannot be used to review a study conducted in an institution with its own in-house IRB.
- Independent researchers (physicians) commonly submit proposal to local community hospitals, university independent IRBs, or state or local government health agency for review.

Evolving agency positions

- Summer 2016 Testimony Caucus on International Narcotics Control
  - http://www.drugcaucus.senate.gov/content/drug-caucus-hearing-barriers-cannabidiol-research-0

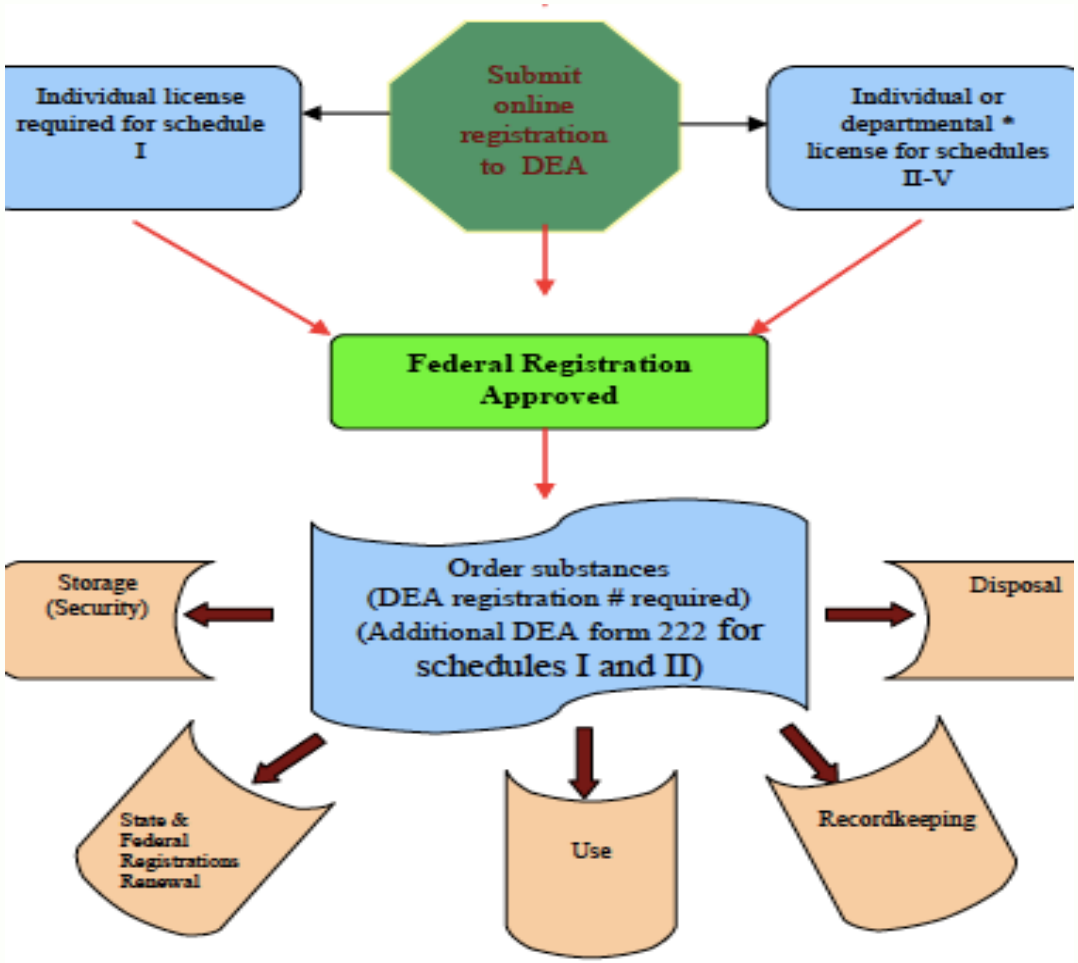
  - DOJ Rannazzisi Testimony 49:00
  - FDA: Throckmorton Testimony 54:20
  - NIDA: Volkow Testimony 59:00

Observational research

- Observational research- where cannabis is obtained and administered by the research participants rather than the researcher
  - Not required to use NIDA cannabis supply
  - Non federally funded/regulated studies may not require IRB review
    - all organizations receiving federal funding would require IRB review
    - Common Rule currently under revision
  - Observational research alone would not lead to FDA approval

The Research Environment – Plant research

- DEA registration still required - reviews
  - qualifications and competency of each practitioner requesting registration
  - merits of the research protocol.



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Hemp Research Exception

- Universities and states may now grow or cultivate industrial hemp if--
- (1) the industrial hemp is grown or cultivated for purposes of research conducted under an agricultural pilot program or other agricultural or academic research; and
- (2) the growing or cultivating of industrial hemp is allowed under the laws of the State in which such institution of higher education or State department of agriculture is located and such research occurs.

References

1. International Convention on Narcotic Drugs.
2. Controlled Substances Act 21 U.S.C. § 823(f).
3. Safe and Drug Free Schools and Communities Act of 1986 89-10 title IV § 4001.
4. *"Human Subjects Research (45 CFR 46)"*. *Office for Human Research Protections*.
5. Memorandum from James M. Cole, Deputy Attorney Gen. to U.S. Att'ys (October 19, 2009). http:/www.justice.gov/sites/default/files/opa/legacy/2009/10/19/medical-marijuana.pdf.
6. Food and Drug Administration. Marijuana research with human subjects. Retrieved from www.fda.gov/NewsEvents/publichealthfocus/ucm421173.thm. Accessed October 30, 2015.
7. Agriculture Act of 2014 Pub. L. 113-79 (2014).
8. National Institute on Drug Abuse NIDA Drug Supply Program. Retrieved from http://www.drugabuse.gov/researchers/research-resources/nida-drug-supply-program Accessed November 24, 2015.
9. Cannabidiol: Barriers to Research and Potential Medical Benefits: Caucus on International Narcotics Control Senate, 114th Cong. (2015) (Testimony of Nora Volkow, Director NIDA).
10. Cannabidiol: Barriers to Research and Potential Medical Benefits: Caucus on International Narcotics Control Senate, 114th Cong. (2015) (Testimony of Joseph T. Rannazzisi, Deputy Assistant Administrator).
11. Cannabidiol: Barriers to Research and Potential Medical Benefits statement before the Caucus on International Narcotics Control, Senate, 114th Cong. (2015) (Testimony of Douglas Throckmorton).
12. Food and Drug Administration. Institutional Review Boards Frequently Asked Questions Information Sheet. Retrieved from http://www.fda.gov/RegulatoryInformation/Guidances/ucm126420.htm .

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Thank you

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Task Force Researching the Medical and Public Health Properties of Cannabis

Report to the Legislature

DRAFT 12-31-2015

NOT FOR CIRCULATION

Oregon Health Authority

Prepared for OHA by Candice Beathard, PhD and

Karen Volmar, JD MPH

Oregon State University

III. Legal Requirements for Cannabis Research

As indicated by the summary of research above, human subjects research on the medicinal properties of cannabis is permitted in the United States. However, it is strictly controlled by federal agencies. As a signatory to the Convention on Narcotic Drugs, the U.S. is required to designate a federal level agency to control production and distribution of cannabis and other narcotic drugs. The U.S. Drug Enforcement Agency (DEA) is the responsible agency for the U.S. and as part of its responsibility, categorizes drugs by their relative level of danger. The DEA continues to place cannabis on Schedule I, which includes drugs with "high abuse potential" and "no accepted medical use"[1]. With that designation, production, transportation, or possession of cannabis not explicitly approved by the DEA is illegal under federal law. With respect to research utilizing Schedule I substances, the Controlled Substance Act also includes explicit requirements specific to research. Researchers wishing to conduct research using a Schedule I substance must first register with the DEA, which determines the qualifications and competency of the researchers as well as the merits of the research protocol[2]. The U.S. Department of Justice (DOJ), however, currently is not prosecuting production, dissemination, or possession of cannabis that has been legalized for medical or recreational use under state laws. In 2009 and 2013, the DOJ issued a series of memoranda that indicated it would defer to state and local enforcement of medicinal cannabis as long as the states implemented "strong and effective regulatory and enforcement systems."[3] The Memorandum identify the agencies enforcement concerns as focusing on eight areas:

1. Preventing the distribution of marijuana to minors;
2. Preventing revenue from the sale of marijuana from going to criminal enterprises, gangs, and cartels;
3. Preventing the diversion of marijuana from states where it is legal under state law in some form to other states;
4. Preventing state-authorized marijuana activity from being used as a cover or pretext for the trafficking or other illegal drugs or other illegal activity;
5. Preventing violence and the use of firearms in the cultivation and distribution of marijuana;
6. Preventing drugged driving and the exacerbation of other adverse public health consequences associated with marijuana use;

7. Preventing growing of marijuana on public lands and the attendant public safety and environmental dangers posed by marijuana production on public lands; and
8. Preventing marijuana possession or use on federal property[3].

These memoranda articulate enforcement priorities and how the DOJ's prosecutorial discretion will be utilized and have provided adequate assurances for some states to enable both medical and recreational marijuana use. In December 2015, Congress also approved language in the 2016 Consolidated Appropriations Act that prohibits the Department of Justice from using its federal funds to interfere with state medical cannabis programs[4]. However, the memoranda and instructions for 2016 federal monies do not change or nullify existing federal laws relating to cannabis. Cannabis remains illegal for any non-federally approved use at the federal level. Furthermore, entities that receive significant federal funding, such as universities and hospitals (which receive monies from NIH or the Medicare Program), agree as a condition to that funding, to comply with federal laws. The Drug Free Schools and Communities Act also places explicit requirements on institutions of higher education to follow federal drug policy laws.[5] With respect to cannabis, using a non-federal government sanctioned cannabis supply remains illegal under federal law and could therefore jeopardize federal funding.

Clinical Research: Cannabis Research Using Human Subjects

Food and Drug Administration New Drug Approval Process

In addition to the control of certain substances through the Controlled Substances Act, the Federal Food, Drug, and Cosmetic Act (FDCA) and National Research Act govern the conduct of drug research involving human subjects and controlled substances. Those laws contain provisions that allow for clinical (human subjects) research with investigational drugs, including Schedule I substances, provided certain steps are taken to protect the rights, safety, and welfare of human subjects. The FDA is responsible for reviewing the safety and efficacy of drug products. The agency requires drug manufactures to submit to a multi-step approval process designed to demonstrate that the drug is safe and effective for its intended clinical use (prescription or over the counter). The process includes both pre-clinical (investigational new drug – IND) and clinical (New drug application – NDA) reviews of the product. The agency is also responsible for ensuring that drug products are manufactured according to good manufacturing processes. The standards of evidence differ somewhat for synthetic or highly

purified drugs compared to botanical drug products. The FDA has indicated it would review cannabis under its botanicals process though highly purified or synthetic components (such as CBD) would be subject to the agencies standard drug approval process. These botanical drug specific guidelines recognize the complex nature of botanicals and require researchers to submit documentation of the identity, quality, strength, potency, and consistency of the botanical (rather than an identification of the active ingredients as required for synthetic or highly purified drugs). (See Appendix A.)

Institutional Review Boards Requirements

Most research involving human subjects must be approved by federally regulated Institutional Review Boards (IRBs) charged with insuring that human subjects are not subject to unreasonable risk as a result of their participation in trials. Federal IRB requirements apply to "all research involving human subjects conducted, supported or otherwise subject to regulation by any federal department or agency."[6] This includes all research funded by the federal government and research intended to support applications for research or marketing of products regulated by FDA. IRBs are registered with the federal government and those reviewing research involving potential drug products operate under Department of Health and Human Services regulations as well as FDA IRB regulations.

In practice, IRB reviews of research protocols involving cannabis and particularly studies involving children are rigorous. As long as cannabis is characterized as a Schedule I drug at the federal level, IRBs should treat it as such and the risks of administration of a drug with high addictive potential must be adequately mitigated in the study design/protocols. Furthermore, the protocols should insure secure storage, handling, and disposal of cannabis products. The existing federally supported trials have developed protocols that adequately insure both the safe storage and transfer as well as safe and proper administration of cannabis in studies, however many researchers have described the process of obtaining IRB approval for studies as lengthy and arduous.

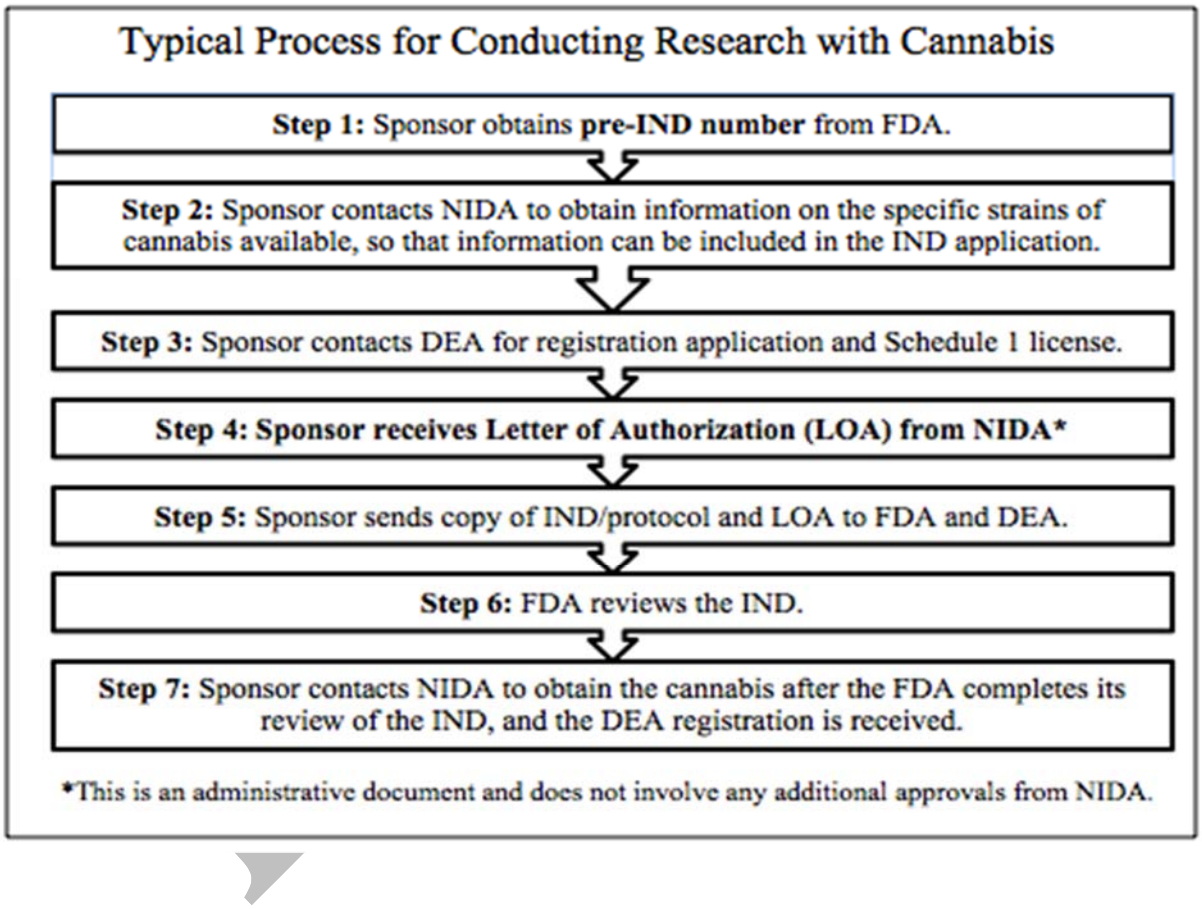
Procuring Cannabis For Clinical Research

Currently, cannabis and its components may be utilized in research if the research has been approved by three federal agencies.[7] Individuals wanting to conduct clinical research with cannabis must:

1) apply to the U.S. Food and Drug Administration (FDA) for investigational new drug application approval (FDA applications also require that research protocols be approved by Institutional Review Boards)
2) obtain a DEA certificate to handle and conduct research on Schedule I substances, and
3) apply to the National Institute on Drug Abuse (NIDA) to receive the federally approved research grade cannabis[8] (See Figure 1 below).

In practice, the enforcement of these requirements has created barriers and delays for researchers.

Figure 1: FDA, DEA, and NIDA Process for Conducting Research With Cannabis



NIDA Drug Supply Program

Pursuant to the CSA, the DEA remains responsible for the control and supervision of cannabis supplies for research. The DEA thus far has only issued one license to supply clinical research grade medical cannabis, designating NIDA as the agency responsible for managing the production and provision of research grade cannabis. NIDA contracts with the University of

Mississippi to manufacture cannabis products for research. That supply is available to researchers, however obtaining cannabis supply has been problematic in practice. NIDA has only approved supply for 16 non-federally funded studies since it began overseeing cannabis production in 1974[9]. Even those researchers who were able to obtain supply indicated that they have experienced significant delays in the process. The supply grown at NIDA is limited.[10] Additionally, some researchers note that the supply is an old cannabis strain that does not allow for evaluation of the more potent strains now available. Anecdotally, researchers with current studies indicate that they have found NIDA responsive to their needs and willing to produce products meeting the needs of research programs. That can, however, be a lengthy process.

The NIDA approval process includes several factors that influence the researcher's ability to obtain cannabis. First, NIDA does prioritize federally funded research. NIH funded studies are first to receive cannabis supplies from NIDA and much of the NIDA supply services addiction/abuse related studies that serve NIDA's own mission[11]. Second, though no explicit preference for closed system research programs (where funding and research institutions are the same organization, like the California program), such systems have been more successful in obtaining supply from NIDA than programs where the funding entity and the researcher are not part of the same entity (i.e. Colorado).

If the state wished to produce cannabis products for research, Oregon can also apply to be the legal supplier of cannabis for NIDA, though the distribution would still be managed by NIDA. NIDA regularly solicits proposals for organizations to produce the NIDA supply of research grade cannabis. The University of Mississippi has held that contract since 1968, most recently winning the competitive bid again in the summer of 2015.[12] Mississippi's current contract runs through 2016 with up to four additional yearlong extensions. University of Massachusetts researchers also attempted to receive approval for a research growing facility from the DEA in the 2000's but were denied even after engaging in a lengthy appeals process and legal battle.[13]

Non Clinical Research (public health/observational)

Researchers-may also conduct research without directly supplying cannabis to enrolled subjects. These types of observational studies may be subject to varying legal requirements. Federally funded research or research being conducted by hospitals or universities will still require IRB approval. However, privately funded research conducted outside of universities and

hospitals that intended to contribute generalizable knowledge but not FDA regulated (i.e. part of an IND or IND application) research may not require federally regulated IRB review.[14] The federal IRB regulations, referred to as "the Common Rule" are currently undergoing significant revisions. One of the proposed changes to the Common Rule would remove the discretion of IRBs to apply different sets of standards to federally funded (or federally IRB review required) studies and non-federally funded studies.

Plant Research

Under the CSA, manufacturers or laboratories wishing to grow or analyze cannabis must also receive approval from the DEA to do so. Separate research registration is the? only requirement of researchers working with Schedule I substances. Currently X## growers are DEA approved to provide research grade cannabis and X## labs. Thus far the DEA has not demonstrated an interest in increasing the number of labs authorized to conduct research on cannabis. Cannabis supplies are also available for analysis through NIDA. NIDA may provide supplies to non-NIH funded, non human subjects research protocols if the scientist can demonstrate both their expertise and the scientific validity and ethical soundness of the research protocol.[15]

Hemp

The 2014 Farm Bill removed industrial hemp from some Schedule I restrictions by allowing states and universities in states where hemp has been legalized, (such as Oregon) to grow hemp crops for agricultural research[16]. Since the farm bill was enacted, at least 14 states have authorized hemp research and the Kentucky Department of Agriculture has established a state Department of Agriculture hemp growing and research program. States organizations wishing to grow hemp may do so without DEA Schedule I manufacturer licensure. However, they may only import foreign hemp seed if licensed as an importer by the DEA. Additionally, in its negotiations with Kentucky, the DEA also has confirmed that the state may distribute hemp seeds to state universities/researchers, as well as private farmers as long private entities also agree to comply with all federal laws[17]. (After consulting with the DEA to establish its state hemp research program, the state of Kentucky had its supply of hemp seeds confiscated by the DEA on the eve of its first planting as Kentucky had not obtained its importer license from the

DEA. The state was able to get is license on an expedited basis from the DEA and was able to plant the seeds imported from Italy.)

The Evolving Federal Landscape

In recognition of increasing interest in research regarding the medicinal benefits of cannabis and its components, some federal agencies have revisited some of the details of those restrictions detailed above, simplifying the processes and increasing the availability of cannabis supply.

- The Department of Health and Human Services recently revised the guidelines, first published in 1999, regarding the provision of cannabis for medical research through NIDA. In the revision, the FDA eliminated the need for a fourth agency – the Public Health Service – approval for research but retained the requirement that supply come from NIDA. [18]
- FDA staff have verbally expressed that the FDA itself does not require that cannabis researchers utilize the NIDA cannabis supply exclusively for FDA regulated studies. FDA would consider applications involving products from other legal suppliers but notes that the Mississippi farm has completed all filings to be on the FDA's Master Drug File. That means that the farm has submitted detailed information on the manufacturing facilities, processes, and materials. Though completion of the Master Drug File process is not required of manufacturers[19], the FDA indicated that it would expect alternative cannabis manufacturers/growers to complete the process. Other suppliers would need to complete the Master Drug File filings http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122886.htm
- NIDA itself and the National Institute of Health are funding research on the therapeutic benefits of cannabis.[20] NIDA also announced in May 2014 that it would significantly increase the supply of clinical research grade cannabis from 21 Kgs per year to 650 Kgs.[21] In June 2015 testimony to congress, NIDA Director Dr. Nora Volkow supported the need for clinical studies of CBD and identified the current research barriers, including the lack of CBD that has been produced under the guidance of Current Good Manufacturing Processes (cGMP) (required for testing in human clinical trials) as well as the variable quality and purity of CBD from state

medical marijuana program sources.[22] (Note: Dr. Volkow also provided significant testimony to congress on the adverse health effects of cannabis a year earlier.)

- Following inquires from members of congress, DEA Deputy Assistant Administrator Joesph T. Rannazzisi testified that the "DOJ and DEA are fully committed to supporting lawful research involving marijuana and CBD by ensuring compliance with the Controlled Substances Act and the Single Convention on Narcotic Drugs. DEA will continue to review the relevant regulations to ensure they are consistent with supporting lawful research. If this review determines that amending the existing regulations governing the Schedule I researcher registration process is necessary to accomplish these goals, DEA would initiate the process to do so.[23] He also indicated a significant increase in approved cannabis researchers. As of June 4, 2015, there were 265 active researchers registered with DEA to conduct bona fide research with marijuana and marijuana extracts that include CBD, and 41 (up from 16 in November 2014) researchers approved to conduct research with CBD on human subjects…. In furtherance of our ongoing efforts to support CBD research, DEA will continue its policy of expediting these applications."[25]

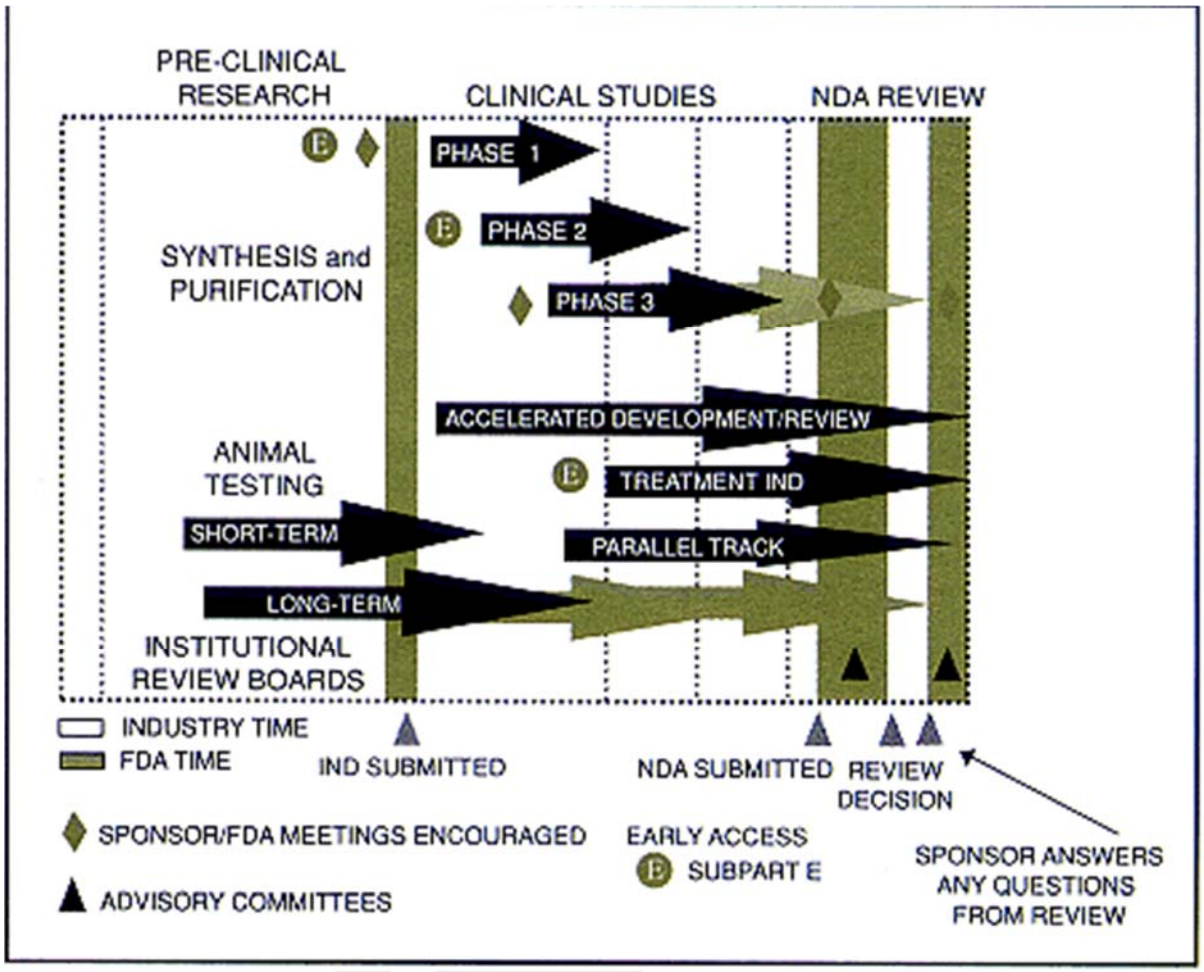
Despite increasing support from DHHS, the FDA, in its most recent statements to Congress re-iterated that the DEA is the lead agency and the FDA will continue to follow its classification of cannabis as a Schedule I substance. Further, the FDA "will continue to play its role in ensuring that any new therapies (including those derived from cannabis) are safe, effective, and manufactured to a high quality, applying the drug development paradigm that continues to provide new medicine that meet these standards for patients[24]". Though the DEA, in compliance with the Obama administration's position, has thus far indicated that it will not interfere with state initiatives and though it has approved significant research work in the area, it has not demonstrated willingness to allow other sources. Currently, only two laboratories are currently approved for bench science on cannabis and its components. . Most approved researchers are evaluating the negative effects of cannabis use. DEA official policy statement documents still posted on the agencies website are also strongly worded and indicate that the DEA continues to find that cannabis possesses no legitimate medical uses[25]. Indeed, DEA

leadership has been widely criticized for its overly strong statements on the issue – referring to medical cannabis as a "joke".[26]

Key areas for discussion

1. Should the state seek approval from the federal government (DEA) to manufacture, transport and study cannabis within the state? Can the state partner with tribes?
2. How could hemp research now legal support the task force's research goals?
3. Should the state encourage entities within Oregon to apply to become a supplier of research grade cannabis for NIDA?
4. Should a state research program require individual researchers to negotiate the federal approval processes for their research?
5. Should the state invest in research program infrastructure in anticipation of greater flexibility at the federal level?
6. Other questions/solutions?

Appendix A: FDA Approval Process



Appendix B: Recommended Resources and Online Information

For more information regarding CMCR completed studies, visit:
http://www.cmcr.ucsd.edu/index.php?option=com_content&view=category&id=41&Itemid=135

For more information regarding Colorado approved grants, visit:
https://www.colorado.gov/pacific/cdphe/approved-medical-marijuana-research-grants

For more information about NIDA approved studies, visit:
http://www.drugabuse.gov/drugs-abuse/marijuana/nida-research-therapeutic-benefits-cannabis-cannabinoids

For more information regarding NIH sponsored studies, visit:
https://era.nih.gov/nih_and_grantor_agencies/other/query_view_and_report.cfm

For more information regarding the National Center for Natural Research, visit:
http://pharmacy.olemiss.edu/ncnpr/research-programs/cannabis-research/

For more information about the FDA drug approval process generally, visit:
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/default.htm

For more information about the process of getting FDA approval for cannabis research, visit:
http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm421173.htm

For more information on the FDA botanical drug approval guidance, visit:
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CenterforDrugEvaluationandResearch/ucm106136.pdf

For more information about NIDA's position and available cannabis supplies, visit:
http://www.drugabuse.gov/drugs-abuse/marijuana/marijuana-research-nida
http://www.drugabuse.gov/researchers/research-resources/nida-drug-supply-program-dsp/marijuana-plant-material-available-nida-drug-supply-program

For more information about the DEA's position, visit:
http://www.dea.gov/docs/marijuana_position_2011.pdf

For more information about federal government policies that limit medical cannabis research, visit:
http://www.brookings.edu/~/media/research/files/papers/2015/10/20-war-on-marijuana-research-hudak-wallack/ending-the-us-governments-war-on-medical-marijuana-research.pdf

For more information about institutional review board regulations,
visit:http://www.hhs.gov/ohrp/humansubjects/commonrule/index.html#

References

[1] Drug Enforcement Administration. (2015). Drug scheduling. Retrieved from http://www.dea.gov/druginfo/ds.shtml. Accessed October 27, 2015.

[2] Controlled Substances Act 21 U.S.C. § 823(f).

[3] Memorandum from James M. Cole, Deputy Attorney Gen. to U.S. Att'ys (October 19, 2009). http:/www.justice.gov/sites/default/files/opa/legacy/2009/10/19/medical-marijuana.pdf.

[4] 2016 Consolidated Appropriations Act, Section 542 http://docs.house.gov/billsthisweek/20151214/CPRT-114-HPRT-RU00-SAHR2029-AMNT1final.pdf

[5] Safe and Drug Free Schools and Communities Act of 1986 89-10 title IV §4001

[6] Department of Health and Human Services. Human Subjects Research (45 CFR 46). Retrieved from http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html

[7] Food and Drug Administration. Marijuana research with human subjects. Retrieved from www.fda.gov/NewsEvents/publichealthfocus/ucm421173.thm. Accessed October 30, 2015.

[8] National Institute on Drug Abuse. NIDA's role in providing marijuana for research. Retrieved from http://www.drugabuse.gov/drugs-abuse/marijuana/nidas-role-in-providing-marijuana-research Accessed October 30, 2015

[9] National Institute on Drug Abuse. Independently funded studies receiving research grade marijuana. Retrieved from http://www.drugabuse.gov/drugs-abuse/marijuana/independently-funded-studies-receving-research-grade-marijuana-1999-to-present Accessed December 3, 2015.

[10] Kovaleski, S. (2014). Medical marijuana research hits wall of U.S. law. New York Times. A4.

[11] National Institute on Drug Abuse – NIDA's role in providing marijuana for research (revised June 2015). Retrieved from http://www.drugabuse.gov/drugs-abuse/marijuana/nidas-role-in-providing-marijuana-research Accessed November 24, 2015.

[12] National Institute on Drug Abuse -- Information on the marijuana farm contract. Retrieved from http://www.drugabuse.gov/drugs-abuse/marijuana/nidas-role-in-providing-marijuana-research/information-marijuana-farm-contract. Accessed October 30, 2015.

[13] Multidisciplinary Association for Psychedelic Studies. DEA lawsuit overview. Retrieved from http://www.maps.org/research/mmj/dea-license/144-dea-lawsuit-overview. Accessed December 18, 2015.

[14] Food and Drug Administration. Institutional Review Boards Frequently Asked Questions. Retrieved from http://www.fda.gov/RegulatoryInformation/Guidances/ucm126420.htm Accessed December 15, 2015.

[15] National Institute on Drug Abuse NIDA Drug Supply Program. Retrieved from http://www.drugabuse.gov/researchers/research-resources/nida-drug-supply-program Accessed November 24, 2015.

[16] Agriculture Act of 2014 Pub. L. 113-79 (2014)

[17] Patton, J. Kentucky Agriculture Department, DEA reach Agreement hemp seeds; planting could come soon. Lexington Herald May 24, 2014 Retrieved from http://www.kentucky.com/news/business/article44489994.html Accessed December 18, 2015.

[18] Center for Medicinal Cannabis Research. Report to the legislature and governor of the

state of California presenting findings pursuant to SB847 which created the CMCR and provided state funding. Retrieved from http://cmcr.ucsd.edu/images/pdfs/cmcr_report_feb17.pdf. Accessed October 15, 2015.

[19] Food and Drug Administration. Master Drug File: Guidelines. Retrieved from http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122886.htm

[20] National Institute on Drug Abuse. NIDA's role in providing marijuana for research. Retrieved from http://www.drugabuse.gov/drugs abuse/marijuana/nidas-role-in-providing-marijuana-research. Accessed October 30, 2015

[21] 80 FR 34693.

[22] Cannabidiol: Barriers to Research and Potential Medical Benefits: Caucus on International Narcotics Control Senate, 114th Cong. (2015) (Testimony of Nora Volkow, Director NIDA).

[23] Cannabidiol: Barriers to Research and Potential Medical Benefits: Caucus on International Narcotics Control Senate, 114th Cong. (2015) (Testimony of Joseph T. Rannazzisi, Deputy Assistant Administrator).

[24] Cannabidiol: Barriers to Research and Potential Medical Benefits statement before the Caucus on International Narcotics Control, Senate, 114th Cong. (2015) (Testimony of Douglas Throckmorton).

[25] Drug Enforcement Administration. The DEA position on marijuana. Retrieved from http://www.dea.gov/docs/marijuana_position_2011.pdf Accessed November 20, 2015.

[26] Dockterman, E. People want DEA chief to resign after he called medical marijuana a joke. Time November 10, 2015. http://time.com/4107603/dea-medical-marijuana-joke-2/

Oregon State Cannabis Research Task Force: Needs and Opportunities

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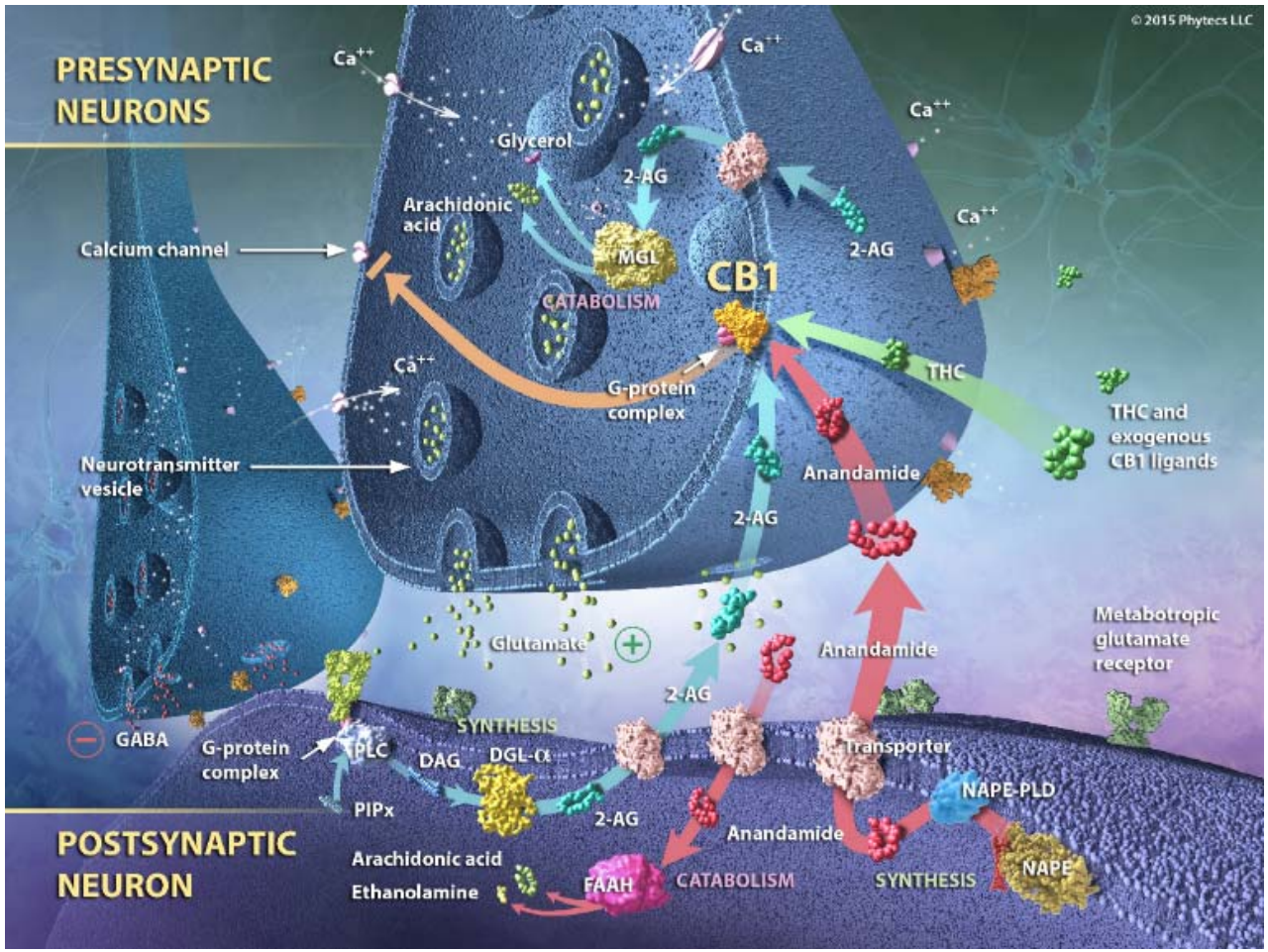
PHYTECS

Cannabinoids: 3 Varieties

- **Phytocannabinoids** (Pate 1994): terpenophenolic 21-C compounds found in the genus *Cannabis* (e.g., THC, CBD)
- **Endocannabinoids** (Di Marzo 1998): natural endogenous compounds binding cannabinoid receptors (e.g., anandamide) whose functions are: **“relax, eat, sleep, forget and protect”**
- **Synthetic cannabinoids** (e.g., ajulemic acid) that also affect cannabinoid receptors

Russo, E.B. 2008. Cannabinoids in management of difficult to control pain. *Therapeutics & Clinical Risk Management* 4(1):245-259.

CB_1 Activation, Synthesis, Catabolism



An internal homeostatic regulatory system of 3 components:

Endocannabinoids (anandamide, 2-AG),

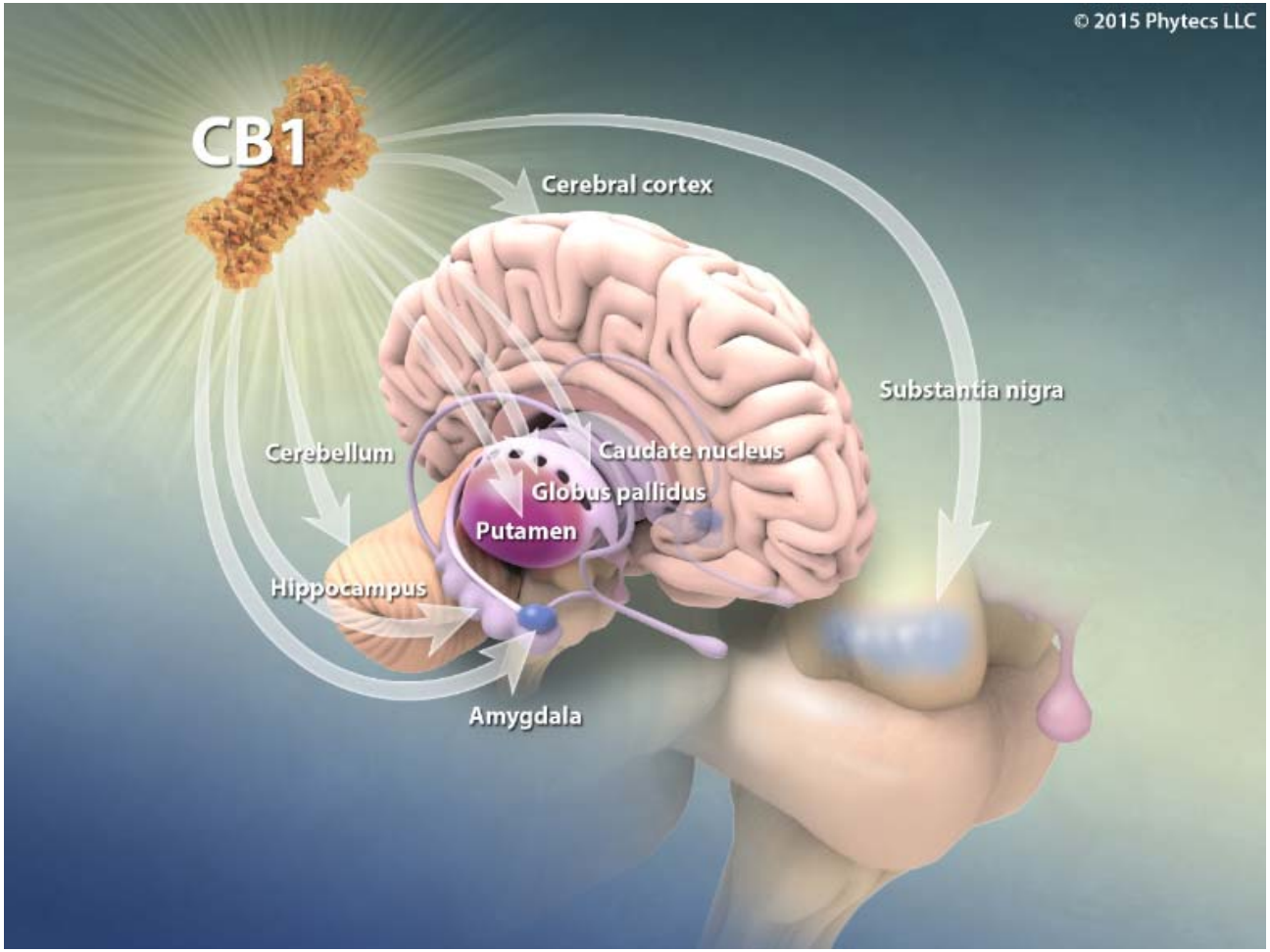
CB_1,CB_2 & TRPV1 receptors,

Their regulatory enzymes

Endocannabinoids are produced on demand, travel in retrograde fashion to inhibit neurotransmitter release.

Active and “inactive” components work together in an “Entourage Effect.”

CB_1 Expression in Brain

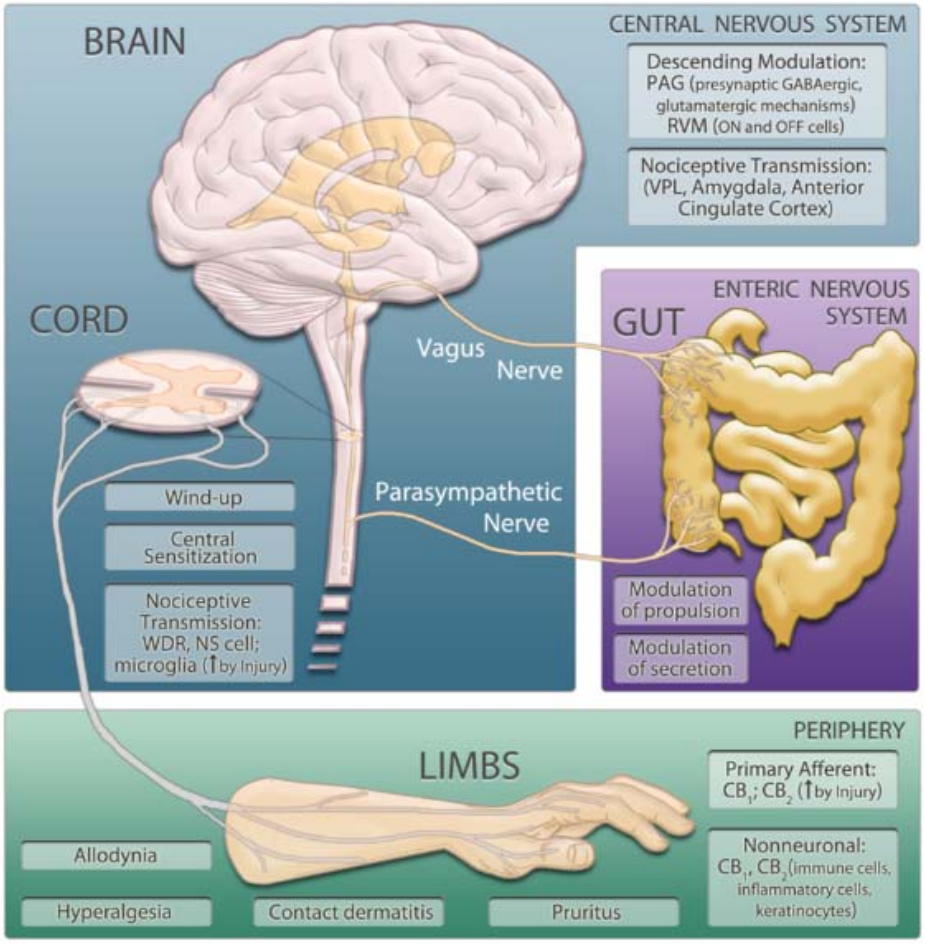


CB_1 is highly expressed in nociceptive areas, cerebellum, limbic system, basal ganglia and reward pathways.

Although prominent in the substantia nigra and periacqueductal grey matter, it is distributed in a limited fashion otherwise in the brainstem, and not in medullary respiratory centers.

The Endocannabinoid System (continued)

Russo EB, Hohmann AG. Role of cannabinoids in pain management. In: Deer T, Gordin V, editors. *Comprehensive Treatment of Chronic Pain by Medical, Interventional and Behavioral Approaches.* New York: Springer; 2013, pp. 181-197.



James Brodie, 2012

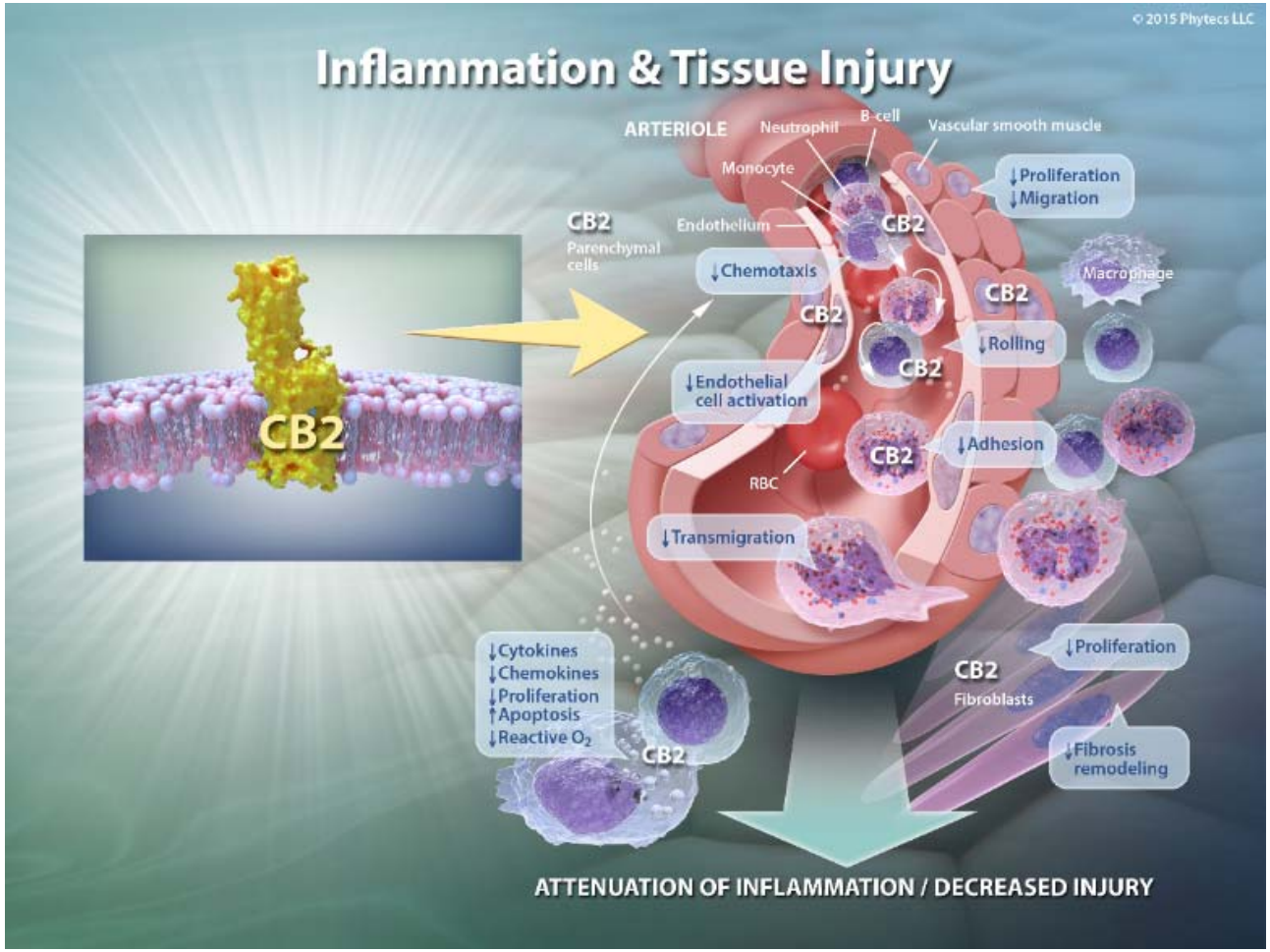
CB_1 is the most abundant G-protein-coupled receptor in the brain, with a major neuromodulatory function.

Role characterized as, “relax, eat, sleep, forget and protect.”

(Di Marzo, 1998)

Modulates pain, movement, emotion, emesis, seizure threshold, et al.

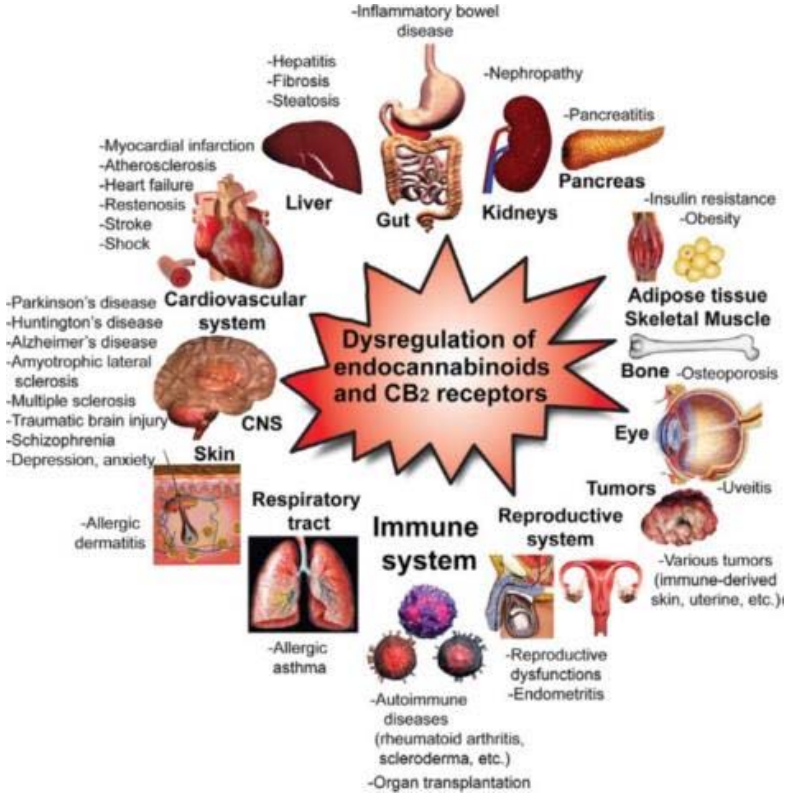
CB_2 and Inflammation

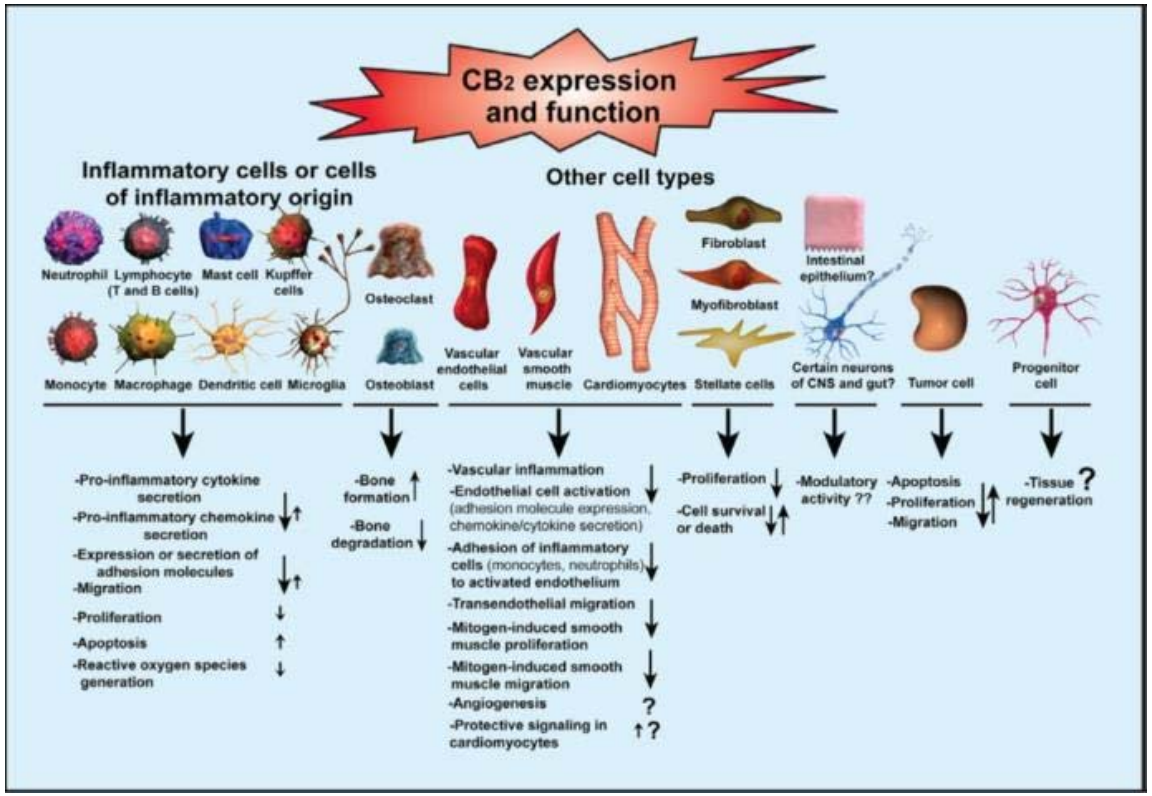


CB_2 is a mainly peripheral, immunomodulatory receptor with an important role in pain and inflammation.

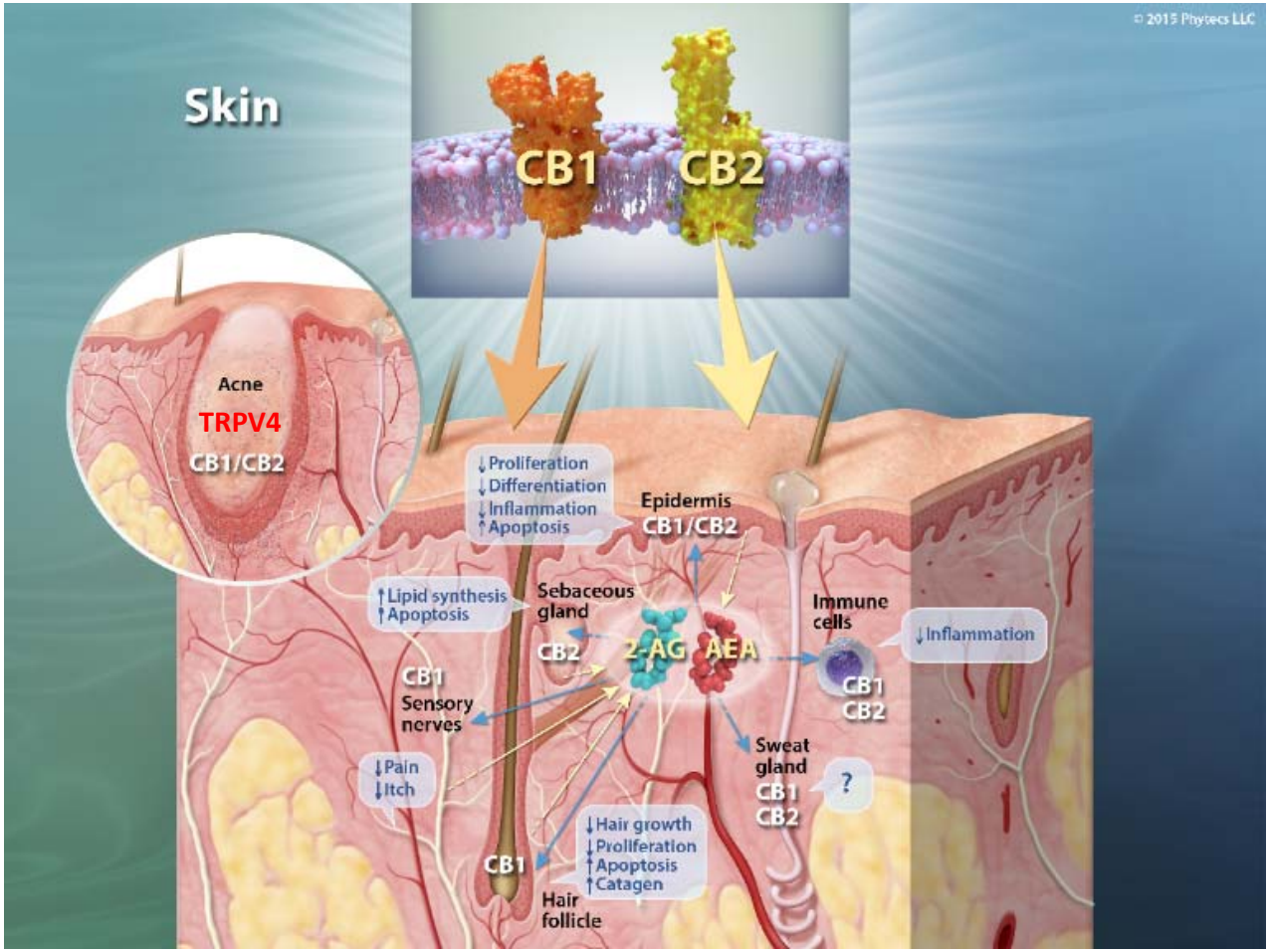
CB_2 agonists also hold great promise in treatment of hepatic fibrosis and related conditions.

Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* 2011.



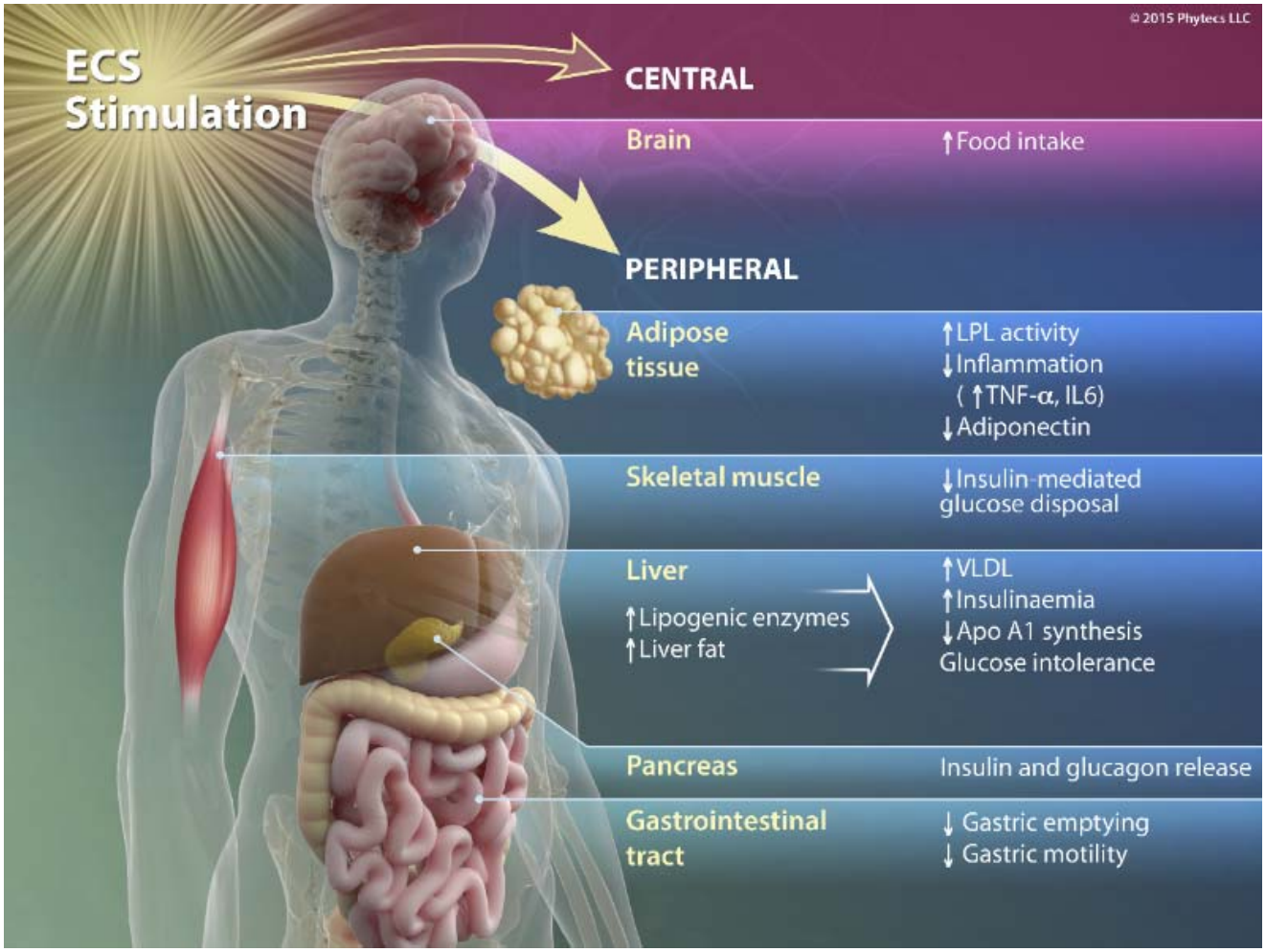


CB_1 and CB_2 in Skin



In addition to its anti-inflammatory and bacteriostatic effects, cannabidiol is a TRPV4 agonist that works as a sebostatic agent in acne (Olah 2014).

ECS Stimulation



In addition to these systems, the ECS is active in cardiac and bone physiology.

Cannabidiol was recently demonstrated to stimulate bone fracture healing (Kogan et al. 2015)

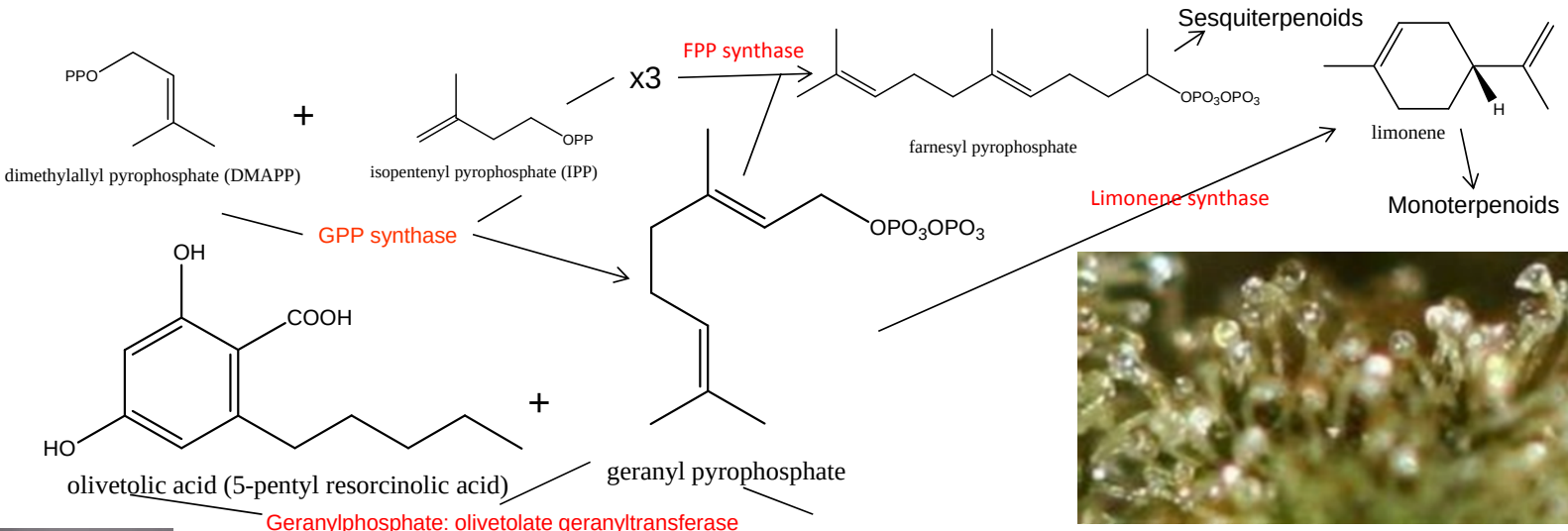
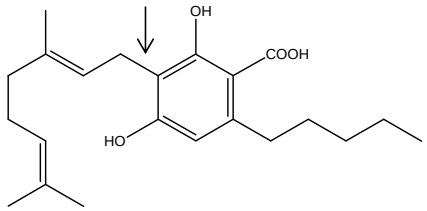
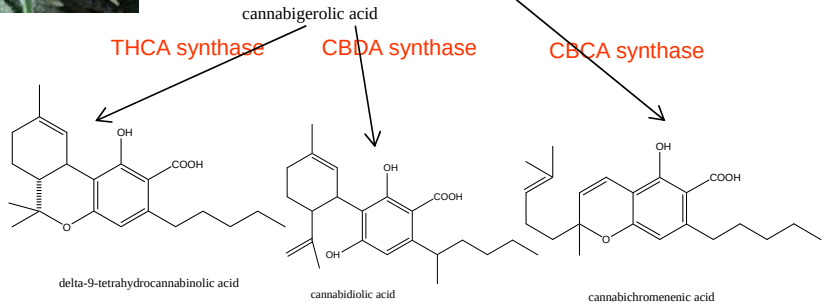


Photo EBR, with permission, Bedrocan





Pentyl Cannabinoid Synthesis

Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects.
Br J Pharmacol 163(7):1344-64, 2011.

Cannabis Dosing: Smoking

- **Illegal in most jurisdictions, especially in public**
- **Provokes intoxication**
- Dose titration not easily achieved
- Inefficient and wasteful of THC
- Polyaromatic hydrocarbons (PAH) produce premalignant cytological changes
- Bronchial irritation inevitable
- **Can not achieve FDA approval as a prescription product**

Pesticides in Smoked Cannabis

Table 3: Recovery of pesticides in smoke condensate.

Sample/residue	μg/gram plant	% Recovery
Water pipe with filters		
Diazinon	589 ± 31.0	0.08
Paclobutrazol	420 ± 32.5	10.2
Bifenthrin	77 ± 34.5	9.00
Permethrin	685 ± 34.9	10.9
Cotton filter		
Diazinon	190 ± 11.0	24.9
Paclobutrazol	109 ± 8.80	30.1
Bifenthrin	20.8 ± 9.16	26.6
Permethrin	134 ± 8.52	25.1
Carbon filter	N/A	N/A
Water pipe w/out filters		
Diazinon	2930 ± 15.1	42.2
Paclobutrazol	2040 ± 11.3	49.5
Bifenthrin	389 ± 10.1	45.4
Permethrin	3760 ± 9.72	59.9
Glass pipe		
Diazinon	4270 ± 12.3	61.5
Paclobutrazol	2789 ± 13.8	67.4
Bifenthrin	516 ± 12.8	60.3
Permethrin	4360 ± 9.70	69.5

Data presented as mean μg pesticide/gram plant material ± relative standard deviation. Sample size of 3 for all measurements.

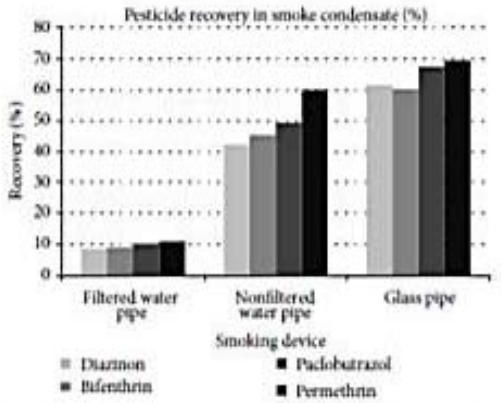
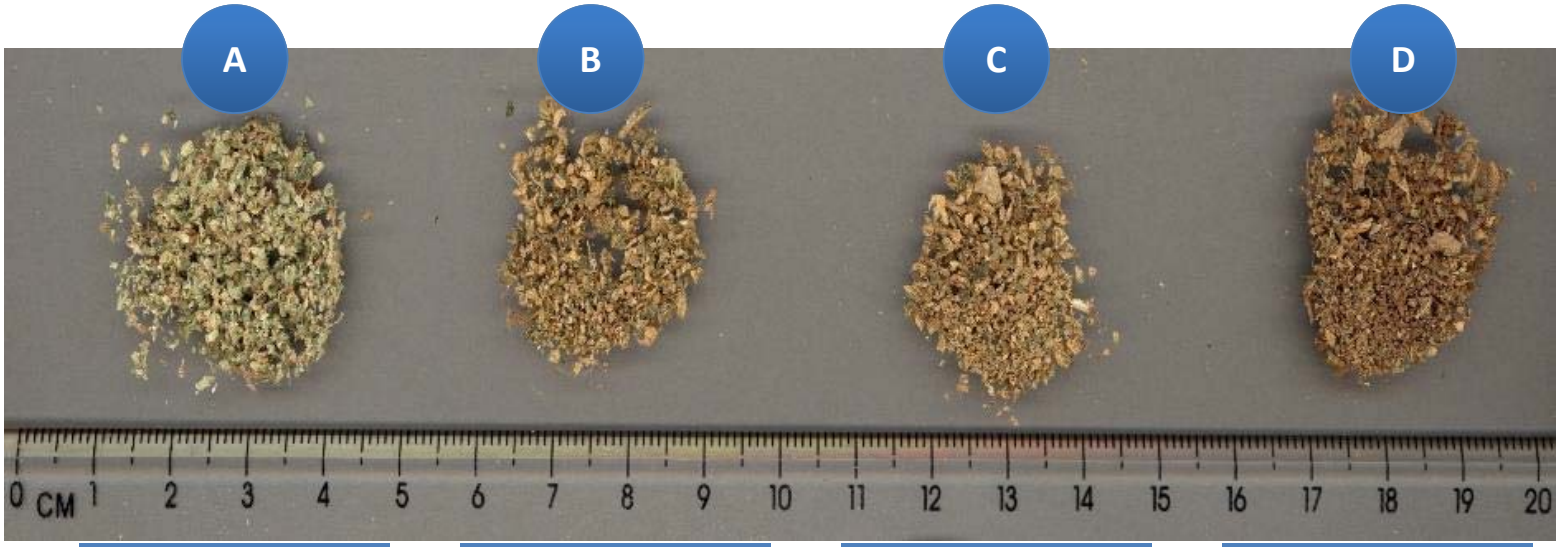


Figure 1: Percent recovery of pesticides from the smoke stream from each device.

- **No EPA tolerances are set for pesticides on smoked crops.**
- Pesticide and growth regulator residues are frequently noted in lab testing of black market cannabis.
- As noted, **~40-70% of toxic residues are residual in smoke.**

Sullivan N, Elzinga S, Raber JC. Determination of pesticide residues in cannabis smoke. *J Toxicol.* 2013:1-6.

Differential Vaporization



Untreated dried cannabis	Post-vaporization **175° C**	Post-vaporization **195° C**	Post-vaporization **230° C**

Vaporization, to date, has not eliminated toxic tar components or ammonia

Only 54% of medical users had tried vaporizers (Hazekamp 2013), and only half, or 27% preferred them.

Poses same regulatory hurdles as smoked cannabis

Cannabis Concentrates, or “Dabs”

Table 1: Detailed description of the five different protocols used for preparation of Cannabis oils.

Preparation step	1) NAPHTHA	2) PETROLEUM ETHER	3) ETHANOL	4) OLIVE OIL I	5) OLIVE OIL II
CANNABIS (g)	5g	5g	5g	5g	10g
SOLVENT (mL)	Naphtha (200 mL)	Petroleum ether (200 mL)	Ethanol (200 mL)	Olive oil (20 mL) + water (70 mL)	Olive oil (100 mL)
EXTRACTION/ FILTRATION	Extraction #1: 5 g cannabis + 100 mL naphtha, agitate 20 min. (a) • Filtration with filter paper • Extraction #2: Same cannabis + 100 mL naphtha, agitate 20 min. (a) • Filtration with filter paper • Combine extracts	Extraction #1: 5 g cannabis + 100 mL petr. ether, agitate 20 min. (a) • Filtration with filter paper • Extraction #2: Same cannabis + 100 mLpetr. ether, agitate 20 min. (a) • Filtration with filter paper • Combine extracts	Extraction #1: 5 g cannabis + 100 mL ethanol, agitate 20 min. (a) • Filtration with filter paper • Extraction #2: Same cannabis + 100 mL ethanol, agitate 20 min. (a) • Filtration with filter paper • Combine extracts	5g cannabis + 20 mL olive oil + 50 mL water. Heat in water bath ~98°C for 60 min. • Before filtration, let it stand to cool off. • Filtrate by pressing (b) • Rinse the plant material with 20 mL of hot water • Filtrate by pressing (b) • Combine extracts	10 g cannabis + 100 mL olive oil. Heat in water bath ~98°C for 120 min. • Before filtration, let it stand to cool off. • Filtrate by pressing (b)
EXTRACT CLEAN-UP	N/A	N/A	(optional): Filter extract over a column filled with activated charcoal	N/A	N/A
EVAPORATION/ SEPARATION	Evaporate solvent in water bath ~98°C under stream of nitrogen gas	Evaporate solvent in water bath ~98°C under stream of nitrogen gas	Evaporate solvent in water bath ~98°C under stream of nitrogen gas	Let the solution stand to separate water and oil. Put it in the freezer (-20°C) overnight	N/A
RECONSTITUTION	Reconstitute residue with EtOH to 100 mL	Reconstitute residue with EtOH to 100 mL	Reconstitute residue with EtOH to 100 mL	Collect upper layer (oil) by pouring it off the frozen water layer	Collect the oil
EXTRACT CONCENTRATION (cannabis/solvent)	5 g/100 mL	5 g/100 mL	5 g/100 mL	5 g/20 mL	10 g/100 mL
DILUTION FACTOR FOR ANALYSIS	20x	20x	20x	100x	40x
FINAL CONCENTRATION (cannabis/solvent)	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL

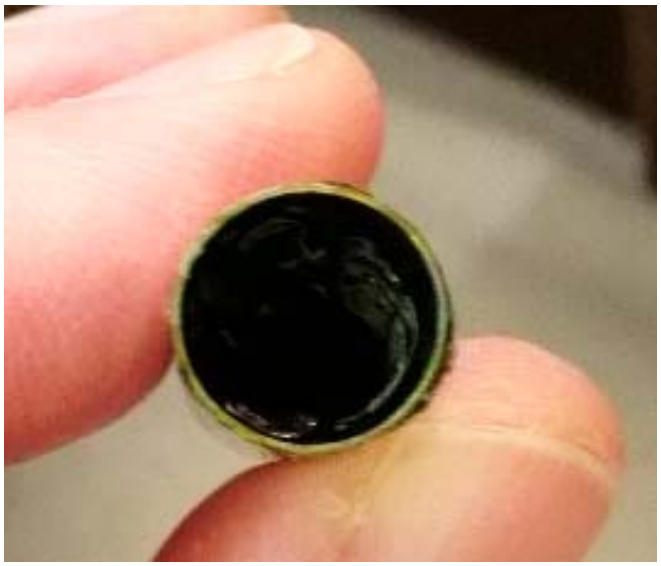
a): agitate by using a shaking platform @ 120 rpm
b): separate oil from plant material by using a French coffee press

Romano LL, Hazekamp A. Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine. *Cannabinoids.* 2013;1:1-11.

- Cannabis is extracted with polar solvents
- Many are flammable and **potentially explosive**
- THC (and contaminants) are highly concentrated by the process
- **“Naptha” and butane are often contaminated and leave toxic residues**
- How high does a patient need to be to have symptom relief?

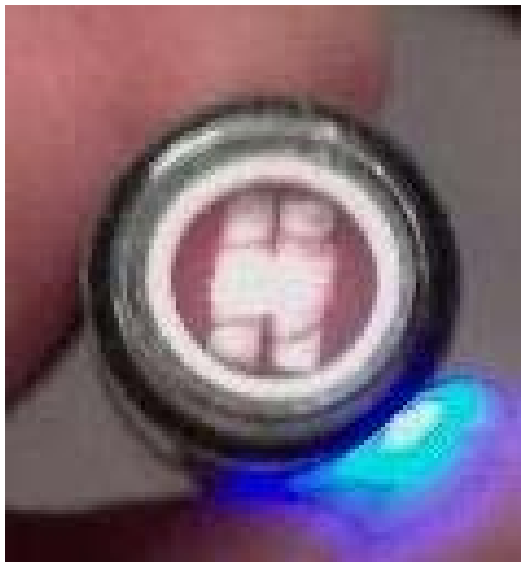
EBR

“Vaporization” of Wax



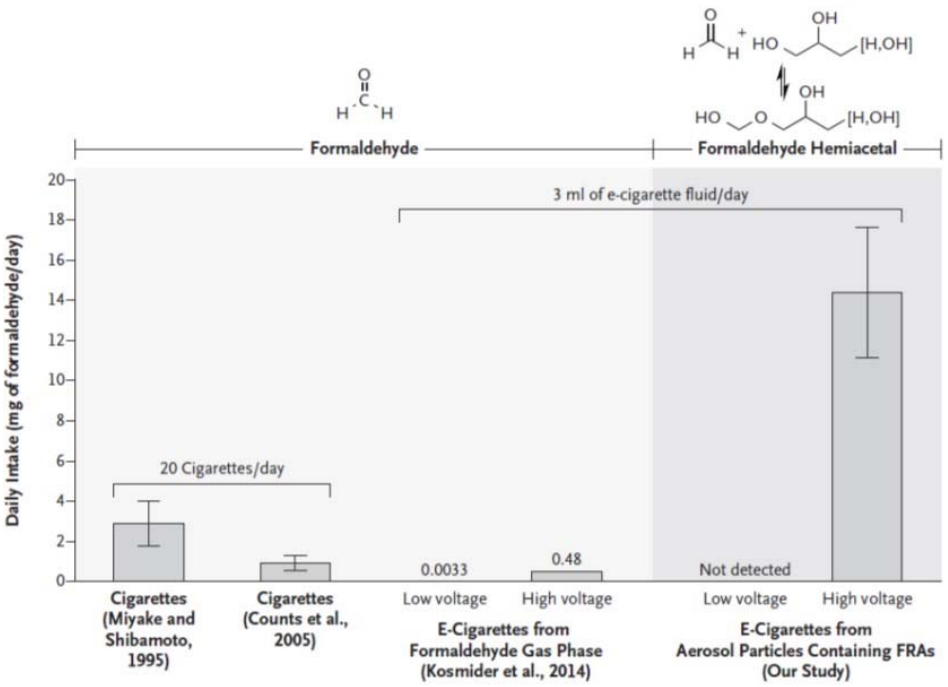
“Wax” in vape pen

Unheated coil



Red hot in seconds

Vape Pens, Propylene Glycol & Formaldehyde



Jensen, R. P. et al. 2015. Hidden formaldehyde in e-cigarette aerosols. *NEJM* 372 (4):392-4.

E-cigarettes use propylene glycol/glycerol as propellant

Under high heat, up to **2%** of this mixture **forms formaldehyde**, a Group 1 carcinogen (Intl. Agency for Research on Cancer)

Risk is as much as 15X that of chronic cigarette smoking.

Cannabis Preparation Array

Sinsemilla buds
Berkeley, California
(photo EBR)

Cannabis confections
Berkeley, California
(photo EBR)

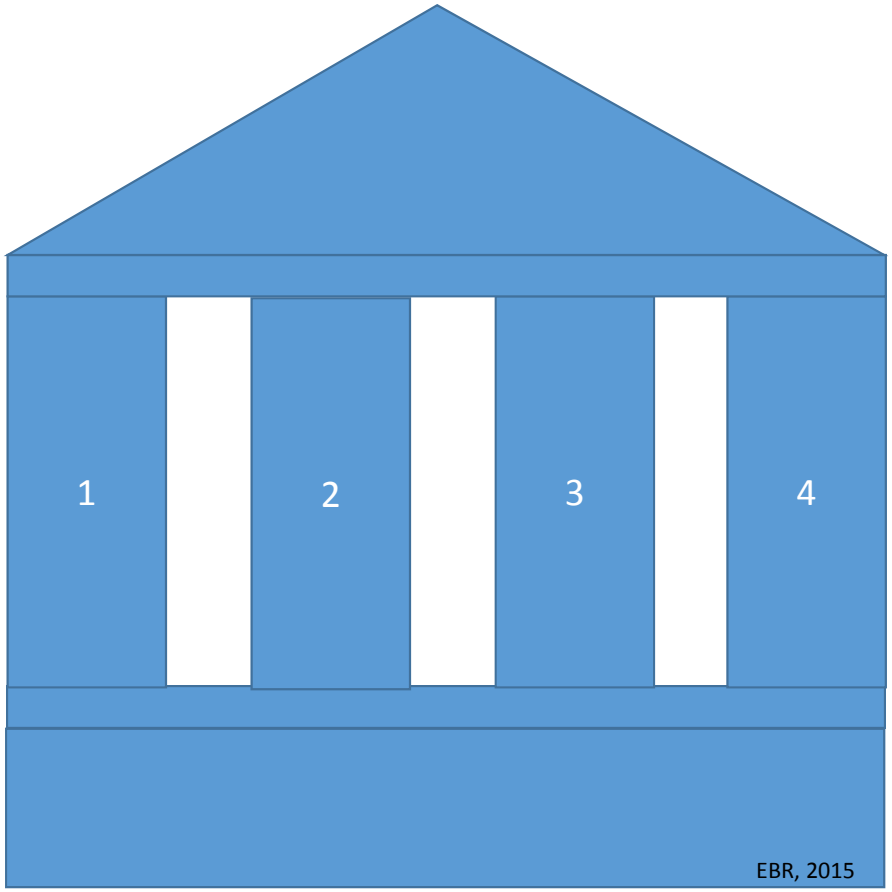
- **No real quality control (USA)**
- **No regulatory approval**
- **Confections attractive to children**

Cannabis-based medicine extract knock-off:
"I Can't Believe It's Not Nabiximols!"
Victoria, BC, Canada
(photo EBR)

How American Cannabis Research Works (or Doesn't)

- All cannabis for medical research originates from NIDA
- No other **domestic** material is permitted, even if meeting GMP standards, except perhaps for observational studies, due to Schedule I restrictions and a unique interpretation of Single Convention Treaty
- Clinical trials require state approval, IRB approval, FDA Investigational New Drug application
- 1999-2014: Additional PHS review, now eliminated, to little practical benefit
- **All studies to date (primarily California) have been small in scope, extremely short in duration, and are unreproducible due to lack availability of standardized material.**

The Four Pillars of a True Medicine



1) Efficacy
2) Safety
3) Standardization
4) Accessibility

A Pharmaceutical Cannabis-based Medicine Must

R_x

- Be standardized, consistent and display a quality equal to any New Chemical Entity
- Possess a practical and suitable delivery system that minimizes patient risk, including intoxication, other aspects of drug abuse liability or serious adverse events (e.g., pulmonary sequelae)
- Have a supply chain that ensures security that it is being distributed to its intended target patients
- Be accessible, meaning available and affordable.

Advantages of Pharmaceutical Prescription Cannabinoids

- Safe and effective **evidence-based** pharmaceutical formulations
- Preparations that physicians may prescribe with confidence, that pharmacists endorse and supply
- Prescriptions that government health services and third party payers will cover

Why Black Market Cannabis Fails These Challenges

- It cannot gain regulatory approval in most nations
- Biochemical variability of chemovars (“strains”)
- Unregulated material may harbor molds, coliform bacteria, pesticides or heavy metals that endanger public health
- The most common delivery system, smoking, imposes risks: chronic cough, phlegm production, bronchitis, and inhalation of pyrolytic by-products
- Cannabis inhalation, whether by smoking or vaporizer produces a rapid peak in serum and brain concentrations that maximizes intoxication and possible reinforcement that are risk factors for drug abuse liability

Standardization

- Evidence that the drug is chemically consistent and identical in structure and dose over time.
- This is a particular challenge for botanicals, but is clearly achievable.

Problems in Cannabis Laboratory Analysis

- Hampered by illegality: Lack of Schedule I permits (USA)
- Lack of uniformity in methodology
- Poor application of due diligence, “dry-lab results”
- Dearth of cannabinoid standards 
- Cannabinoids are tough to assay properly 
- Terpenoids are even tougher 

Medical Efficacy: Levels of Evidence

Level	Type of evidence
I	Large RCTs with clear cut results
II	Small RCTs with unclear results
III	Cohort and case-control studies
IV	Historical cohort or case-control studies
V	Case series, studies with no controls

Sackett DL. Rules of evidence and clinical recommendations on the use of antithrombotic agents. *Chest* 1989;95:2S–4S

Randomized Clinical Trials: The *Sine qua non*

- Phase I: Testing drug in normals to assess tolerability, dosing, pharmacokinetics
- Phase II: Testing in specific disease or condition with small numbers, assessing safety and efficacy (e.g., Epidiolex (CBD) for intractable seizures)
- Phase III: Testing in condition with large numbers assessing safety and efficacy (e.g., Sativex (THC/CBD et al.) for spasticity or cancer pain)

Abrams DI, et al. Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo-controlled trial. *Neurology.* 2007;68(7):515-21.

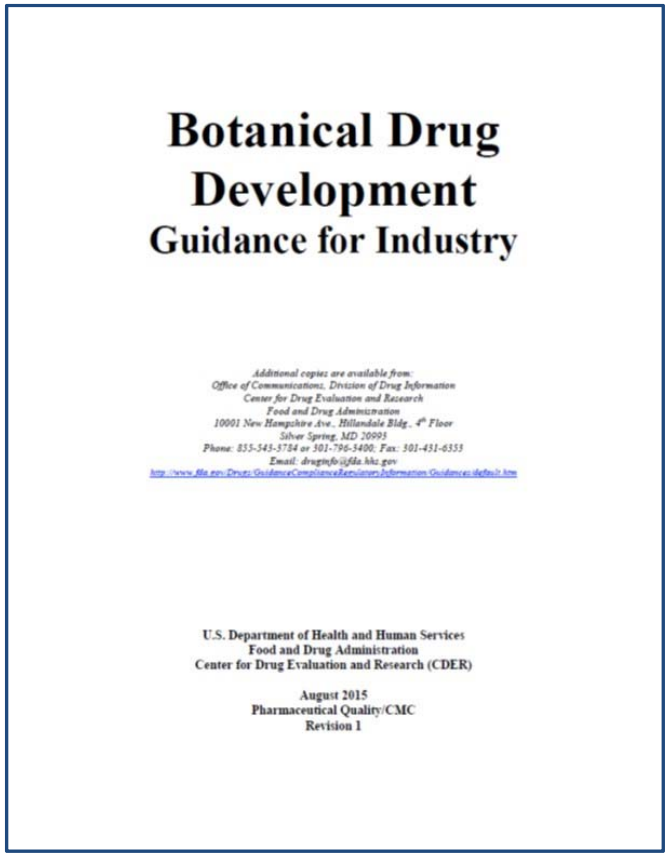
- Smoked NIDA cannabis in **50 subjects** TID for **5 days**
- **All required to have previous cannabis smoking experience**
- Results
  - decreased daily pain (p=0.03)
  - hyperalgesia (p=0.05)
  - 52% with >30% pain reduction vs. placebo (p=0.04)
- **AEs in smoking group (psychoactive effects) were prominent, and to a degree that would preclude FDA approval.**

	Adjusted estimates	
	Cannabis, mean (95% CI)	Placebo, mean (95% CI)
Anxiety*	0.25 (0.14, 0.44)	0.10 (0.05, 0.22)
Sedation†	0.54 (0.36, 0.81)	0.08 (0.04, 0.17)
Disorientation†	0.16 (0.07, 0.34)	0.01 (0.00, 0.04)
Paranoia	0.13 (0.03, 0.45)	0.04 (0.01, 0.14)
Confusion†	0.17 (0.07, 0.39)	0.01 (0.00, 0.06)
Dizziness†	0.15 (0.07, 0.31)	0.02 (0.01, 0.05)
Nausea	0.11 (0.04, 0.30)	0.03 (0.01, 0.14)

Side effects were rated three times daily on a 0 to 3 scale (0 = none, 1 = mild, 2 = moderate, 3 = severe).

* p, 0.05; † $p < 0.001$.

Food and Drug Administration. *Guidance for industry: Botanical drug Development: Guidance for Industry.* US Government. 2015.



- Quality
  - Product Composition
  - Characterization
  - Quantification of components
  - Standardization / Consistency
  - Stability / Storage
- Safety
  - Animal data, including:
    - Carcinogenicity
    - Reproductive toxicology
    - Chronic toxicology
    - Genotoxicology
    - Safety pharmacology
  - Clinical data
    - Several hundred patient-years of data required
    - Reports of all adverse events (mild/moderate/severe – related and unrelated)
    - Immediate regulatory notification of serious adverse events
- Efficacy
  - Multiple Phase II & Phase III placebo-controlled clinical trials for indication

A blueprint for botanical drug development.

Bottom line:
A botanical pharmaceutical must prove:
Biochemical Standardization
Safety
Efficacy

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm458484.pdf

Nabiximols Oromucosal Extract

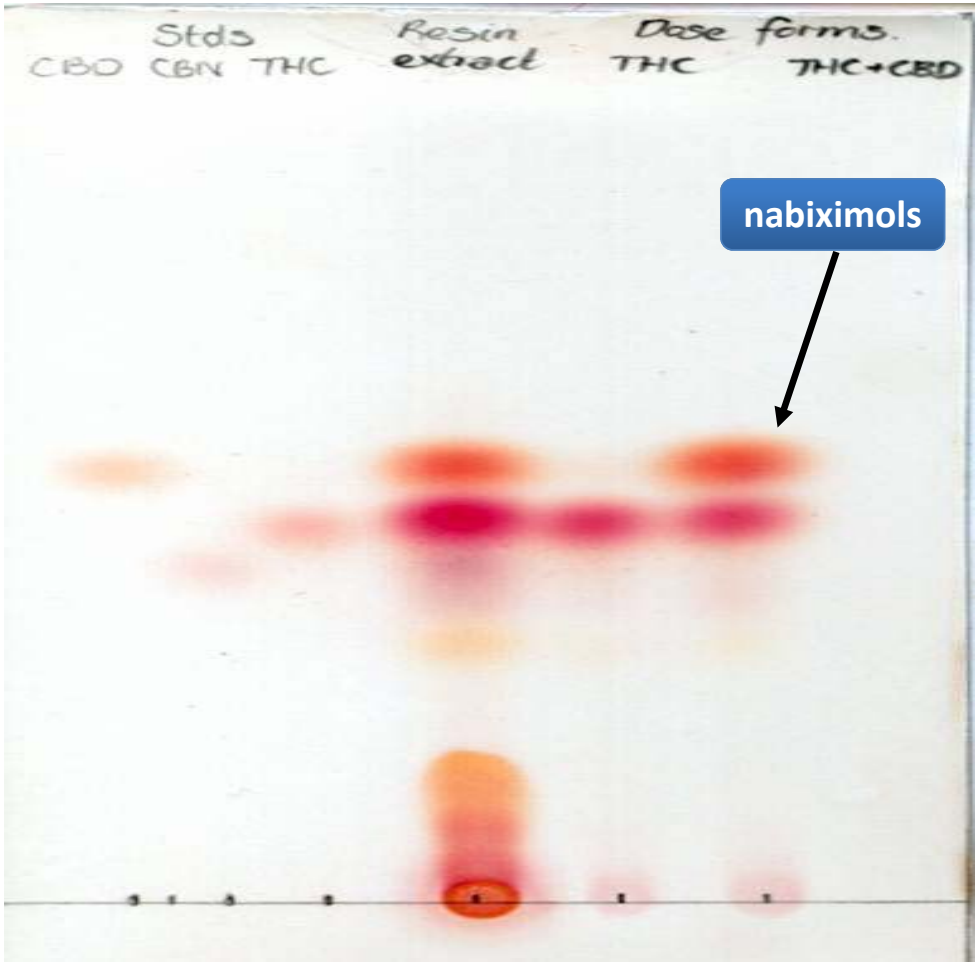
- 1:1 combination from two clonal cannabis chemovars yielding a high THC extract and a high CBD extract.
- A botanical drug substance (BDS) of defined composition with controlled reproducibility batch to batch.
- USAN: nabiximols (accepted as a unitary formulation)
- THC and CBD comprise some 70% (w/w) of the total BDS, with minor cannabinoids, terpenoids (most GRAS), and other minor components (also GRAS).
- each 100 µL pump-action spray provides 2.7mg of THC and 2.5mg of CBD, the minor components, plus ethanol, propylene glycol excipients, and peppermint as flavoring/masking agent.

- Intermediate onset
- Allows dose titration
- Acceptable to patients

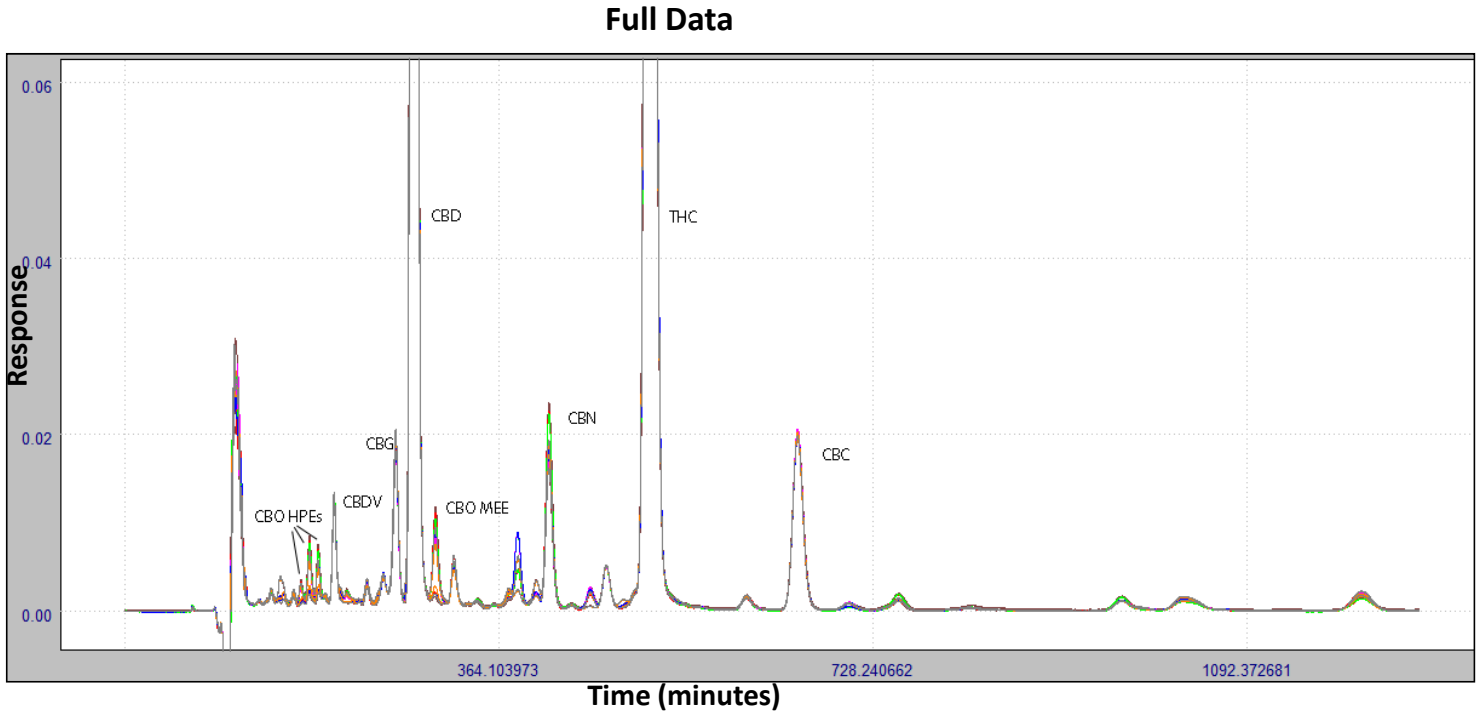
GWP Good Agricultural Practice (GAP)

- Grown in organic compost (“leaf mold”)
- Female clones from mother plant assure biochemical consistency
- Fertilization prevented
- Climate-controlled (temperature, light cycles, humidity)
- Integrated Pest Management (IPM) without pesticides of any kind

Thin Layer Chromatography of Cannabis Resin vs. nabiximols



Biochemical Fingerprinting of nabiximols



Overlay of cannabinoid chromatographic profiles from 25 batches of nabiximols over 9 years

(courtesy of Peter Gibson, PhD, GW Pharmaceuticals)

This level of standardization is necessary to pass the CMC (Chemistry, Manufacturing and Control) standards at FDA

Nabiximols Efficacy: Multiple Sclerosis Clinical Trials

Study Code	Study Details	Key Efficacy Result	P-value	Reference
Phase II *(Randomised, Double-Blind, Placebo Controlled Studies)*				
GWN19902	Symptoms of MS and other nervous system conditions (n=25)	Improvement in Spasticity (VAS)	<0.05	Wade DT et al. Clin Rehab. 2003
GWMS0001	MS Symptoms (n=160)	Improvement in Spasticity (VAS)	0.001	Wade DT et al. Multiple Sclerosis 2004
Phase III *(Randomised, Double-Blind, Placebo Controlled Studies)*				
X **GWCL0403**	MS, Spasticity (n=337)	Improvement in Spasticity (NRS)	0.22 0.035 (PP)	Collin C et al. Neurol Res. 2010
GWMS0106	MS, Spasticity (n=189)	Improvement in Spasticity (NRS)	0.048	Collin C et al. Eur J Neurol. 2007
GWSP0604	MS, Spasticity (n= (A) -572, (B) -241)	Improvement in Spasticity (NRS)	p=0.0002	Novotna J et al. Eur J Neurol 2011
GWSP0702	MS, Spasticity (n=36) *Randomised Withdrawal Study Design*	Time to treatment failure (NRS)	p=0.013	Notcutt W et al. Multiple Sclerosis 2011
GWSP1172	MS Spasticity (n=121) 12 month RCT	GIC	P<0.0001	ECTRIMS 2013
Long Term Extension Studies (Open Label)				
GWMS0001	Open label extension study (n=137)	Long term efficacy(NRS)	N/A	Wade DT et al. Mult Scler 2007
GWEXT0102	Open label extension study (n=507)	Long term efficacy(NRS)	N/A	

Randomized Controlled Trials of nabiximols in Pain

N=	Indication	Duration/Type	Outcome/References
20	Neurogenic pain	Series of 2-week N-of-1 crossover blocks	Improvement with high-THC extract and nabiximols on VAS pain vs. placebo ($p<0.05$), symptom control best with nabiximols ($p<0.0001$) [Wade et al. 2003]
24	Chronic intractable pain	12 weeks, series of N-of-1 crossover blocks	VAS pain improved over placebo ($p<0.001$) especially in MS ($p<0.0042$) [Notcutt et al. 2004]
48	Brachial Plexus Avulsion	6 weeks in 3 two-week crossover blocks	Benefits noted in Box Scale-11 pain scores with high-THC extract ($p=0.002$) and nabiximols ($p=0.005$) over placebo [Berman et al. 2004]
66	Central Neuropathic Pain in MS	5 weeks	Numerical Rating Scale (NRS) analgesia improved over placebo ($p=0.009$) [Rog et al. 2005]
125	Peripheral Neuropathic Pain	5 weeks	Improvements in NRS pain levels ($p=0.004$), dynamic allodynia ($p=0.042$), and punctuate allodynia ($p=0.021$) vs. placebo [Nurmikko et al. 2007]
56	Rheumatoid Arthritis	Nocturnal dosing for 5 weeks	Improvements over placebo morning pain on movement ($p=0.044$), morning pain at rest ($p=0.018$), DAS-28 ($p=0.002$), and SF-MPQ pain at present ($p=0.016$) [Blake et al. 2006]
117	Pain after spinal injury	10 days	NSD in NRS pain scores, but improved Brief Pain Inventory ($p=0.032$), and Patients Global Impression of Change ($p=0.001$) (unpublished)
177	Intractable cancer pain	2 weeks	Improvements in NRS analgesia vs placebo ($p=0.0142$), THC extract NSD [Johnson, 2010 #6899]
135	Intractable lower urinary tract symptoms in MS	8 weeks	Improved bladder severity symptoms including pain over placebo ($p=0.001$) (unpublished)
360	Intractable cancer pain	5 weeks/DB	CRA of lower and middle dose cohorts improved over placebo ($p=0.006$)/GWCA0701) (Johnson 2010)

X

Adapted from: Russo EB, Hohmann AG. Role of cannabinoids in pain management. In: Deer T, Gordin V, editors. *Comprehensive Treatment of Chronic Pain by Medical, Interventional and Behavioral Approaches.* New York: Springer. 2013:181-197.

Smoked cannabis RCTs in pain: total 3 patient-years

Nabiximols RCTs and other monitoring total >6000 patient-years just in older published studies.

GW Pharmaceuticals Product Pipeline

The Placebo Effect

- The mere act of being in a clinical trial generates a certain degree of subjective improvement.
- Estimates of correct drug assignment up to 75% in cannabis RCTs (Ellis 2009)
- This is aggravated when:
  - The RCT lacks objective measures.
  - The tested drug is psychoactive (e.g., antidepressants, cannabis).
  - The tested drug has a reputation as “miraculous,” e.g., the common perception of cannabis.

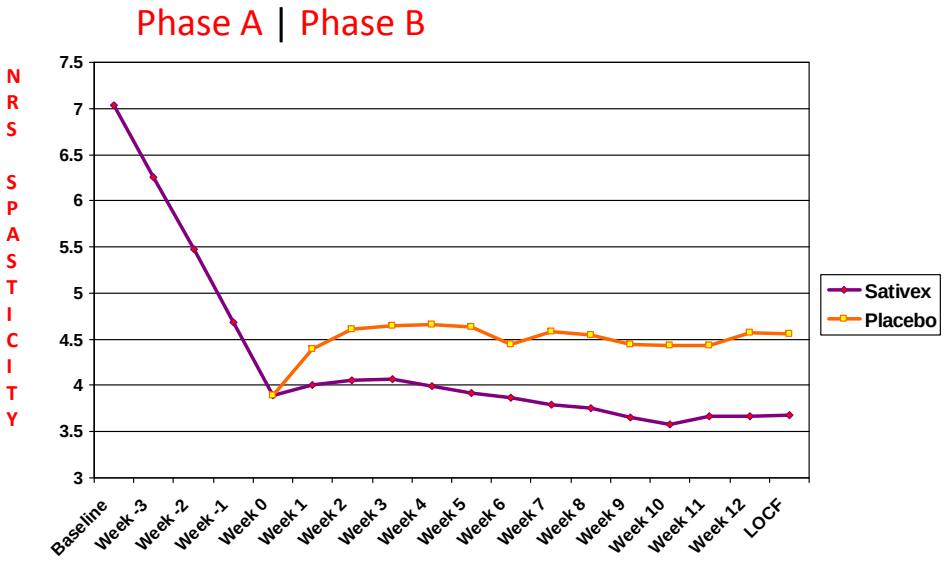
Wright S, Duncombe P, Altman DG. Assessment of blinding to treatment allocation in studies of a cannabis-based medicine (Sativex®) in people with multiple sclerosis: a new approach. *Trials.* 2012;13:189.

- No statistical differences were observed in the incidence of Euphoric Mood among patients with prior experience of cannabis vs. those who were cannabis-naïve (3% in each instance)
- No differences were noted in the two groups with respect to efficacy of symptom control with nabiximols (supporting the efficacy of blinding).
- This was considered as effective blinding, in marked contrast to smoked cannabis studies

Tuttle, A. H. et al. 2015. Increasing placebo responses over time in U.S. clinical trials of neuropathic pain. *Pain, 156*(12), 2616-2626.

- Between 1990 & 2013, placebo responses increased significantly (p=0.002).
- While drug responses in pain initially decreased an average of 34.7% from baseline and were stable over time, producing 16.5% greater analgesia than placebo, or 1 point decrease in NRS, by 2013, treatment advantage decreased (p=0.0003) with only an 8.9% decrease in pain over baseline.
- Placebo responses increased with sample size (p=0.001), and study length (p=0.05), the worst differences by far in the USA.
- FDA requires 12 week RCTs in Phase III in accordance with IMMPACT **(Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials)** Guidelines, but this may be counterproductive!

Cannabis and Spasticity: Overcoming the Placebo Effect with a Randomized Withdrawal Design



Mean 48% improvement in spasticity on nabiximols over 16 weeks

Novotna J et al. *Eur J Neurol* 2011

- All patients begun on nabiximols (unbeknownst to them).
- After 4 weeks, only responders continued.
- At resupply visit, half received nabiximols at prior dose.
- Half received same number of sprays of placebo.
- Mirrors approaches in clinical practice.

Other Strategies to Reduce Placebo Effect in Cannabis-Based Medicine RCTs

- Utilize non-inhaled cannabis preparations (slower pharmacokinetics, avoiding peak serum/brain levels)
- Limit patient expectations: “This drug may or may not help you.”
- Treat patients in a neutral fashion.
- Avoid ancillary benefits (e.g., free massages at the dispensary).
- Utilize a slower delivery technique, i.e., not inhalation.
- Utilize preparations that attenuate THC psychoactivity with cannabidiol and terpenoid buffers.

Oregon, and the Future of Cannabis Research

- Federal roadblocks
- Clinical Research
- Basic Science Research
- Agricultural Research
  - Food
  - Fiber

Issues: Federal Roadblocks to Cannabis Research

- Rules created by successive administrations totally opposed to the concept of medicinal cannabis
- It's cannabis, not "marihuana"
- FDA not the problem (imho), as they "play by the book"
- Cannabis as Schedule I forbidden medicine
- MDs and NPs as gatekeepers, often with no educational foundation, and vast legal disincentives
- Available consumer info on chemovars woefully inadequate (e.g., strain names are essentially meaningless across jurisdictions with THC & CBD content specified without terpenoid profile)
- Many patients leery of restrictions

Clinical Cannabis Research in Oregon

- **Pros:**
- Oregonian legalization will open doors:
  - Attitude Shift
  - Scheduling issues reduced, or not?
- Good genetics available in state to develop and formulate medicines
- Interested and informed clinicians available, if sparsely, in some areas
- Approved conditions: Alzheimer disease, cachexia, cancer, chronic pain, glaucoma, HIV/AIDS, nausea, muscular spasms, PTSD, seizures, others subject to review.
- Estimated 71K current patients

- **Cons:**
- Large state with rural sprawl
- Centralized tertiary medical care a problem for recruiting rural clientele to RCTs
- Weather a hindrance in winter
- Professional ignorance and hostility inhibit clinical trial recruitment
- Why should patients enter a clinical trial when cannabis is already available?
- Brainwashed clinicians & political hierarchy
- Federal roadblocks persist: Is it possible to do top quality clinical research with non-NIDA cannabis?

Cannabis Basic Science Research

- **Needs:**
- Breeding expertise
- **Genetics**
- **Genomics**
- Ascertaining cannabinoid and terpenoid metabolic pathways and control (never done!)
- Optimizing cannabis component ratios
- Seed commerce

- **Issues:**
- Organic culture vs. GMO
- Pesticides
- Heavy Metals
- Microbiological contaminants
- Knowledge deficit in medical schools
- Opposition of hierarchies (insurers/medical societies)

Clinical Research Priorities

- Pain and Inflammation, particularly unstudied conditions
- Arthritis, both rheumatoid and osteoarthritis
- Inflammatory bowel disease (Crohn, ulcerative colitis)
- Metabolic syndrome/insulin resistance
- Dermatology: acne, psoriasis, contact dermatitis
- Neuroprotection in dementia, TBI, CVA
- Optimizing ECS health
- Lifestyle and nutritional research

} **Reduce cannabis need**

Prospective Cannabis Research in Oregon

What is needed:

1) Standardized GMP cannabis with appropriate cannabinoid & terpenoid profiles
2) Genuine Phase II and III clinical trials meeting FDA standards for pharmaceutical development
3) Parallel research on GMP-grade OTC products (topicals & skin care cosmeceuticals)

What is not needed:

1) More case-studies
2) More surveys
3) Additional NIDA-supplied studies that cannot be reproduced or advance therapeutics
4) Wasted public funds

Agricultural Hemp Research (possible under Oregon Senate Bill 676 of 2009

- **Food:**
- **Optimizing hemp seed nutrition**
- **Fiber Hemp**
- **Roots research for medicine and cosmetics**

Experimental plot, University of Kentucky EBR

Hemp Seed Nutrition

Photo: EBR

- Possibly the single most nutritionally complete food on earth, and powerful anti-inflammatory
- Contains all essential amino acids
- 35% protein, as digestible edestin
- 35% oil, rich in essential fatty acids (EFA) in 3:1 ω6:ω3 ratio:
  - 75% linoleic acid (LA, ω-6)
  - 25% linolenic acid (LNA, ω-3)
  - 9% gamma-linolenic acid (GLA, ω-6) (Callaway 2004)

Cannabis Root Components

EBR

Triterpenoids

Alkaloids

Sterols et al.

Roots: Basic Observations

- Ancient and folk contentions concerning therapeutic approaches with cannabis root are borne out by modern testing indicating strong anti-inflammatory and analgesic effects. Some evidence also supports activity against cancers, and as a febrifuge.
- The triterpenoids, friedelin and epifriedelanol, seem the best candidates.
- Little work has been done on root alkaloids.

Oregon & Cannabis: 10 Years Hence

The Crystal Ball
by
John William Waterhouse

- A disproportionate over-achiever in basic science?
- Great opportunities in clinical cannabis therapeutics, fiber production, hemp seed nutrition and cannabis-based cosmetics

Role of Cannabinoids in Pain Management

18

Ethan B. Russo and Andrea G. Hohmann

Key Points

- Cannabinoids are pharmacological agents of endogenous (endocannabinoids), botanical (phytocannabinoids), or synthetic origin.
- Cannabinoids alleviate pain through a variety of receptor and non-receptor mechanisms including direct analgesic and anti-inflammatory effects, modulatory actions on neurotransmitters, and interactions with endogenous and administered opioids.
- Cannabinoid agents are currently available in various countries for pain treatment, and even cannabinoids of botanical origin may be approvable by FDA, although this is distinctly unlikely for smoked cannabis.
- An impressive body of literature supports cannabinoid analgesia, and recently, this has been supplemented by an increasing number of phase I–III clinical trials.

Introduction

Plants and Pain

It is a curious fact that we owe a great deal of our insight into pharmacological treatment of pain to the plant world [1]. Willow bark from *Salix* spp. led to development of aspirin and eventual elucidation of the analgesic effects of prostaglandins and their role in inflammation. The opium poppy (*Papaver somniferum*) provided the prototypic narcotic analgesic morphine, the first alkaloid discovered, and stimulated the much later discovery of the endorphin and enkephalin systems. Similarly, the pharmacological properties of cannabis (*Cannabis sativa*) prompted the isolation of Δ^9-tetrahydrocannabinol (THC), the major psychoactive ingredient in cannabis, in 1964 [2]. It is this breakthrough that subsequently prompted the more recent discovery of the body's own cannabis-like system, the endocannabinoid system (ECS), which modulates pain under physiological conditions. Pro-nociceptive mechanisms of the endovanilloid system were similarly revealed by phytochemistry of capsaicin, the pungent ingredient in hot chile peppers (*Capsicum annuum* etc.), which activates transient receptor potential vanilloid receptor-1 (TRPV1). Additional plant products such as the mints and mustards activate other TRP channels to produce their physiological effects.

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The Endocannabinoid System

There are three recognized types of cannabinoids: (1) the phytocannabinoids [3] derived from the cannabis plant, (2) synthetic cannabinoids (e.g., ajulemic acid, nabilone, CP55940, WIN55, 212-2) based upon the chemical structure of THC or other ligands which bind cannabinoid receptors, and (3) the endogenous cannabinoids or endocannabinoids. Endocannabinoids are natural chemicals such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) found in animals whose basic functions are "relax, eat, sleep, forget, and protect" [4]. The endocannabinoid system encompasses the endocannabinoids themselves, their biosynthetic and catabolic enzymes, and their corresponding receptors [5]. AEA is hydrolyzed by the enzyme fatty-acid amide hydrolase (FAAH) into breakdown products arachidonic acid and ethanolamine [6]. By contrast, 2-AG is hydrolyzed primarily by the enzyme monoacylglycerol lipase (MGL) into breakdown products arachidonic acid and glycerol [7] and to a lesser extent by the enzymes ABHD6 and ABHD12. FAAH, a

T.R. Deer et al. (eds.), *Comprehensive Treatment of Chronic Pain by Medical, Interventional, and Integrative Approaches*,
DOI 10.1007/978-1-4614-1560-2_18, © American Academy of Pain Medicine 2013

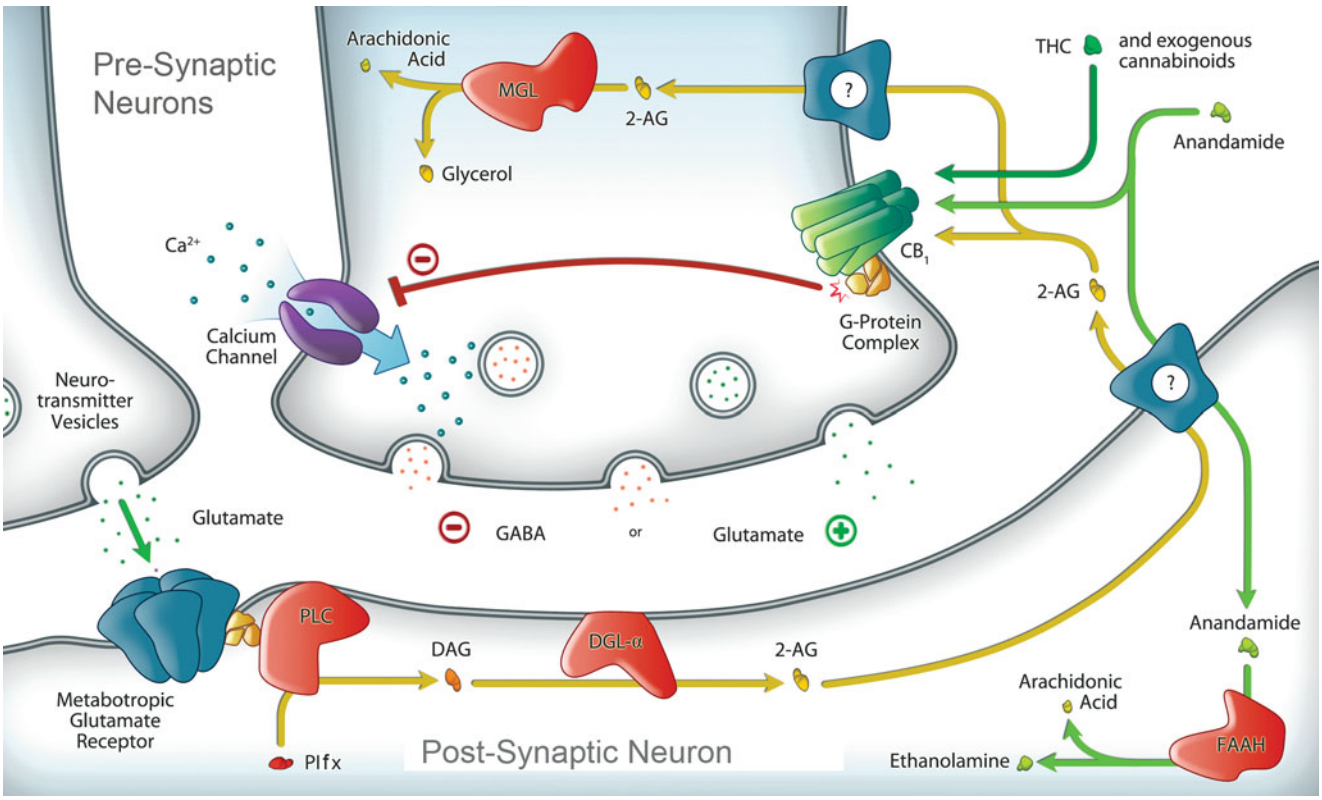

Fig. 18.1 Putative mechanism of endocannabinoid-mediated retrograde signaling in the nervous system. Activation of metabotropic glutamate receptors (*mGluR*) by glutamate triggers the activation of the phospholipase C (*PLC*)-diacylglycerol lipase (*DGL*) pathway to generate the endocannabinoid 2-arachidonoylglycerol (*2-AG*). First, the 2-AG precursor diacylglycerol (*DAG*) is formed from PLC-mediated hydrolysis of membrane phospholipid precursors (*PIPx*). DAG is then hydrolyzed by the enzyme DGL-α to generate 2-AG. 2-AG is released from the postsynaptic neuron and acts as a retrograde signaling molecule. Endocannabinoids activate presynaptic CB_1 receptors which reside on terminals of glutamatergic and GABAergic neurons. Activation of CB_1 by 2-AG, anandamide, or exogenous cannabinoids (e.g., tetrahydrocannabinol, *THC*) inhibits calcium influx in the presynaptic terminal, thereby inhibiting release of the primary neurotransmitter (i.e., glutamate or GABA) from the synaptic vesicle. Endocannabinoids are then rapidly deactivated by transport into cells (via a putative endocannabinoid transporter) followed by intracellular hydrolysis. 2-AG is metabolized by the enzyme monoacylglycerol lipase (*MGL*), whereas anandamide is metabolized by a distinct enzyme, fatty-acid amide hydrolase (*FAAH*). Note that MGL co-localizes with CB_1 in the presynaptic terminal, whereas FAAH is localized to postsynaptic sites. The existence of an endocannabinoid transporter remains controversial. Pharmacological inhibitors of either endocannabinoid deactivation (e.g., FAAH and MGL inhibitors) or transport (i.e., uptake inhibitors) have been developed to exploit the therapeutic potential of the endocannabinoid signaling system in the treatment of pain (Figure by authors with kind assistance of James Brodie, GW Pharmaceuticals)

postsynaptic enzyme, may control anandamide levels near sites of synthesis, whereas MGL, a presynaptic enzyme [8], may terminate 2-AG signaling following CB_1 receptor activation. These enzymes also represent therapeutic targets because inhibition of endocannabinoid deactivation will increase levels of endocannabinoids at sites with ongoing synthesis and release [9]. The pathways controlling formation of AEA remain poorly understood. However, 2-AG is believed to be formed from membrane phospholipid precursors through the sequential activation of two distinct enzymes, phospholipase C and diacylglycerol lipase-α. First, PLC catalyzes formation of the 2-AG precursor diacylglycerol (DAG) from membrane phosphoinositides. Then, DAG is hydrolyzed by the enzyme diacylglycerol lipase-α (DGL-α) to generate 2-AG [199].

There are currently two well-defined cannabinoid receptors, although additional candidate cannabinoid receptors have also been postulated. CB_1, a seven transmembrane spanning G-protein-coupled receptor inhibiting cyclic AMP release, was identified in 1988 [10]. CB_1 is the primary neuromodulatory receptor accounting for psychopharmacological effects of THC and most of its analgesic effects [11]. Endocannabinoids are produced on demand in postsynaptic cells and engage presynaptic CB_1 receptors through a retrograde mechanism [12]. Activation of presynaptic CB_1 receptors then acts as a synaptic circuit breaker to inhibit neurotransmitter release (either excitatory or inhibitory) from the presynaptic neuron (*vide infra*) (Fig. 18.1). CB_2 was identified in 1992, and while thought of primarily as a peripheral immunomodulatory receptor, it also has important

effects on pain. The role of CB_2 in modulating persistent inflammatory and neuropathic pain [13] has been recently reviewed [14, 15]. Activation of CB_2 suppresses neuropathic pain mechanisms through nonneuronal (i.e., microglia and astrocytes) and neuronal mechanisms that may involve interferon-gamma [16]. THC, the prototypical classical cannabinoid, is a weak partial agonist at both CB_1 and CB_2 receptors. Transgenic mice lacking cannabinoid receptors (CB_1, CB_2, GPR55), enzymes controlling endocannabinoid breakdown (FAAH, MGL, ABHD6), and endocannabinoid synthesis (DGL-α, DGL-β) have been generated [17]. These knockouts have helped elucidate the role of the endocannabinoid system in controlling nociceptive processing and facilitated development of inhibitors of endocannabinoid breakdown (FAAH, MGL) as novel classes of analgesics.

A Brief Scientific History of Cannabis and Pain

Centuries of Citations

Cannabis has been utilized in one form or another for treatment of pain for longer than written history [18–21]. Although this documentation has been a major preoccupation of the lead author [22–25], and such information can provide provocative direction to inform modern research on treatment of pain and other conditions, it does not represent evidence of form, content, or degree that is commonly acceptable to governmental regulatory bodies with respect to pharmaceutical development.

Anecdotes Versus Modern Proof of Concept

While thousands of compelling stories of efficacy of cannabis in pain treatment certainly underline the importance of properly harnessing cannabinoid mechanisms therapeutically [26, 27], prescription analgesics in the United States necessitate Food and Drug Administration (FDA) approval. This requires a rigorous development program proving consistency, quality, efficacy, and safety as defined by basic scientific studies and randomized controlled trials (RCT) [28] and generally adhering to recent IMMPACT recommendations [29], provoking our next question.

Can a Botanical Agent Become a Prescription Medicine?

Most modern physicians fail to recognize that pharmacognosy (study of medicinal plants) has led directly or indirectly to an estimated 25 % of modern pharmaceuticals [30]. While the plethora of available herbal agents yield an indecipherable cacophony to most clinicians and consumers alike, it is certainly possible to standardize botanical agents and facilitate their recommendation based on sound science [31]. Botanical medicines can even fulfill the rigorous dictates of the FDA and attain prescription drug status via a clear roadmap in the form of a blueprint document [32], henceforth termed the *Botanical Guidance*: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070491.pdf. To be successful and clinically valuable, botanicals, including cannabis-based medicines, must demonstrate the same quality, clinical analgesic benefit, and appropriately safe adverse event profile as available new chemical entities (NCE) [28].

The Biochemical and Neurophysiological Basis of Pain Control by Cannabinoids

Neuropathic Pain

Thorough reviews of therapeutic effects of cannabinoids in preclinical and clinical domains have recently been published [33, 34]. In essence, the endocannabinoid system (ECS) is active throughout the CNS and PNS in modulating pain at spinal, supraspinal, and peripheral levels. Endocannabinoids are produced on demand in the CNS to dampen sensitivity to pain [35]. The endocannabinoid system is operative in such key integrative pain centers as the periaqueductal grey matter [36, 37], the ventroposterolateral nucleus of the thalamus [38], and the spinal cord [39, 40]. Endocannabinoids are endogenous mediators of stress-induced analgesia and fear-conditioned analgesia and suppress pain-related phenomena such as windup [41] and allodynia [42]. In the periphery and PNS [13], the ECS has key effects in suppressing both hyperalgesia and allodynia via CB_1 [43] and CB_2 mechanisms (Fig. 18.2). Indeed, pathological pain states have been postulated to arise, at least in part, from a dysregulation of the endocannabinoid system.

Antinociceptive and Anti-inflammatory Pain Mechanisms

Beyond the mechanisms previously mentioned, the ECS plays a critical role in peripheral pain, inflammation, and hyperalgesia [43] through both CB_1 and CB_2 mechanisms. CB_1 and CB_2 mechanisms are also implicated in regulation of contact dermatitis and pruritus [44]. A role for spinal CB_2 mechanisms, mediated by microglia and/or astrocytes, is also revealed under conditions of inflammation [45]. Both THC and cannabidiol (CBD), a non-euphoriant phytocannabinoid common in certain cannabis strains, are potent anti-inflammatory antioxidants with activity exceeding that of

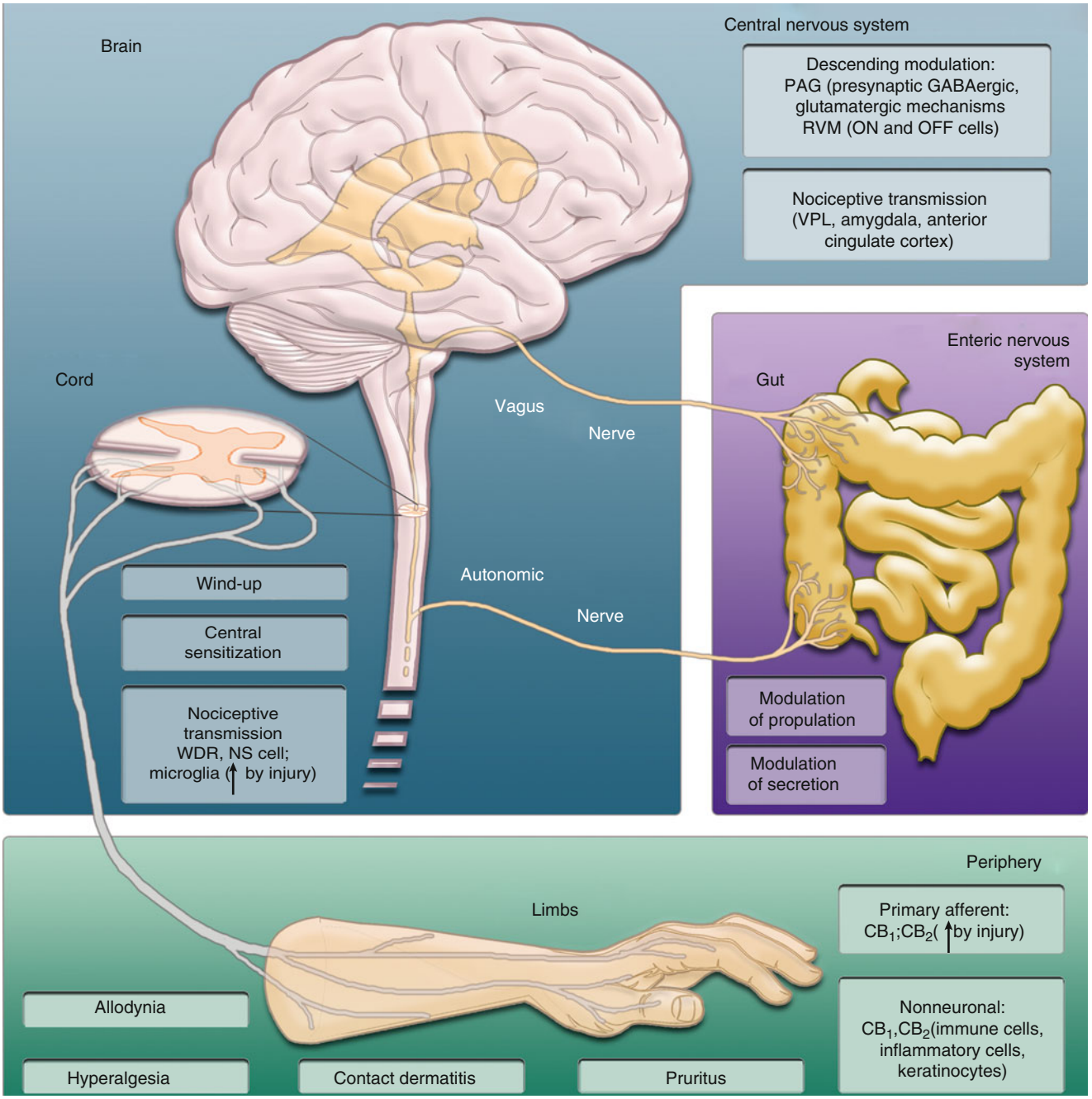

Fig. 18.2 Cannabinoids suppress pain and other pathophysiological (e.g., contact dermatitis, pruritis) and physiological (e.g., gastrointestinal transit and secretion) processes through multiple mechanisms involving CB_1 and CB_2 receptors. Peripheral, spinal, and supraspinal sites of cannabinoid actions are shown. In the periphery, cannabinoids act through both neuronal and nonneuronal mechanisms to control inflammation, allodynia, and hyperalgesia. CB_1 and CB_2 have been localized to both primary afferents and nonneuronal cells (e.g., keratinocytes, microglia), and expression can be regulated by injury. In the spinal cord, cannabinoids suppress nociceptive transmission, windup, and central sensitization by modulating activity in the ascending pain pathway of the spinothalamic tract, including responses of wide dynamic range (*WDR*) and nociceptive specific (*NS*) cells. Similar processes are observed at rostral levels of the neuraxis (e.g., ventroposterolateral nucleus of the thalamus, amygdala, anterior cingulate cortex). Cannabinoids also actively modulate pain through descending mechanisms. In the periaqueductal gray, cannabinoids act through presynaptic glutamatergic and GABAergic mechanisms to control nociception. In the rostral ventromedial medulla, cannabinoids suppress activity in ON cells and inhibit the firing pause of OFF cells, in response to noxious stimulation to produce antinociception (Figure by authors with kind assistance of James Brodie, GW Pharmaceuticals)

vitamins C and E via non-cannabinoid mechanisms [46]. THC inhibits prostaglandin E-2 synthesis [47] and stimulates lipooxygenase [48]. Neither THC nor CBD affects COX-1 or COX-2 at relevant pharmacological dosages [49].

While THC is inactive at vanilloid receptors, CBD, like AEA, is a $TRPV_1$ agonist. Like capsaicin, CBD is capable of inhibiting fatty-acid amide hydrolase (FAAH), the enzyme which hydrolyzes AEA and other fatty-acid amides that do not bind to cannabinoid receptors. CBD additionally inhibits AEA reuptake [50] though not potently. Thus, CBD acts as an endocannabinoid modulator [51], a mechanism that various pharmaceutical firms hope to emulate with new chemical entities (NCEs). CBD inhibits hepatic metabolism of THC to 11-hydroxy-THC, which is possibly more psychoactive, and prolongs its half-life, reducing its psychoactivity and attenuating attendant anxiety and tachycardia [51]; antagonizes psychotic symptoms [52]; and attenuates appetitive effects of THC [53] as well as its effects on short-term memory [54]. CBD also inhibits tumor necrosis factor-alpha (TNF-α) in a rodent model of rheumatoid arthritis [55]. Recently, CBD has been demonstrated to enhance adenosine receptor A2A signaling via inhibition of the adenosine transporter [56].

Recently, GPR18 has been proposed as a putative CBD receptor whose function relates to cellular migration [57]. Antagonism of GPR18 (by agents such as CBD) may be efficacious in treating pain of endometriosis, among other conditions, especially considering that such pain may be endocannabinoid-mediated [58]. Cannabinoids are also very active in various gastrointestinal and visceral sites mediating pain responses [59, 60].

Cannabinoid Interactions with Other Neurotransmitters Pertinent to Pain

As alluded to above, the ECS modulates neurotransmitter release via retrograde inhibition. This is particularly important in NMDA-glutamatergic mechanisms that become hyperresponsive in chronic pain states. Cannabinoids specifically inhibit glutamate release in the hippocampus [61]. THC reduces NMDA responses by 30–40 % [46]. Secondary and tertiary hyperalgesia mediated by NMDA [62] and by calcitonin gene-related peptide [40] may well be targets of cannabinoid therapy in disorders such as migraine, fibromyalgia, and idiopathic bowel syndrome wherein these mechanisms seem to operate pathophysiologically [63], prompting the hypothesis of a "clinical endocannabinoid deficiency." Endocannabinoid modulators may therefore restore homeostasis, leading to normalization of function in these pathophysiological conditions. THC also has numerous effects on serotonergic systems germane to migraine [64], increasing its production in the cerebrum while decreasing reuptake [65]. In fact, the ECS seems to modulate the trigeminovascular system of migraine pathogenesis at vascular and neurochemical levels [66–68].

Cannabinoid-Opioid Interactions

Although endocannabinoids do not bind to opioid receptors, the ECS may nonetheless work in parallel with the endogenous opioid system with numerous areas of overlap and interaction. Pertinent mechanisms include stimulation of beta-endorphin by THC [69] as well as its ability to demonstrate experimental opiate sparing [70], prevent opioid tolerance and withdrawal [71], and rekindle opioid analgesia after loss of effect [72]. Adjunctive treatments that combine opioids with cannabinoids may enhance the analgesic effects of either agent. Such strategies may permit lower doses of analgesics to be employed for therapeutic benefit in a manner that minimizes incidence or severity of adverse side effects.

Clinical Trials, Utility, and Pitfalls of Cannabinoids in Pain

Evidence for Synthetic Cannabinoids

Oral dronabinol (THC) has been available as the synthetic Marinol® since 1985 and is indicated for nausea associated with chemotherapy and appetite stimulation in HIV/AIDS. Issues with its cost, titration difficulties, delayed onset, and propensity to induce intoxicating and dysphoric effects have limited clinical application [73]. It was employed in two open-label studies of chronic neuropathic pain in case studies in 7 [74] and 8 patients [75], but no significant benefit was evident and side effects led to prominent dropout rates (average doses 15–16.6 mg THC). Dronabinol produced benefit in pain in multiple sclerosis [76], but none was evident in postoperative pain (Table 18.1) [77]. Dronabinol was reported to relieve pruritus in three case-report subjects with cholestatic jaundice [78]. Dronabinol was assessed in 30 chronic noncancer pain patients on opioids in double-blind crossover single-day sessions vs. placebo with improvement [79], followed by a 4-week open-label trial with continued improvement (Table 18.1). Associated adverse events were prominent. Methodological issues included lack of prescreening for cannabinoids, 4 placebo subjects with positive THC assays, and 58 % of subjects correctly guessing Marinol dose on test day. An open-label comparison in polyneuropathy examined nabilone patients with 6 obtaining 22.6 % mean pain relief after 3 months, and 5 achieving 28.6 % relief after 6 months, comparable to conventional agents [80]. A pilot study of Marinol in seven spinal cord injury patients with neuropathic pain saw two withdraw, and the remainder appreciate no greater efficacy than with diphenhydramine [81].

Table 18.1 Randomized controlled trials of cannabinoids in pain

Agent	*N*=	Indication	Duration/type	Outcomes/reference
Ajulemic acid	21	Neuropathic pain	7 day crossover	Visual analogue pain scales improved over placebo ($p=0.02$)/Karst et al. [92]
Cannabis, smoked	50	HIV neuropathy	5 days/DB	Decreased daily pain ($p=0.03$) and hyperalgesia ($p=0.05$), 52 % with >30 % pain reduction vs. placebo ($p=0.04$)/Abrams et al. [94]
Cannabis, smoked	23	Chronic neuropathic pain	5 days/DB	Decreased pain vs. placebo only at 9.4 % THC level ($p=0.023$)/Ware et al. [98]
Cannabis, smoked	38	Neuropathic pain	Single dose/DBC	NSD in pain except at highest cannabis dose ($p=0.02$), with prominent psychoactive effects/Wilsey et al. [95]
Cannabis, smoked	34	HIV neuropathy	5 days /DB	DDS improved over placebo ($p=0.016$), 46 % vs. 18 % improved >30 %, 2 cases toxic psychosis/Ellis et al. [97]
Cannabis, vaporized	21	Chronic pain on opioids	5 days/DB	27 % decrement in pain/Abrams et al. [118]
Cannador	419	Pain due to spasm in MS	15 weeks	Improvement over placebo in subjective pain associated with spasm ($p=0.003$)/Zajicek et al. [120]
Cannador	65	Postherpetic neuralgia	4 weeks	No benefit observed/Ernst et al. [122]
Cannador	30	Postoperative pain	Single doses, daily	Decreasing pain intensity with increased dose ($p=0.01$)/Holdcroft et al. [123]
Marinol	24	Neuropathic pain in MS	15–21 days/DBC	Median numerical pain ($p=0.02$), median pain relief improved ($p=0.035$) over placebo/Svendsen et al. [76]
Marinol	40	Postoperative pain	Single dose/DB	No benefit observed over placebo/Buggy et al. [77]
Marinol	30	Chronic pain	3 doses, 1 day/DBC	Total pain relief improved with 10 mg ($p<0.05$) and 20 mg ($p<0.01$) with opioids, AE prominent/Narang et al. [79]
Nabilone	41	Postoperative pain	3 doses in 24 h/DB	NSD morphine consumption. Increased pain at rest and on movement with nabilone 1 or 2 mg/Beaulieu [85]
Nabilone	31	Fibromyalgia	2 weeks/DBC	Compared to amitriptyline, nabilone improved sleep, decrease wakefulness, had no effect on pain, and increased AE/Ware et al. [90]
Nabilone	96	Neuropathic pain	14 weeks/DBC vs. dihydrocodeine	Dihydrocodeine more effective with fewer AE/Frank et al. [88]
Nabilone	13	Spasticity pain	9 weeks/DBC	NRS decreased 2 points for nabilone ($p<0.05$)/Wissel et al. [87]
Nabilone	40	Fibromyalgia	4 weeks/DBC	VAS decreased in pain, Fibromyalgia Impact Questionnaire, and anxiety over placebo (all, $p<0.02$)/Skrabek et al. [89]
Sativex	20	Neurogenic pain	Series of 2-week N-of-1 crossover blocks	Improvement with Tetranabinex and Sativex on VAS pain vs. placebo ($p<0.05$), symptom control best with Sativex ($p<0.0001$)/Wade et al. [132]
Sativex	24	Chronic intractable pain	12 weeks, series of N-of-1 crossover blocks	VAS pain improved over placebo ($p<0.001$) especially in MS ($p<0.0042$)/Notcutt et al. [133]
Sativex	48	Brachial plexus avulsion	6 weeks in 3 two-week crossover blocks	Benefits noted in Box Scale-11 pain scores with Tetranabinex ($p=0.002$) and Sativex ($p=0.005$) over placebo/Berman et al. [134]
Sativex	66	Central neuropathic pain in MS	5 weeks	Numerical Rating Scale (NRS) analgesia improved over placebo ($p=0.009$)/Rog et al. [135]

(continued)

Table 18.1 (continued)

Agent	*N*=	Indication	Duration/type	Outcomes/reference
Sativex	125	Peripheral neuropathic pain	5 weeks	Improvements in NRS pain levels ($p=0.004$), dynamic allodynia ($p=0.042$), and punctuate allodynia ($p=0.021$) vs. placebo/Nurmikko et al. [136]
Sativex	56	Rheumatoid arthritis	Nocturnal dosing for 5 weeks	Improvements over placebo morning pain on movement ($p=0.044$), morning pain at rest ($p=0.018$), DAS-28 ($p=0.002$), and SF-MPQ pain at present ($p=0.016$)/Blake et al. [138]
Sativex	117	Pain after spinal injury	10 days	NSD in NRS pain scores, but improved Brief Pain Inventory ($p=0.032$), and Patients' Global Impression of Change ($p=0.001$) (unpublished)
Sativex	177	Intractable cancer pain	2 weeks	Improvements in NRS analgesia vs. placebo ($p=0.0142$), Tetranabinex NSD/ Johnson et al. [139]
Sativex	135	Intractable lower urinary tract symptoms in MS	8 weeks	Improved bladder severity symptoms including pain over placebo ($p=0.001$) [200]
Sativex	360	Intractable cancer pain	5 weeks/DB	CRA of lower and middle-dose cohorts improved over placebo ($p=0.006$)/ [201]

Nabilone, or Cesamet®, is a semisynthetic analogue of THC that is about tenfold more potent, and longer lasting [82]. It is indicated as an antiemetic in chemotherapy in the USA. Prior case reports in neuropathic pain [83] and other pain disorders [84] have been published. Sedation and dysphoria are prominent associated adverse events. An RCT of nabilone in 41 postoperative subjects dosed TID actually resulted in increased pain scores (Table 18.1) [85]. An uncontrolled study of 82 cancer patients on nabilone noted improved pain scores [86], but retention rates were limited. Nabilone improved pain ($p<0.05$) vs. placebo in patients with mixed spasticity syndromes in a small double-blind trial (Table 18.1) [87], but was without benefits in other parameters. In a double-blind crossover comparison of nabilone to dihydrocodeine (schedule II opioid) in chronic neuropathic pain (Table 18.1) [88], both drugs produced marginal benefit, but with dihydrocodeine proving clearly superior in efficacy and modestly superior in side-effect profile. In an RCT in 40 patients of nabilone vs. placebo over 4 weeks, it showed significant decreases in VAS of pain and anxiety (Table 18.1) [89]. A more recent study of nabilone vs. amitriptyline in fibromyalgia yielded benefits on sleep, but not pain, mood, or quality of life (Table 18.1) [90]. An open-label trial of nabilone vs. gabapentin found them comparable in pain and other symptom relief in peripheral neuropathic pain [91].

Ajulemic acid (CT3), another synthetic THC analogue in development, was utilized in a phase II RCT in peripheral neuropathic pain in 21 subjects with apparent improvement (Table 18.1) [92]. Whether or not ajulemic acid is psychoactive is the subject of some controversy [93].

Evidence for Smoked or Vaporized Cannabis

Few randomized controlled clinical trials (RCTs) of pain with smoked cannabis have been undertaken to date [94–97]. One of these [96] examined cannabis effects on experimental pain in normal volunteers.

Abrams et al. [94] studied inpatient adults with painful HIV neuropathy in 25 subjects in double-blind fashion to receive either smoked cannabis as 3.56 % THC cigarettes or placebo cigarettes three times daily for 5 days (Table 18.1). The smoked cannabis group had a 34 % reduction in daily pain vs. 17 % in the placebo group ($p=0.03$). The cannabis cohort also had a 52 % of subjects report a >30 % reduction in pain scores over the 5 days vs. 24 % in the placebo group ($p=0.04$) (Table 18.1). The authors rated cannabis as "well tolerated" due to an absence of serious adverse events (AE) leading to withdrawal, but all subjects were cannabis experienced. Symptoms of possible intoxication in the cannabis group including anxiety (25 %), sedation (54 %), disorientation (16 %), paranoia (13 %), confusion (17 %), dizziness (15 %), and nausea (11 %) were all statistically significantly more common than in the placebo group. Despite these findings, the authors stated that the values do not represent any serious safety concern in this short-term study. No discussion in the article addressed issues of the relative efficacy of blinding in the trial.

Wilsey et al. [95] examined neuropathic pain in 38 subjects in a double-blind crossover study comparing 7 % THC cannabis, 3.5 % THC cannabis, and placebo cigarettes via a complex cumulative dosing scheme with each dosage given

once, in random order, with at least 3 day intervals separating sessions (Table 18.1). A total of 9 puffs maximum were allowed over several hours per session. Authors stated, "Psychoactive effects were minimal and well-tolerated, but neuropsychological impairment was problematic, particularly with the higher concentration of study medication." Again, only cannabis-experienced subjects were allowed entry. No withdrawals due to AE were reported, but 1 subject was removed due to elevated blood pressure. No significant differences were noted in pain relief in the two cannabis potency groups, but a significant separation of pain reduction from placebo ($p=0.02$) was not evident until a cumulative 9 puffs at 240 min elapsed time. Pain unpleasantness was also reduced in both active treatment groups ($p<0.01$). Subjectively, an "any drug effect" demonstrated a visual analogue scale (VAS) of 60/100 in the high-dose group, but even the low-dose group registered more of a "good drug effect" than placebo ($p<0.001$). "Bad drug effect" was also evident. "Feeling high" and "feeling stoned" were greatest in the high-dose sessions ($p<0.001$), while both high- and low-dose differentiated significantly from placebo ($p<0.05$). Of greater concern, both groups rated impairment as 30/100 on VAS vs. placebo ($p=0.003$). Sedation also demarcated both groups from placebo ($p<0.01$), as did confusion ($p=0.03$), and hunger ($p<0.001$). Anxiety was not considered a prominent feature in this cannabis-experienced population. This study distinguished itself from some others in its inclusion of specific objective neuropsychological measures and demonstrated neurocognitive impairment in attention, learning, and memory, most noteworthy with 7 % THC cannabis. No commentary on blinding efficacy was included.

Ellis et al. [97] examined HIV-associated neuropathic pain in a double-blind trial of placebo vs. 1–8 % THC cannabis administered four times daily over 5 days with a 2-week washout (Table 18.1). Subjects were started at 4 % THC and then titrated upward or downward in four smoking sessions dependent upon their symptom relief and tolerance of the dose. In this study, 96 % of subjects were cannabis-experienced, and 28 out of 34 subjects completed the trial. The primary outcome measure (Descriptor Differential Scale, DDS) was improved in the active group over placebo ($p=0.016$), with >30 % relief noted in 46 % of cannabis subjects vs. 18 % of placebo. While most adverse events (AE) were considered mild and self-limited, two subjects had to leave the trial due to toxicity. One cannabis-naïve subject was withdrawn due to "an acute cannabis-induced psychosis" at what proved to be his first actual cannabis exposure. The other subject suffered intractable cough. Pain reduction was greater in the cannabis-treated group ($p=0.016$) among completers, as was the proportion of subjects attaining >30 % pain reduction (46 % vs. 18 %, $p=0.043$). Blinding was assessed in this study; whereas placebo patients were inaccurate at guessing the investigational product, 93 % of those receiving cannabis guessed correctly. On safety issues, the authors stated that the frequency of some nontreatment-limiting side effects was greater for cannabis than placebo. These included concentration difficulties, fatigue, sleepiness or sedation, increased duration of sleep, reduced salivation, and thirst.

A Canadian study [98] examined single 25-mg inhalations of various cannabis potencies (0–9.4 % THC) three times daily for 5 days per cycle in 23 subjects with chronic neuropathic pain (Table 18.1). Patients were said to be cannabis-free for 1 year, but were required to have some experience of the drug. Only the highest potency demarcated from placebo on decrements in average daily pain score (5.4 vs. 6.1, $p=0.023$). The most frequent AE in the high-dose group were headache, dry eyes, burning sensation, dizziness, numbness, and cough, but with "high" or "euphoria" reported only once in each cannabis potency group.

The current studies of smoked cannabis are noteworthy for their extremely short-term exposure and would be of uncertain relevance in a regulatory environment. The IMMPACT recommendations on chronic neuropathic pain clinical trials that are currently favored by the FDA [29] generally suggest randomized controlled clinical trials of 12-week duration as a prerequisite to demonstrate efficacy and safety. While one might assume that the degree of pain improvement demonstrated in these trials could be maintained over this longer interval, it is only reasonable to assume that cumulative adverse events would also increase to at least some degree. The combined studies represent only a total of 1,106 patient-days of cannabis exposure (Abrams: 125, Wilsey: 76, Ellis: 560, Ware 345) or 3 patient-years of experience. In contrast, over 6,000 patient-years of data have been analyzed for Sativex between clinical trials, prescription, and named-patient supplies, with vastly lower AE rates (data on file, GW Pharmaceuticals) [28, 99]. Certainly, the cognitive effects noted in California-smoked cannabis studies figure among many factors that would call the efficacy of blinding into question for investigations employing such an approach. However, it is also important to emphasize that unwanted side effects are not unique to cannabinoids. In a prospective evaluation of specific chronic polyneuropathy syndromes and their response to pharmacological therapies, the presence of intolerable side effects did not differ in groups receiving gabapentinoids, tricyclic antidepressants, anticonvulsants, cannabinoids (including nabilone, Sativex), and topical agents [80]. Moreover, no serious adverse events were related to any of the medications.

The current studies were performed in a very select subset of patients who almost invariably have had prior experience of cannabis. Their applicability to cannabis-naïve populations is, thus, quite unclear. At best, the observed benefits might possibly accrue to some, but it is eminently likely that candidates for such therapy might refuse it on any number of

grounds: not wishing to smoke, concern with respect to intoxication, etc. Sequelae of smoking in therapeutic outcomes have had little discussion in these brief RCTs [28]. Cannabis smoking poses substantial risk of chronic cough and bronchitic symptoms [100], if not obvious emphysematous degeneration [101] or increase in aerodigestive cancers [102]. Even such smoked cannabis proponents as Lester Grinspoon has acknowledged are the only well-confirmed deleterious physical effect of marihuana is harm to the pulmonary system [103]. However, population-based studies of cannabis trials have failed to show any evidence for increased risk of respiratory symptoms/chronic obstructive pulmonary disease [100] or lung cancer [102] associated with smoking cannabis.

A very detailed analysis and comparison of mainstream and sidestream smoke for cannabis vs. tobacco smoke was performed in Canada [104]. Of note, cannabis smoke contained ammonia (NH_3) at a level of 720 μg per 775 mg cigarette, a figure 20-fold higher than that found in tobacco smoke. It was hypothesized that this finding was likely attributable to nitrate fertilizers. Formaldehyde and acetaldehyde were generally lower in cannabis smoke than in tobacco, but butyraldehyde was higher. Polycyclic aromatic hydrocarbon (PAH) contents were qualitatively similar in the comparisons, but total yield was lower for cannabis mainstream smoke, but higher than tobacco for sidestream smoke. Additionally, NO, NO_x, hydrogen cyanide, and aromatic amines concentrations were 3–5 times higher in cannabis smoke than that from tobacco. Possible mutagenic and carcinogenic potential of these various compounds were mentioned. More recently, experimental analysis of cannabis smoke with resultant acetaldehyde production has posited its genotoxic potential to be attributable to reactions that produce DNA adducts [105].

Vaporizers for cannabis have been offered as a harm reduction technique that would theoretically eliminate products of combustion and associated adverse events. The Institute of Medicine (IOM) examined cannabis issues in 1999 [106], and among their conclusions was the following (p. 4): "Recommendation 2: Clinical trials of cannabinoid drugs for symptom management should be conducted with the goal of developing rapid-onset, reliable, and safe delivery systems." One proposed technique is vaporization, whereby cannabis is heated to a temperature that volatilizes THC and other components with the goal of reducing or eliminating by-products of combustion, including potentially carcinogenic polycyclic aromatic hydrocarbons, benzene, acetaldehyde, carbon monoxide, toluene, naphthaline, phenol, toluene, hydrogen cyanide, and ammonia. Space limitations permit only a cursory review of available literature [107–115].

A pilot study of the Volcano vaporizer vs. smoking was performed in the USA in 2007 in 18 active cannabis consumers, with only 48 h of presumed abstinence [116]. NIDA 900-mg cannabis cigarettes were employed (1.7, 3.4, and 6.8 % THC) with each divided in two, so that one-half would be smoked or vaporized in a series of double-blind sessions. The Volcano vaporizer produced comparable or slightly higher THC plasma concentrations than smoking. Measured CO in exhaled vapor sessions diminished very slightly, while it increased after smoking ($p<0.001$). Self-reported visual analogue scales of the associated high were virtually identical in vaporization vs. smoking sessions and increased with higher potency material. A contention was advanced that the absence of CO increase after vaporization can be equated to "little or no exposure to gaseous combustion toxins." Given that no measures of PAH or other components were undertaken, the assertion is questionable. It was also stated that there were no reported adverse events. Some 12 subjects preferred the Volcano, 2 chose smoking, and 2 had no preference as to technique, making the vaporizer "an acceptable system" and providing "a safer way to deliver THC."

A recent [202, 117] examined interactions of 3.2 % THC NIDA cannabis vaporized in the Volcano in conjunction with opioid treatment in a 5-day inpatient trial in 21 patients with chronic pain (Table 18.1). All subjects were prior cannabis smokers. Overall, pain scores were reduced from 39.6 to 29.1 on a VAS, a 27 % reduction, by day 5. Pain scores in subjects on morphine fell from 34.8 to 24.1, while in subjects taking oxycodone, scores dropped from 43.8 to 33.6.

The clinical studies performed with vaporizers to date have been very small pilot studies conducted over very limited timeframes (i.e., for a maximum of 5 days). Thus, these studies cannot contribute in any meaningful fashion toward possible FDA approval of vaporized cannabis as a delivery technique, device, or drug under existing policies dictated by the *Botanical Guidance* [32]. It is likewise quite unlikely that the current AE profile of smoked or vaporized cannabis would meet FDA requirements. The fact that all the vaporization trials to date have been undertaken only in cannabis-experienced subjects does not imply that results would generalize to larger patient populations. Moreover, there is certainly no reason to expect AE profiles to be better in cannabis-naïve patients. Additionally, existing standardization of cannabis product and delivery via vaporization seem far off the required marks. Although vaporizers represent an alternate delivery method devoid of the illegality associated with smoked cannabis, the presence of toxic ingredients such as PAH, ammonia, and acetaldehyde in cannabis vapor are unlikely to be acceptable to FDA in any significant amounts. Existing vaporizers still lack portability or convenience [28]. A large Internet survey revealed that only 2.2 % of cannabis users employed vaporization as their primary cannabis intake method [118]. While studies to date have established that lower temperature vaporization in the Volcano, but not necessarily other devices, can reduce the relative amounts of noxious by-products of combustion, it has yet to be demonstrated that they are totally eliminated. Until or unless this goal is achieved, along with

requisite benchmarks of herbal cannabis quality, safety, and efficacy in properly designed randomized clinical trials, vaporization remains an unproven technology for therapeutic cannabinoid administration.

Evidence for Cannabis-Based Medicines

Cannador is a cannabis extract in oral capsules, with differing THC:CBD ratios [51]. Cannador was utilized in a phase III RCT of spasticity in multiple sclerosis (CAMS) (Table 18.1) [119]. While no improvement was evident in the Ashworth Scale, reduction was seen in spasm-associated pain. Both THC and Cannador improved pain scores in follow-up [120]. Cannador was also employed for postherpetic neuralgia in 65 patients, but without success (Table 18.1) [121, 122]. Slight pain reduction was observed in 30 subjects with postoperative pain (CANPOP) not receiving opiates, but psychoactive side effects were notable (Table 18.1).

Sativex® is a whole-cannabis-based extract delivered as an oromucosal spray that combines a CB_1 and CB_2 partial agonist (THC) with a cannabinoid system modulator (CBD), minor cannabinoids, and terpenoids plus ethanol and propylene glycol excipients and peppermint flavoring [51, 123]. It is approved in Canada for spasticity in MS and under a Notice of Compliance with Conditions for central neuropathic pain in multiple sclerosis and treatment of cancer pain unresponsive to opioids. Sativex is also approved in MS in the UK, Spain, and New Zealand, for spasticity in multiple sclerosis, with further approvals expected soon in some 22 countries around the world. Sativex is highly standardized and is formulated from two *Cannabis sativa* chemovars predominating in THC and CBD, respectively [124]. Each 100 µl pump-action oromucosal spray of Sativex yields 2.7 mg of THC and 2.5 mg of CBD plus additional components. Pharmacokinetic data are available [125–127]. Sativex effects begin within an interval allowing dose titration. A very favorable adverse event profile has been observed in the development program [27, 128]. Most patients stabilize at 8–10 sprays per day after 7–10 days, attaining symptomatic control without undue psychoactive sequelae. Sativex was added to optimized drug regimens in subjects with uncontrolled pain in every RCT (Table 18.1). An Investigational New Drug (IND) application to study Sativex in advanced clinical trials in the USA was approved by the FDA in January 2006 in patients with intractable cancer pain. One phase IIB dose-ranging study has already been completed [201]. Available clinical trials with Sativex have been independently assessed [129, 130].

In a phase II study of 20 patients with neurogenic symptoms [131], significant improvement was seen with both Tetranabinex (high-THC extract without CBD) and Sativex on pain, with Sativex displaying better symptom control ($p<0.0001$), with less intoxication (Table 18.1).

In a phase II study of intractable chronic pain in 24 patients [132], Sativex again produced the best results compared to Tetranabinex ($p<0.001$), especially in MS ($p<0.0042$) (Table 18.1).

In a phase III study of brachial plexus avulsion ($N=48$) [133], pain reduction with Tetranabinex and Sativex was about equal (Table 18.1).

In an RCT of 66 MS subjects, mean Numerical Rating Scale (NRS) analgesia favored Sativex over placebo (Table 18.1) [134].

In a phase III trial ($N=125$) of peripheral neuropathic pain with allodynia [135], Sativex notably alleviated pain levels and dynamic and punctate allodynia (Table 18.1).

In a safety-extension study in 160 subjects with various symptoms of MS [136], 137 patients showed sustained improvements over a year or more in pain and other symptoms [99] without development of any tolerance requiring dose escalation or withdrawal effects in those who voluntarily discontinued treatment suddenly. Analgesia was quickly reestablished upon Sativex resumption.

In a phase II RCT in 56 rheumatoid arthritis sufferers over 5 weeks with Sativex [137], medicine was limited to only 6 evening sprays (16.2 mg THC + 15 mg CBD). By study end, morning pain on movement, morning pain at rest, DAS-28 measure of disease activity, and SF-MPQ pain all favored Sativex (Table 18.1).

In a phase III RCT in intractable cancer pain on opioids ($N=177$), Sativex, Tetranabinex THC-predominant extract, and placebo were compared [138] demonstrating strongly statistically significant improvements in analgesia for Sativex only (Table 18.1). This suggests that the CBD component in Sativex was necessary for benefit.

In a 2-week study of spinal cord injury pain, NRS of pain was not statistically different from placebo, probably due to the short duration of the trial, but secondary endpoints were positive (Table 18.1). Additionally, an RCT of intractable lower urinary tract symptoms in MS also demonstrated pain reduction (Table 18.1).

The open-label study of various polyneuropathy patients included Sativex patients with 3 obtaining 21.56 % mean pain relief after 3 months (2/3 > 30 %), and 4 achieving 27.6 % relief after 6 months (2/4 > 30 %), comparable to conventional agents [80].

A recently completed RCT of Sativex in intractable cancer pain unresponsive to opioids over 5 weeks was performed in 360 subjects (Table 18.1). Results of a Continuous Response Analysis (CRA) showed improvements over placebo in the low-dose ($p=0.08$) and middle-dose cohorts ($p=0.038$) or combined ($p=0.006$). Pain NRS improved over placebo in the low-dose ($p=0.006$) and combined cohorts ($p=0.019$).

Sleep has improved markedly in almost all Sativex RCTs in chronic pain based on symptom reduction, not a hypnotic effect [139].

The adverse event (AE) profile of Sativex has been quite benign with bad taste, oral stinging, dry mouth, dizziness, nausea, or fatigue most common, but not usually prompting discontinuation [128]. Most psychoactive sequelae are early and transient and have been notably lowered by more recent application of a slower, less aggressive titration schedule. While no direct comparative studies have been performed with Sativex and other agents, AE rates were comparable or greater with Marinol than with Sativex employing THC dosages some 2.5 times higher, likely due to the presence of accompanying CBD [28, 51]. Similarly, Sativex displayed a superior AE profile compared to smoked cannabis based on safety-extension studies of Sativex [28, 99], as compared to chronic use of cannabis with standardized government-supplied material in Canada for chronic pain [140] and the Netherlands for various indications [141, 142] over a period of several months or more. All AEs are more frequent with smoked cannabis, except for nausea and dizziness, both early and usually transiently reported with Sativex [27, 28, 128]. A recent meta-analysis suggested that serious AEs associated with cannabinoid-based medications did not differ from placebo and thus could not be attributable to cannabinoid use, further reinforcing the low toxicity associated with activation of cannabinoid systems.

Cannabinoid Pitfalls: Are They Surmountable?

The dangers of COX-1 and COX-2 inhibition by nonsteroidal anti-inflammatory drugs (NSAIDS) of various design (e.g., gastrointestinal ulceration and bleeding vs. coronary and cerebrovascular accidents, respectively) [143, 144] are unlikely to be mimicked by either THC or CBD, which produce no such activity at therapeutic dosages [49].

Natural cannabinoids require polar solvents and may be associated with delayed and sometimes erratic absorption after oral administration. Smoking of cannabis invariably produces rapid spikes in serum THC levels; cannabis smoking attains peak levels of serum THC above 140 ng/ml [145, 146], which, while desirable to the recreational user, has no necessity or advantage for treatment of chronic pain [28]. In contrast, comparable amounts of THC derived from oromucosal Sativex remained below 2 ng/ml with much lower propensity toward psychoactive sequelae [28, 125], with subjective intoxication levels on visual analogue scales that are indistinguishable from placebo, in the single digits out of 100 [100]. It is clear from RCTs that such psychoactivity is not a necessary accompaniment to pain control. In contrast, intoxication has continued to be prominent with oral THC [73].

In comparison to the questionable clinical trial blinding with smoked and vaporized cannabis discussed above, all indications are that such study blinding has been demonstrably effective with Sativex [147, 148] by utilizing a placebo spray with identical taste and color. Some 50 % of Sativex subjects in RCTs have had prior cannabis exposure, but results of two studies suggest that both groups exhibited comparable results in both treatment efficacy and side effect profile [134, 135].

Controversy continues to swirl around the issue of the potential dangers of cannabis use medicinally, particularly its drug abuse liability (DAL). Cannabis and cannabinoids are currently DEA schedule I substances and are forbidden in the USA (save for Marinol in schedule III and nabilone in schedule II) [73]. This is noteworthy in itself because the very same chemical compound, THC, appears simultaneously in schedule I (as THC), schedule II (as nabilone), and schedule III (as Marinol). DAL is assessed on the basis of five elements: intoxication, reinforcement, tolerance, withdrawal, and dependency plus the drug's overall observed rates of abuse and diversion. Drugs that are smoked or injected are commonly rated as more reinforcing due to more rapid delivery to the brain [149]. Sativex has intermediate onset. It is claimed that CBD in Sativex reduces the psychoactivity of THC [28]. RCT AE profiles do not indicate euphoria or other possible reinforcing psychoactive indicia as common problems with its use [99]. Similarly, acute THC effects such as tachycardia, hypothermia, orthostatic hypotension, dry mouth, ocular injection, and intraocular pressure decreases undergo prominent tachyphylaxis with regular usage [150]. Despite that observation, Sativex has not demonstrated dose tolerance to its therapeutic benefits on prolonged administration, and efficacy has been maintained for up to several years in pain conditions [99].

The existence or severity of a cannabis withdrawal syndrome remains under debate [151, 152]. In contrast to reported withdrawal sequelae in recreational users [153], 24 subjects with MS who volunteered to discontinue Sativex after a year or more suffered no withdrawal symptoms meeting Budney criteria. While symptoms such as pain recurred after some 7–10 days without Sativex, symptom control was rapidly reattained upon resumption [99].

Finally, no known abuse or diversion incidents have been reported with Sativex to date (March 2011). Formal DAL studies of Sativex vs. Marinol and placebo have been completed and demonstrate lower scores on drug liking and similar measures at comparable doses [155].

Cognitive effects of cannabis also remain at issue [155, 156], but less data are available in therapeutic applications. Studies of Sativex in neuropathic pain with allodynia have revealed no changes vs. placebo on Sativex in portions of the Halstead-Reitan Battery [135], or in central neuropathic pain in MS [134], where 80 % of tests showed no significant differences. In a recent RCT of Sativex vs. placebo in MS patients, no cognitive differences of note were observed

[157]. Similarly, chronic Sativex use has not produced observable mood disorders.

Controversies have also arisen regarding the possible association of cannabis abuse and onset of psychosis [156]. However, an etiological relationship is not supported by epidemiological data [158–161], but may well be affected by dose levels and duration, if pertinent. One may speculate that lower serum levels of Sativex combined with antipsychotic properties of CBD [52, 162, 163] might attenuate such concerns. Few cases of related symptoms have been reported in SAFEX studies of Sativex.

Immune function becomes impaired in experimental animals at cannabinoid doses 50–100 times necessary to produce psychoactive effects [164]. In four patients smoking cannabis medicinally for more than 20 years, no changes were evident in leukocyte, CD4, or CD8 cell counts [155]. MS patients on Cannador demonstrated no immune changes of note [165] nor were changes evident in subjects smoking cannabis in a brief trial in HIV patients [166]. Sativex RCTs have demonstrated no hematological or immune dysfunction.

No effects of THC extract, CBD extract, or Sativex were evident on the hepatic cytochrome P450 complex [167] or on human CYP450 [168]. Similarly, while Sativex might be expected to have additive sedative effects with other drugs or alcohol, no significant drug-drug interactions of any type have been observed in the entire development program to date.

No studies have demonstrated significant problems in relation to cannabis affecting driving skills at plasma levels below 5 ng/ml of THC [169]. Four oromucosal sprays of Sativex (exceeding the average single dose employed in therapy) produced serum levels well below this threshold [28]. As with other cannabinoids in therapy, it is recommended that patients not drive nor use dangerous equipment until accustomed to the effects of the drug.

Future Directions: An Array of Biosynthetic and Phytocannabinoid Analgesics

Inhibition of Endocannabinoid Transport and Degradation: A Solution?

It is essential that any cannabinoid analgesic strike a compromise between therapeutic and adverse effects that may both be mediated via CB_1 mechanisms [34]. Mechanisms to avoid psychoactive sequelae could include peripherally active synthetic cannabinoids that do not cross the blood-brain barrier or drugs that boost AEA levels by inhibiting fatty-acid amide hydrolase (FAAH) [170] or that of 2-AG by inhibiting monoacylycerol lipase (MGL). CBD also has this effect [50] and certainly seems to increase the therapeutic index of THC [51].

In preclinical studies, drugs inhibiting endocannabinoid hydrolysis [171, 172] and peripherally acting agonists [173] all show promise for suppressing neuropathic pain. AZ11713908, a peripherally restricted mixed cannabinoid agonist, reduces mechanical allodynia with efficacy comparable to the brain penetrant mixed cannabinoid agonist WIN55,212-2 [173]. An irreversible inhibitor of the 2-AG hydrolyzing enzyme MGL suppresses nerve injury-induced mechanical allodynia through a CB_1 mechanism, although these anti-allodynic effects undergo tolerance following repeated administration [172]. URB937, a brain impermeant inhibitor of FAAH, has recently been shown to elevate anandamide outside the brain and suppress neuropathic and inflammatory pain behavior without producing tolerance or unwanted CNS side effects [171]. These observations raise the possibility that peripherally restricted endocannabinoid modulators may show therapeutic potential as analgesics with limited side-effect profiles.

The Phytocannabinoid and Terpenoid Pipeline

Additional phytocannabinoids show promise in treatment of chronic pain [123, 163, 174]. Cannabichromene (CBC), another prominent phytocannabinoid, also displays anti-inflammatory [175] and analgesic properties, though less potently than THC [176]. CBC, like CBD, is a weak inhibitor of AEA reuptake [177]. CBC is additionally a potent TRPA1 agonist [178]. Cannabigerol (CBG), another phytocannabinoid, displays weak binding at both CB_1 and CB_2 [179, 180] but is a more potent GABA reuptake inhibitor than either THC or CBD [181]. CBG is a stronger analgesic, anti-erythema, and lipooxygenase agent than THC [182]. CBG likewise inhibits AEA uptake and is a TRPV1 agonist [177], a TRPA1 agonist, and a TRPM8 antagonist [178]. CBG is also a phospholipase A2 modulator that reduces PGE-2 release in synovial cells [183]. Tetrahydrocannabivarin, a phytocannabinoid present in southern African strains, displays weak CB_1 antagonism [184] and a variety of anticonvulsant activities [185] that might prove useful in chronic neuropathic pain treatment. THCV also reduced inflammation and attendant pain in mouse experiments [187]. Most North American [187] and European [188, 189] cannabis strains have been bred to favor THC over a virtual absence of other phytocannabinoid components, but the latter are currently available in abundance via selective breeding [124, 190].

Aromatic terpenoid components of cannabis also demonstrate pain reducing activity [123, 163]. Myrcene displays an opioid-type analgesic effect blocked by naloxone [191] and reduces inflammation via PGE-2 [192]. β-Caryophyllene displays anti-inflammatory activity on par with phenylbutazone via PGE-1 [193], but contrasts by displaying gastric cytoprotective activity [194]. Surprisingly, β-caryophyllene has proven to be a phytocannabinoid in its own right as a selective CB_2 agonist [195]. α-Pinene inhibits PGE-1 [196], and linalool acts as a local anesthetic [197].

Summary

Basic science and clinical trials support the theoretical and practical basis of cannabinoid agents as analgesics for chronic pain. Their unique pharmacological profiles with multimodality effects and generally favorable efficacy and safety profiles render cannabinoid-based medicines promising agents for adjunctive treatment, particularly for neuropathic pain. It is our expectation that the coming years will mark the advent of numerous approved cannabinoids with varying mechanisms of action and delivery techniques that should offer the clinician useful new tools for treating pain.

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Current Status and Future of Cannabis Research

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Although cannabis is primarily viewed by the public as a recreational drug or agent of abuse, its medical application spans recorded history.[1,2] Evolution has yielded a cannabis plant that produces a family of some 100 chemicals called phytocannabinoids ("plant cannabinoids"), many of which have distinct and valuable therapeutic effects.[3,4]

Cannabis is a versatile herb that can produce a variety of medicinal preparations with distinct pharmacologic properties, depending on the content of cannabinoids and other phytochemicals, many of which possess synergistic effects.[4] The best known plant cannabinoid is tetrahydrocannabinol (THC), the primary psychoactive agent in cannabis, responsible for the preponderance of the cannabis "high"; however, it is also a powerful analgesic,[5] muscle relaxant,[6] and antinausea agent,[7] among myriad other effects. Coming to greater recognition is its analogue sister, cannabidiol (CBD), which distinguishes itself by its lack of intoxication and its ability to complement the pain relief, antiemetic, anticonvulsant,[8] and other benefits of THC, while modulating and attenuating its associated side effects (anxiety, tachycardia, et al.).[4,9–13]

Cannabis is a versatile herb that can produce a variety of medicinal preparations with distinct pharmacologic properties.

To gain regulatory approval of a cannabis-based product, pursuing the dietary supplement/botanical path—as opposed to the pharmaceutical one—may be an option for certain preparations. Dietary supplements rarely contain substances with abuse potential, and manufacturers and vendors of such products can make only "structure and function" claims (e.g., "promotes heart health"), rather than medical claims. Therefore, it is probably unlikely that cannabis preparations with a notable amount of THC could be treated as dietary supplements. However, nonpsychoactive cannabinoids, such as CBD could be descheduled (i.e., removed from the federal Controlled Substances Act [CSA]) and developed and marketed as botanical supplements.

Cannabis exerts its effects through a variety of receptor and nonreceptor mechanisms. All vertebrates tested to date harbor an endogenous cannabinoid system (ECS),[14] a regulator of physiological homeostasis whose function has been summarized as "relax, eat, sleep, forget, and protect."[15] The ECS has three components: endocannabinoids, biosynthetic and catabolic enzymes, and two cannabinoid receptors—CB1, the "psychoactive" neuromodulator that is the most abundant G-protein coupled receptor in the brain, and CB2, a nonpsychoactive immunomodulatory and anti-inflammatory receptor most abundant in the periphery.[14,16]

Although various surveys support the idea that the American public already accepts the medical utility of cannabis and is acting upon that belief in ever higher numbers, the U.S. Food and Drug Administration (FDA) requires more rigorous proof. Additionally, a survey of Colorado family physicians found that; "Despite a high prevalence of use in Colorado, most family physicians are not convinced of marijuana's health benefits and believe its use carries risks. Nearly all agreed on the need for further medical education about medical marijuana."[17]

If cannabis-based medicines are to overcome prejudice and gain greater trust from physicians, their production must be standardized and their contents proven safe and efficacious in randomized clinical trials (RCTs) that follow accepted scientific method and are the *sine qua non* of regulatory bodies such as the FDA[18] However, botanical cannabis is highly inconsistent and variable in its chemical composition.

Procedures for standardization of plant-based medicines have been formally presented in the U.S., providing an FDA blueprint for their regulatory approval in the "Guidance for Industry: Botanical Drug Products."[19] Meanwhile, although cannabis smoking may not be epidemiologically linked to lung cancer,[20] it is responsible for chronic cough, sputum, and cytological changes,[21,22] which render smoked cannabis an impossible candidate for approval as a prescription product in most jurisdictions.

Anecdotal claims for efficacy of crude cannabis hold no sway for the FDA.[18] There is a relative paucity of published RCT data for inhaled cannabis: the existing trials for pain total only three patient-years of data, whereas the corresponding figure for nabiximols (Sativex®, GW Pharmaceuticals), a standardized oromucosal extract spray combining THC, CBD, and other cannabis components, exceeds 6,000 patient-years of data in published studies of pain, or a two thousand-fold difference.[5] The latter is also approved in 26 countries for treatment of spasticity in multiple sclerosis, and is currently completing clinical trials for opioid-resistant cancer pain in the U.S. and elsewhere.[23–25] This agent has fulfilled criteria of safety and consistency, and has not been abused or diverted to any degree in more than 30,000 patient-years of recorded usage.

Regulatory Challenges and Solutions

The FDA has responsibility for assessing human research and evaluating data from clinical studies. Such research is initiated by an individual researcher in an investigator-initiated trial (IIT) or by a pharmaceutical company. In both situations, an Investigational New Drug (IND) application containing one or more protocols must be presented to, and allowed by, the FDA.[26]

For industry-sponsored programs, the FDA requires a range of nonclinical/preclinical studies and then clinical trials to demonstrate that the product meets the FDA's exacting standards of quality, safety, and efficacy in a particular patient population.

The FDA has clarified that it will allow both IITs and RCT development programs with cannabis or cannabis-derived products. Examples of such IITs have been completed and published.[27,28] An industry-sponsored development program is also progressing with a cannabis-derived product.[29] Finally, FDA has promulgated "expanded access" regulations in the *Code of Federal Regulations* in 21 CFR sections 312.310, 312.315, and 312.320, allowing seriously ill patients who lack conventional treatment options and clinical trial opportunities to be treated with an investigational product on a compassionate access basis. More than 300 children

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with various types of medication-resistant epilepsies have been allowed by FDA to receive treatment with a cannabis-derived (but purified) CBD product under such expanded access programs.[30]

Studies involving herbal cannabis must obtain the material from the National Institute on Drug Abuse (NIDA), which is the sole federally lawful source of research-grade cannabis. NIDA has contracted with the University of Mississippi to grow cannabis (of various cannabinoid ratios and potencies) for research.[31,32]

FDA has approved at least two products based on botanical extracts; however, FDA has not previously approved any raw botanical/herbal material as a prescription medicine. Such material would face regulatory challenges, such as achieving adequate purity, displaying batch-to-batch standardization, and identifying an appropriate method of delivery (i.e., one that would supply a precise and reproducible dose without the production of toxic by-products).

Cannabis, THC, and products containing botanically or synthetically derived cannabinoids found in the cannabis plant are classified under Schedule I of the federal CSA. The CSA contains five schedules corresponding to a substance's abuse potential and medical usefulness.

Schedule I and II substances are subject to strict security, recordkeeping, and other measures. Substances in Schedule I have "no currently accepted medical use in the U.S." and a high potential for abuse. Substances in Schedule II also have a high potential for abuse, but have an "accepted medical use," a phrase given specific meaning by the federal Drug Enforcement Administration (DEA) and upheld by federal courts:

1. The drug's chemistry must be known and reproducible;
2. There must be adequate safety studies;
3. There must be adequate and well-controlled studies proving efficacy;
4. The drug must be accepted by qualified experts; and
5. The scientific evidence must be widely available.[33]

If FDA approves a cannabis-derived product, such approval constitutes "accepted medical use," and that product will then be moved to a less stringent schedule. Although a substance and a product containing that substance are in the same schedule, "differential" scheduling is possible. For example, Marinol, a product comprising synthetic THC in sesame oil, is classified in Schedule III, whereas other forms of THC remain in Schedule I.[34] This may serve as precedent if a cannabis-derived product is FDA approved and rescheduled, although cannabis may remain in Schedule I.

Cannabis's (and THC's) Schedule I status means there are additional hurdles to overcome to conduct research in the U.S. As provided in 21 CFR section 1301.13, a physician who holds a DEA registration (license) to prescribe controlled substances in Schedules II–V may conduct research within those schedules as a "coincident activity" to his or her existing registration, with no further approval from the DEA.

However, to conduct research with a Schedule I substance, an investigator must secure a Schedule I research registration from DEA (which is substance- and protocol-specific), and (often) a Schedule I research license from the state-controlled drugs agency. These additional steps can add three to six months to the time required before an investigator can begin the research project.

A specific medical product cannot be prescribed by physicians and dispensed by pharmacists unless the FDA has approved that product (the "compounding pharmacy" exception is very limited). Therefore, even if cannabis were moved to Schedule II, physicians could not automatically prescribe it directly to patients. Although the NIDA single-source supply is the only domestic source, cannabis-derived products may be manufactured in Europe or elsewhere, and the finished product may be imported into the U.S. for research or ultimately for commercial distribution following FDA approval.[35]

Current Status of Clinical Cannabinoid Medicine

Due to the obstacles involved in human clinical research using cannabis, widespread use in the clinical setting has preceded well-established data on dosage, delivery systems, safety, and efficacy. In states that have legalized medical cannabis, about 0.77% of the population use cannabis with the recommendation of a medical provider.[36]

Although various surveys support the idea that the American public already accepts the medical utility of cannabis and is acting upon that belief in ever higher numbers, the U.S. Food and Drug Administration (FDA) requires more rigorous proof.

Cannabinoids are considered nonlethal and have a wide range of effective and tolerated dosages. Many patients use medical cannabis in a harm-reduction paradigm to decrease or discontinue the use of prescribed and illicit substances.[37] Also, the growing number of medical providers accepting cannabis as a viable treatment option[38] may attest to observed or suspected clinical efficacy. Meanwhile, observational studies can inform the emerging clinical practice of cannabinoid medicine, while guiding the development of clinical experimental design.[39]

One of this article's authors has observed clinical responses in his patient population in oral doses beginning as low as 0.1 mg cannabinoids/kg body weight/day, whereas some find optimal benefits at doses as high as 25 mg/kg/day. This wide dosing range is complicated by a biphasic dose-response curve, where lower doses may exhibit greater efficacy and tolerability than higher doses, as seen in a clinical trial of nabiximols for poorly controlled chronic pain in opioid-treated cancer patients.[24]

Another clinical trial of inhaled cannabis for neuropathic pain found low-potency (3.5% THC) and high-potency (7% THC) cannabis to have equivalent analgesic properties.[27] Biphasic dose-response effects may be due to subjects' sensitization to cannabinoids at lower doses and tolerance building at higher doses. This hypothesis is supported by preclinical studies in which administration of exogenous cannabinoids both upregulate endocannabinoid system function at acute and lower doses via increased endocannabinoid production,[40] cannabinoid receptor expression,[41] and cannabinoid receptor affinity,[42] and downregulate endocannabinoid system function upon persistent agonism via membrane receptor endosome internalization.[43]

Bidirectional effects are often related to dosage,[44,45] with high doses of cannabinoids potentially causing symptoms usually ameliorated by lower dosages. The mindset of the cannabis user and setting in which the cannabis use takes place also influence bidirectional effects; anxious subjects tend to become less anxious and more euphoric, nonanxious individuals tend to become somewhat more anxious,[46] and stressful environments can precipitate adverse emotional responses.[47]

Polymorphisms have been associated with variable responses to cannabis, including protective effects on development of cannabis dependence in adolescents,[48] intensity of withdrawal and craving during cannabis abstinence,[49] and white matter volume deficits and cognitive impairments in schizophrenic heavy cannabis users.[50]

Cannabis use history also complicates clinical response, with cannabis-naïve patients demonstrating more frequent adverse effects[51] and regular users demonstrating less psychotomimetic, perceptual altering, amnestic, and endocrine effects.[52]

Another factor to note is that physicians often lack training in using botanical medicines, and endocannabinoid physiology is still absent from most medical school curricula. Many legal cannabis patients receive permission to use cannabis from their physician, but must rely on formula selection and dosing instructions provided by cannabis growers or dispensary staff with little training or experience.

Properly interpreting observational data on medical cannabis patients requires an understanding of the chemical composition and potency of the cannabis preparations used, and of the pharmacokinetics of the delivery system employed. Laboratories offering third-party chemical analysis of herbal cannabis preparations under industry-published standards[53] can be found in most states that allow the use of medical cannabis.[54]

Due to the obstacles involved in human clinical research using cannabis, widespread use in the clinical setting has preceded well-established data on dosage, delivery systems, safety, and efficacy.

Conclusion

The endocannabinoid system regulates physiologic homeostasis and is an exciting target for disease management and health promotion. Cannabis-based preparations are poised to become an accepted option in mainstream medicine, with broad support from preclinical models, patient testimonials, and more recently, human clinical trials.

However, numerous regulatory, botanical, and pharmacologic factors challenge the collection and interpretation of clinical data on the efficacy of cannabinoid therapies. The understanding of an individual's optimal dosing and delivery method of cannabinoids for various ailments is still emerging, and must be guided by both observational and experimental data.

Clinical researchers can overcome the challenges inherent in cannabinoid therapeutics and help elucidate solutions for a wide variety of prevalent health challenges.

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Studies involving herbal cannabis must obtain the material from the National Institute on Drug Abuse (NIDA), which is the sole federally lawful source of research-grade cannabis.

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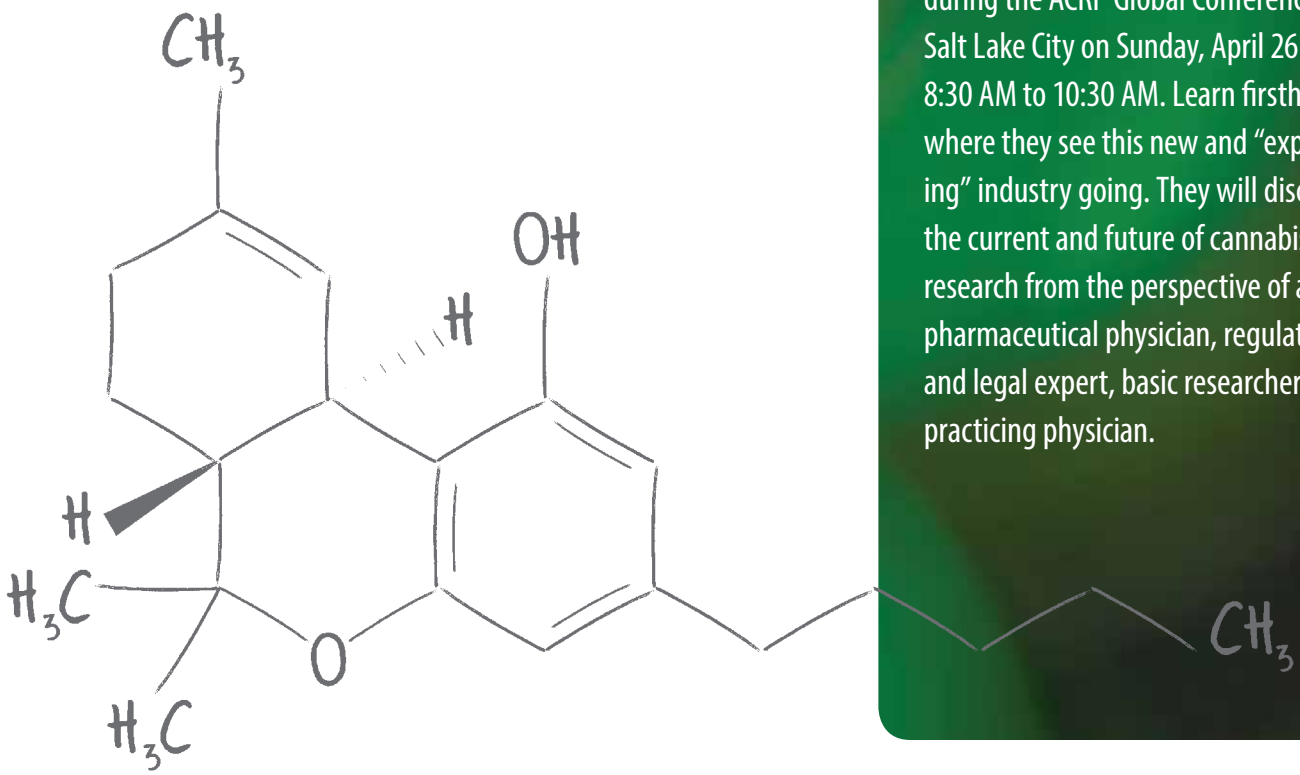

Clear the Mud: Current and Future of Cannabis Research.

The authors of this article will be joined by Sean McAllister, PhD, to speak at a two-hour session presented during the ACRP Global Conference in Salt Lake City on Sunday, April 26 from 8:30 AM to 10:30 AM. Learn firsthand where they see this new and "exploding" industry going. They will discuss the current and future of cannabis research from the perspective of a pharmaceutical physician, regulatory and legal expert, basic researcher, and practicing physician.

Medical Marijuana: Clearing Away the Smoke

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Abstract: Recent advances in understanding of the mode of action of tetrahydrocannabinol and related cannabinoid ingredients of marijuana, plus the accumulating anecdotal reports on potential medical benefits have spurred increasing research into possible medicinal uses of cannabis. Recent clinical trials with smoked and vaporized marijuana, as well as other botanical extracts indicate the likelihood that the cannabinoids can be useful in the management of neuropathic pain, spasticity due to multiple sclerosis, and possibly other indications. As with all medications, benefits and risks need to be weighed in recommending cannabis to patients. We present an algorithm that may be useful to physicians in determining whether cannabis might be recommended as a treatment in jurisdictions where such use is permitted.

Keywords: Cannabis, chronic pain, pain.

INTRODUCTION

In this article we review evidence that cannabis may be useful as medicine. We discuss potential indications for its use and provide an algorithm to guide medicinal cannabis recommendations.

The reasons for a revival of interest in medicinal cannabis are multiple, and beyond the scope of this review, but include increasing anecdotal and clinical study reports of potential benefit, advances in understanding of the endocannabinoid signaling system upon which cannabis acts, as well as growing public acceptance that cannabis should be available as a medicine if a physician recommends it.

BRIEF REVIEW OF PAST CLINICAL STUDIES ON MEDICINAL CANNABINOIDS

As recently as a decade ago a review of the world literature on the status of the efficacy and safety of cannabinoids for pain and spasticity revealed that only nine randomized studies of acceptable quality had been conducted [1]. All of these were single dose studies comparing oral synthetic THC (or cannabinoid analogs or congeners) to codeine or placebo. Two were "N of 1" randomized trials and two were of very small samples of acute post-operative pain. The remaining trials primarily addressed chronic cancer-related pain. Taken as a group it appeared that oral cannabinoids (e.g., THC 10mg) outperformed placebo and were analgesically equivalent to codeine 60mg; higher doses (THC 20mg) were comparable to codeine 120mg, but had a much higher incidence of adverse effects, particularly sedation [2]. Authoritative reviews judged cannabinoids as being unlikely to have a role in acute pain management, but suggested there was enough evidence for efficacy in chronic neuropathic pain and muscle spasticity to warrant further research [1].

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RECENT STUDIES ON MEDICINAL CANNABIS

In the past decade, the scope and rigor of research has increased dramatically. This research has employed cannabis, cannabis-based extracts, and synthetic cannabinoids delivered by smoking, vaporization, oral, and sublingual or mucosal routes.

Studies on Smoked Cannabis

Smoking cannabis provides rapid and efficient delivery of THC to brain. THC can be detected immediately in plasma after the first puff of a cigarette; peak concentrations occur within 10 minutes, then decrease to approximately 60% of peak by 15 minutes and 20% of peak by 30 minutes, but there can be wide inter-individual variation in concentrations achieved [3]. Rapid onset and predictable decay means that self-titration of dosing is attainable.

Chronic Pain

A series of randomized clinical trials at the University of California Center for Medicinal Cannabis Research (CMCR) investigated the short-term efficacy of smoked cannabis for neuropathic pain. Sponsored by the State of California Medical Marijuana Research Act of 1999, and conducted under the auspices of the Department of Health and Human Services, the National Institute on Drug Abuse, and the Food and Drug Administration, this research allocated participants to smoke cannabis cigarettes containing from 1% to 8% THC by weight (4 to 32 mg THC) or to placebo cannabis cigarettes from which THC had been extracted. The total daily dose of THC ranged from 4 mg to 128 mg. Two trials enrolled patients with painful HIV peripheral neuropathy [4, 5]; one consisted of mixed neuropathic pain due to peripheral or central dysfunction of the nervous system (i.e., com-

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plex regional pain syndrome, peripheral neuropathy, and traumatic focal nerve or spinal cord injury) [6]. Patients were allowed to continue their usual regimen of analgesics. Results consistently indicated that cannabis significantly reduced pain intensity, with patients reporting 34%-40% decrease on cannabis compared to 17-20% on placebo. Moreover a significantly greater proportion of individuals reported at least 30% reduction in pain on cannabis (46%-52%) compared to placebo (18%-24%) [4-6], which is relevant since 30% decrease in pain intensity is generally associated with reports of improved life quality [7]. The number needed-to-treat to achieve a 30% reduction in pain intensity was 3.5-4.5, a range achieved by standard non-opioid analgesics (i.e., noradrenergic antidepressants and anticonvulsants). Interestingly "medium" dose cannabis cigarettes (3.5% THC) were as effective as higher dose (7% THC) [6]. In this same vein, a fourth trial employing an experimental model of neuropathic pain (intradermal injection of capsaicin) in healthy volunteers suggested that there may be a "therapeutic window" or optimal dose for smoked cannabis: low dose cigarettes (2% THC) had no analgesic effect, high dose (8%) was associated with reports of significant pain increase, and medium dose cannabis cigarettes (4% THC) provided significant analgesia [8]. Separately, another recent placebo-controlled, cross-over study of neuropathic pain due to surgery or injury examined the effect of 25 mg doses of smoked cannabis at various potencies (2.5%, 6%, and 9.4% THC by weight), administered three times daily for 14 days [9]. Results suggested that although lower potency dosing was ineffective, 9.4% THC produced modest but significant analgesic effects compared to placebo, in a sample selected for failure to respond to conventional therapy.

Studies of Oral Preparations.

Oral preparations are available as synthetic THC (dronabinol, Marinol^{R}) and a synthetic analog of THC (nabilone, Cesamet^{R}). Absorption from the gut is slower and exhibits a delayed peak plasma concentration compared to smoking with bioavailability ranging from about 5-20% of dose; peak concentrations occur 1-6 hours after ingestion, with a magnitude approximately 10% of that achieved with smoking [3].

Chronic Pain

Most research using oral preparations has targeted neuropathic pain and spasticity associated with multiple sclerosis (MS). These randomized trials suggest that dronabinol (up to 25 mg daily) significantly reduces pain compared to placebo (50% "improved" on dronabinol compared to 30% on placebo, $p < .05$) [10], with a number-needed-to-treat for 50% pain reduction of 3.5, which is in the range of efficacy observed for standard non-opioids [11]. Effects on spasticity are mixed: there may be no observable change in examiner-rated muscle tone, but patients report significant relief [10].

There is less research with nabilone, although one three-week randomized crossover trial reported that nabilone 2mg provided modest analgesia, comparable to dihydrocodeine 240mg daily in neuropathic pain [12].

Nausea-Emesis and Appetite Stimulation

Although serotonin receptor (5 HT3) antagonists (e.g., ondansetron, Zofran^{R}) and Substance P/neurokinin-1 (NK-1) receptor antagonists (e.g., aprepitant, Emend^{R}) are the mainstays for treatment, dronabinol and nabilone are also FDA-approved for control of acute and delayed nausea and emesis due to cancer chemotherapy. Meta-analyses indicate these cannabinoids are equivalent to or more effective than metoclopraminde and neuroleptics, but their side effect profile is less favorable in terms of sedation, dizziness, dysphoria, hypotension, and anxiety [13, 14]. There are no head-to-head comparisons of cannabinoids with serotonin 5 HT3 receptor or Substance P/NK-1 receptor antagonists.

Anorexia, early satiety, weight loss and cachexia are prevalent in late stage cancer and advanced HIV disease, but there are few effective treatments. Trials in AIDS patients with clinically significant weight loss indicated that dronabinol 5mg daily significantly outperformed placebo in terms of short term appetite enhancement (38% vs. 8% at 6 weeks), and that these effects persisted for up to 12 months [15, 16], but were not accompanied by significant differences in weight gain, perhaps because of disease-associated energy wasting. The major practical limitations are the accompanying psychoactive side effects, and the problems of oral administration (eg, delayed onset of action, variable absorption, extended duration of effects).

Studies on Cannabis-based Extracts

Outside the US, extracts of whole plant are licensed and available in capsules (Cannador^{R}), with the main constituents being THC and the non-psychoactive plant cannabinoid, cannabidiol, in a ratio of 2:1. Rectal suppositories are also used to deliver THC hemisuccinate. Several small to medium-sized, randomized, controlled trials in MS suggest improvements in pain and perceived spasticity at daily doses of THC ranging from 7.5mg to 27.5mg [10, 17, 18]. In some trials [19] but not others [10, 20] observer-assessed spasticity also improved.

Studies with Alternative Delivery Systems

The hazards of smoking and the pharmacokinetic limitations of ingestion of cannabinoids has led to a search for alternative systems of administration. One alternative is devices which vaporize cannabis leaves by heating the plant product to below the temperature of combustion (175-225 degrees C), permitting inhalation of volatilized gases minus hazardous pyrroles produced by burning. Preliminary work using plant material with a range of THC content (e.g., 1-7% THC) suggests that there is rapid onset, with peak concentrations and six-hour area under the plasma concentration curves comparable to those achieved by smoking [21]. Vaporization is not a perfect solution since carbon monoxide is formed, but levels are significantly lower than with smoking [21]. Clinical trials are currently in progress at the CMCR assessing the efficacy of vaporized cannabis as an analgesic in chronic neuropathic pain.

Sublingual delivery systems of whole cannabis plant extract, which employ metered spray devices to deliver measured doses of THC (2.7mg) and cannabidiol (2.5mg), are undergoing Phase IIb/III trials in the US, and are licensed elsewhere for cancer pain and multiple sclerosis-associated neuropathic pain and spasticity (nabiximols, Sativex^{R}). The apparent advantages of such systems are known cannabinoid

concentrations, predetermined dosing aliquots, and time-out systems which may help prevent overuse. Some placebo-controlled trials suggest significant analgesia in neuropathic pain due to multiple sclerosis [22] and mixed neuropathy (e.g., post-herpetic, traumatic, vascular neuropathies, [23] but others do not [20]. Other controlled trials suggest efficacy for cancer-related pain inadequately responsive to opioid analgesia [24]. Responders participating in the open label extension phases of controlled trials appear to maintain analgesia on one-year follow-up [23].

In regard to spasticity in multiple sclerosis, a recent meta-analysis combining three trials with nabiximols in over 600 patients noted that mean intensity of patient rated spasticity was significantly reduced compared to placebo [20, 25, 26], and that the proportion of "responders" (30% reduction) was also significantly greater, with about 37% on the cannabinoid compared to 26% on placebo experiencing relief. Those reporting relief of spasticity seemed to maintain their gains over one year follow-up [27]. As with other studies noted above, observer-rated spasticity is often not reduced [20, 25, 28]; however, a recent CMCR study did find a significant reduction in observed spasticity among those administered active smoked marijuana vs. placebo marijuana [58].

PRESERVATION OF MASKING IN CLINICAL TRIALS

Because of the acute psychoactive effects of the experimental agent there is understandable concern that blinding cannot be preserved in placebo-controlled clinical trials of cannabinoids, particularly with cross-over designs. Few studies assess masking, but two cross-over trials tested maintenance of the blind by asking participants to "guess" assignment at different points of the study. Results suggest that participants, whether they are naïve or experienced cannabis users, are in the first week of a trial no more likely than by chance to guess assignment [5, 9]. With continued exposures rates of correct guesses exceed 75%, but exceed chance only in a high potency arm (9%) [9]. In another study correct guessing was related to two factors: whether the subject received placebo or cannabis first; and when during the study the participant guessed assignment [5]. Among individuals randomized to receive placebo first, guessing was no better than chance through the end of the first treatment week, whereas the majority of those randomized to receive cannabis first correctly guessed their treatment assignment at all time points. Furthermore, by the conclusion of the study, when all subjects had been given the opportunity to compare the cannabis placebo and treatments, even those randomized to receive placebo first correctly guessed their treatment assignment [5]. This raised the possibility that some of the pain reduction was placebo driven. Secondary analyses to assess whether correct treatment guessing influenced treatment responses showed that in the placebo group during the first treatment week, when guessing was no better than chance, cannabis still provided pain relief superior to that of placebo. This finding suggests that although placebo effects were present, treatment effects were independent [5].

RISKS AND MANAGEMENT OF MEDICINAL USE OF CANNABINOIDS

Acutely and over the longer term cannabis may have unwanted systemic and psychoactive adverse effects that must be taken into consideration in chronic pain populations, who have high rates of co-occurring medical illness (eg, cardiovascular disease) and co-morbid psychiatric and substance use disorders. In general these effects are dose-related, are of mild to moderate severity, appear to decline over time, and are reported less frequently in experienced than in naïve users. Reviews suggest the most frequent side effects are dizziness or lightheadedness (30%-60%), dry mouth (10%-25%), fatigue (5%-40%), muscle weakness (10%-25%), myalgia (25%), and palpitations (20%) [17]. Cough and throat irritation are reported in trials of smoked cannabis [9]. Tachycardia and postural hypotension are infrequent but caution is warranted in patients with cardiovascular disease, and possibly younger adults who intend to embark on very vigorous physical activity. At higher doses, sedation and ataxia with loss of balance are frequent. Participants in some but not all studies report euphoria: the relative absence of psychoactive effect has been attributed to the observation that plasma concentrations obtained in clinical trials are often <25% of those achieved by "recreational" users (eg, 25ng/ml vs >100ng/ml) [9]. After repeated smoked or oral marijuana doses, tolerance is rapidly acquired (in two to 12 days) to many of its adverse effects, e.g., cardiovascular, autonomic, and many subjective and cognitive effects [29]. After exposure is stopped, tolerance is lost with similar rapidity.

There is little systematic data on timeline to tolerance of either adverse or therapeutic effects, like analgesia. Concerns have long been voiced that rapid tolerance to adverse effects might portend tolerance to beneficial effects [29]. Data from studies using oral sprays of cannabinoids or dronabinol in multiple sclerosis report that individuals can reduce the incidence and severity of adverse effects by downward self-titration without loss of analgesia [17]. Other studies in this population note that overall the incidence and severity of adverse effects diminishes over time without evidence of tolerance to analgesic effects [20, 22]. Yet it is rare that clinical trials of cannabinoids extend follow-up beyond 12 weeks, leaving questions on maintenance of gains or need for dose escalation unanswered [10, 26]. One study with 12-month follow-up concluded there may be sustained analgesia for pain associated with multiple sclerosis, where about 30% of cannabinoid-treated participants report continued "improvement" at 12 months compared to about 15% on placebo [30] on doses conservatively limited to a maximum of 25mg THC daily. This suggests that pain relief may be sustained without dose increases. But the study design was not intended to determine the proportion of patients who experienced diminution of effect, or whether dose escalation, even within the set boundary, was needed for maintenance of efficacy.

There are risks to be considered in assessing the potential of cannabinoid therapeutics. Cannabis, like other analgesics, can be associated with dependence and a withdrawal syndrome, occurring in a dose-dependent fashion [29]. Under controlled conditions in healthy, experienced users of marijuana, withdrawal from a "low" daily dose (ie, oral THC 10 mg every 3-4 hours for 5-21 days) commences within 12 hours, is diminished by 24 hours, and is complete in 48 to 72 hours [29]. Other short term experiments with oral THC (20 to 30mg four times daily) and smoked cannabis (1% and 3% THC cigarettes four times daily) reveal an abstinence syn-

drome characterized by anxiety, irritability-restlessness, insomnia, stomach pain and decreased appetite [31, 32], with mood effects more prominent at the higher dosages. In research specifically designed to establish the time line of abstinence among regular heavy users (4 cigarettes daily), symptoms peak at 2 to 3 days, and persist for up to 2 weeks, although sleep disturbance may continue for up to 6 weeks [33]. In light of abstinence effects, standard practice in clinical trials administering a maximum of 25 mg THC daily is to use a tapering scheme to conclude therapy, with a 20% per day dose reduction [30]. Patients discontinuing higher dose cannabinoids for analgesia might warrant a longer tapering regimen, but this has not been studied.

Fatal overdose with cannabis alone has not been reported. In terms of acute drug interactions, additive effects of cannabis, anticholinergics, and CNS depressants should expected (e.g., increased sedation, dizziness, dry mouth, confusion). Cannabinoids are metabolized by several enzyme systems, including Cytochrome P450 (CYP 2C9, CYP 3A4) and can induce or inhibit CYP 3A4, but there is little evidence of important drug-drug interactions based on CYP 450 systems. Smoking itself (e.g., cannabis or tobacco) induces CYP 1A2, and may increase clearance of some antipsychotics (e.g., olanzapine, clozapine) and antidepressants (e.g., some tricyclics, mirtazepine) [34, 35]. Overall then, the acute medical risks of THC as used in clinical trials are rather low.

There can be adverse psychiatric side effects. THC intoxication and euphoria can be disturbing, particularly to elderly patients. Anxiety and panic attacks occur, as do frank psychotic reactions (principally paranoia), as well as so-called "paradoxical" effects of dysphoria, dejection, and depressed mood [36, 37]. Although unlikely to be a factor in the application of cannabinoids for pain, there is concern that early adolescent use of cannabis may heighten later risk of psychosis [36, 38], and evidence that genetic variation (single nucleotide polymorphisms) heightens vulnerability [39].

Acute cannabinoid intoxication adversely impacts processing speed, attention, learning and recall, perception of time and velocity, reaction time and psychomotor abilities in a dose-dependent fashion [40]. Formal neuropsychological testing in clinical trials reveals mild impairment at usual analgesic doses [6, 23]. While cannabis can acutely impair skills required to drive motor vehicles in a dose-related fashion, epidemiological data are inconclusive with regard to the association of traffic accidents and use of cannabis [41]. There is speculation that cannabis use is associated with increased awareness of impairment (e.g., altered perception of time and speed), which results in compensatory behavioral strategies. What is clearer from experimental and epidemiologic data is that driving under the influence of both alcohol and cannabis in combination confers greater risk of accidents than the risk of either drug alone [41].

The longer-term health risks of cannabis are unclear, and the evidence is based on non-medical use [42]. Long-term use of inhaled cannabis may be associated with dependence and increased respiratory symptoms; but some epidemiological studies have not found more lung disease in long-term users, once the effects of tobacco are accounted [43]. Long-term use of inhaled cannabis has not been associated with increased risk of lung or gastrointestinal cancers [44], although a meta-analysis found evidence of premalignant changes in the respiratory tract [45]. There is some evidence that among individuals with pre-existing cardiac disease, cannabis users have an increased risk of myocardial infarction in the hour after smoking cannabis compared to non-users [46]. A recent meta-analysis showed no major residual effects on neurocognitive functioning in long term daily-users of cannabis [47]. THC rapidly crosses the placenta and accumulates in breast milk of nursing mothers [3], but there is no systematic evidence of direct or behavioral teratogenicity.

In reviewing the possible acute and long term adverse effects of cannabinoids as therapeutic agents one needs also to be mindful that other agents that are used for treatment of pain or spasticity also have adverse effects. Opioids produce sedation, nausea, constipation and dependence, withdrawal from which results in serious abstinence syndrome with much more severe effects – e.g. severe autonomic, gastrointestinal, and psychiatric – than the rather mild cannabis withdrawal phenomena. Tricyclic antidepressants and antiepileptic drugs commonly prescribed for chronic pain have psychotropic (e.g. sedation), anticholinergic (e.g. constipation, dizziness, palpitations, visual disturbance, urinary retention), and neuromuscular effects. Drugs for spasticity produce sedation (e.g. baclofen), hypotension (e.g. tizanidine) and serious interactions with antibiotics (e.g. tizanidine and ciprofloxacin). Benzodiazepines that are sometimes prescribed for spasticity can produce sedation, psychomotor incoordination, memory lapses, and paradoxical reactions, as well as dependence and withdrawal syndromes. Opioids and sedative-hypnotics are also drugs of abuse, and their ability to induce physiological dependence and serious withdrawal states exceed those of cannabis. Therefore, judgements on relative benefits and risks of cannabinoids as medicines need to be viewed within the broader context of risk-benefit of other agents as well [48].

PATIENT SELECTION FOR CANNABINOID THERAPY

Oral THC (eg, dronabinol) is FDA-approved as a second line agent for chemotherapy-associated nausea and emesis, and appetite stimulation. Dronabinol (and nabilone) have some evidence of efficacy for chronic neuropathic pain; whole plant cannabis extracts delivered by capsule or oral-mucosal spray has been approved in Europe for analgesia in neuropathic pain and control of painful muscle spasticity. Patient selection for these agents would seem to be rather straightforward, and focus on therapeutic response to conventional treatments, consideration of possible psychotropic (eg, sedation effects if combined with alcohol) and cardiovascular effects, risk of dependence and an abstinence syndrome, and acknowledgement that there is narrow empirical basis for efficacy compared to standard treatments. Patients should be educated about expected adverse effects. The pharmacokinetics of orally administered cannabinoids would seem to decrease likelihood of diversion or abuse.

On the other hand, prescription of inhaled cannabis for medical purposes is legal in some US jurisdictions, and neurologic consultants, who are likely to be asked about the advisability of prescribing or recommending "medical mari-

juana," may be justifiably uncertain of how to proceed. There are no published consensus statements or systematic approaches to identify candidates for "medical marijuana" or guide treatment; although some regulatory agencies, such as the Medical Board and the Office of the Attorney General of California have proposed guidelines (Table **1**).

It should be noted that the evidence for efficacy is based primarily on relatively few short-term studies with small sample sizes of selected, mostly neuropathic pain conditions (ie, Phase II/III trials). "Medical cannabis", now available from dispensaries in some jurisdictions, is not subject to governmental standardization, and its constituents and potency are consequently unknown. Moreover, the mean potency of marijuana seized by federal and state authorities has more than doubled over the past 15 years to about 6% THC, well over 20% of confiscated plants have a potency exceeding 9%, and some specimens exceed 25% THC [49]. Thus, cannabis obtained from dispensaries or other sources may have potency far exceeding that used in clinical trials described in this review. Furthermore, cannabis elicits concerns among regulators, clinicians, and patients regarding issues of misuse, abuse, and other liabilities. With these facts in mind, a potentially useful framework for evaluating advisability of medical marijuana are guidelines released by professional pain societies concerning prescription of long-term opioid therapy for chronic, non-cancer pain [50, 51]. The guidelines are framed by several questions. One question regards not only the legality of cannabis, but the standard of practice in the clinician's community, since either prescription or recommendation for use of cannabis is outside of "conventional practice." As with prescription of opioids there are potential issues of legal liability [52]. A second question asks whether other treatments offer a more favorable risk-benefit ratio. The answer depends upon a careful differential diagnosis, identification of a potentially responsive pain syndrome (e.g., the strongest evidence is for neuropathic pain), consideration of other approaches (e.g., disease-modifying treatment, ablative interventions, other analgesics like anticonvulsants, noradrenergic antidepressants, opioids, or nonsteroidals, and cognitive-behavioral or rehabilitative therapy, or complementary treatments). A third question is whether there are medical and psychiatric risks. The shorter-term medical risks of cannabis are relatively low overall. Risks of hypotension and tachycardia should be evaluated in patients with cardiovascular diseases, as these may be associated with elevated risk of cannabis-associated myocardial infarction. Elderly patients with neurocognitive impairment may be predisposed to adverse effects of cannabis on memory and concentration, while even intact older individuals may be susceptible to over-sedation, and falls due to ataxia. The intoxicating effects of cannabis may be disturbing. A history of severe anxiety or paranoia on prior exposure to cannabis should be sought and would be a contraindication; since patients with serious mental illness (bipolar disorder or schizophrenia) may be particularly vulnerable to these adverse effects, they are unlikely to be candidates.

Moreover, there must be assessment of the potential for misuse, abuse, or addiction. This requires a careful examination for history of substance use disorders, and psychiatric illness, perhaps supplemented by formal psychiatric consultation. Screening questionnaires, such as the Screener and Opioid Assessment for Patients with Pain (SOAPP-R) [53], have been validated in chronic pain clinic populations to stratify patients into "lower" or "higher" risk of future opioid-related aberrant behaviors, and suitably modified might be applicable to assessment of risks for cannabis misuse. Most pain experts consider sobriety a foundation of successful pain treatment. Cannabis use is prevalent in chronic pain patients prescribed opioids and may be associated with current or future opioid misuse [54]. Patients screened "at risk" for aberrant opioid use, or a history of cannabis or other substance use disorders usually would not be considered eligible for medicinal cannabis. There might be exceptions. For example, there is some evidence that patients with sustained remission from alcohol dependence (ie, sober for $\geq$ 5 years) are at no greater risk for developing a new onset substance abuse problem than non-alcohol controls, so this population would not necessarily be excluded automatically [55]. Also, a recent randomized trial suggests highly-structured approaches may result in successful analgesia and restoration of function without aberrant opioid use in "high risk" patients prescribed opioids for chronic pain [56]. Such methods, which involve a regimen of systematic urine toxicology testing, use of compliance checklists to evaluate for "red flags" of non-adherence to the program (eg, unsanctioned dose escalations, illicit drug use), and enrollment in a substance misuse counseling [56], might be adapted for use in high-risk candidates for medical marijuana. Before embarking on a trial of medical marijuana in patients with prior history of substance use disorders, it would be prudent to

Table 1. Medical Board/Office of the Attorney General of California Guidelines for Medical Marijuana

Physicians Recommending Medical Marijuana Need to:
1 Take a history and conduct a good faith examination of the patient;
2 Develop a treatment plan with objectives;
3 Provide informed consent, including discussion of side effects;
4 Periodically review the treatment's efficacy;
5 Obtain consultations, as necessary; and
6 Keep proper records supporting the decision to recommend the use of medical marijuana.
http://www.mbc.ca.gov/board/media/releases_2004_05-13_marijuana.html
http://ag.ca.gov/cms_attachments/press/pdfs/n1601_medicalmarijuanaguidelines.pdf

establish a similar routine of urine toxicology testing, compliance checklists, and co-enrollment in a formal substance abuse treatment facility, just as is recommended for a trial of opioid analgesics [52]. Finally, chronic pain may be associated with major depression, which complicates treatment, and which must be diagnosed and independently treated for successful pain management. All of these factors being considered, if the decision is made to proceed, the clinician must formulate and document a treatment plan and the patient's agreement to abide by whatever guidelines are established. The clinical "trial" would entail establishing a therapeutic "dose," appropriately monitoring for adverse effects and misuse, and assessing outcome in terms of pain, mood, and function. Based on the literature of efficacy in neuropathic pain, there could be evidence of an effect within a minimum of two weeks. Response rates have been noted to increase, however, between two and four weeks in previous neuropathic pain registration trials for gabapentin and duloxetine [57]; one might select a longer duration in difficult-to-treat cases. Considering all these factors, one would then decide with the patient whether continued treatment is warranted. A possible algorithm to guide physician decision-making is presented in Fig. (**1**).

CONCLUSION

Evidence is accumulating that cannabinoids may be useful medicine for certain indications. Control of nausea and vomiting and the promotion of weight gain in chronic inanition are already licensed uses of oral THC (dronabinol cap-

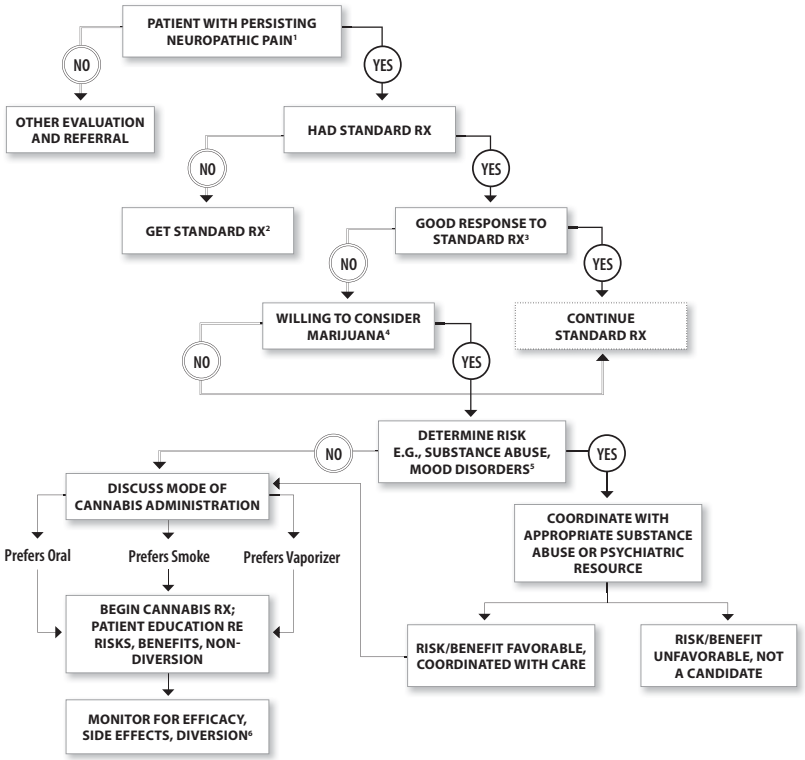

Fig. (1). A decision tree approach for physicians who may be considering recommending medicinal cannabis to a patient. This decision tree suggests some key points that a physician may need to consider in making his/her determination. In this case, a patient is assumed to present with persistent neuropathic pain. Initially, a determination needs to be made that the patient's signs and symptoms are indeed consistent with this diagnosis. Assuming a patient does not respond favorably to more standard treatments (e.g., antidepressants, anticonvulsants, etc), or cannot tolerate those, and the patient is willing to consider medicinal cannabis, the physician needs to determine risk versus benefit. Among these considerations is whether there is a history of substance abuse or serious psychiatric disorder that might be exacerbated by medicinal cannabis. Even if such risks exists, this does not necessarily preclude the use of medicinal cannabis; rather coordination with appropriate substance abuse and psychiatric resources is necessary, and based on such consultation a risk benefit ratio can be determined. In patients in whom the ratio appears favorable, the physician needs to discuss alternative modes of cannabis administration which may include oral, smoked, or vaporized systems. Once risks and benefits are evaluated and discussed with the patient, cannabis treatment may commence as with other psychotropic medications, with attention being paid to side effects as well as efficacy. In addition, there needs to be attentiveness to potential for misuse and diversion, which can then trigger a decision to discontinue.

Key

1. Daily or almost daily pain with typical neuropathic characteristics for at least 3 months; affects life quality.
2. Standard Rx = e.g., antidepressants, anticonvulsants; opioids; nonsteroidal anti-inflammatory drugs.
3. For example, at least 30% reduction in pain intensity.
4. Consider past experience, possible past history of side effects; willingness to smoke.
5. Determine history of substance abuse. If yes, or at "high risk" aberrant for drug behavior; proceed with close observation; possibly coordinate with substance abuse treatment program.
6. Efficacy = at least 30% reduction in pain intensity.

sules). Recent research indicates that cannabis may also be effective in the treatment of painful peripheral neuropathy and muscle spasticity from conditions such as multiple sclerosis [58]. Other indications have been proposed, but adequate clinical trials have not been conducted. As these therapeutic potentials are confirmed, it will be useful if marijuana and its constituents can be prescribed, dispensed, and regulated in a manner similar to other medications that have psychotropic effects and some abuse potential. Given that we do not know precisely which cannabinoids or in which combinations achieve the best results, larger and more representative clinical trials of the plant product are warranted. Because cannabinoids are variably and sometimes incompletely absorbed from the gut, and bioavailability is reduced by extensive first pass metabolism, such trials should include delivery systems that include smoking, vaporization, and oral mucosal spray in order to achieve predictable blood levels and appropriate titration. Advances in understanding the medical indications and limitations of cannabis in its various forms should facilitate the regulatory and legislative processes.

The classification of marijuana as a Schedule I drug as well as the continuing controversy as to whether or not cannabis is of medical value [59] are obstacles to medical progress in this area. Based on evidence currently available the Schedule I classification is not tenable; it is not accurate that cannabis has no medical value, or that information on safety is lacking. It is true cannabis has some abuse potential, but its profile more closely resembles drugs in Schedule III (where codeine and dronabinol are listed). The continuing conflict between scientific evidence and political ideology will hopefully be reconciled in a judicious manner [60, 61]. In the meantime, the decision to recommend this treatment in jurisdictions where use of medical marijuana is already permitted needs to be based on a careful assessment that includes proper diagnosis of a condition for which there is evidence that cannabis may be effective, along with consideration as to response to more standard treatments. Prior substance abuse history, psychiatric comorbidity, and other factors need to be weighed in a risk benefit analysis. Part of this analysis should consider that the potential longer-term harms of the cannabinoids are not fully understood: these include abuse and a dependence syndrome, adverse psychiatric and medical effects in vulnerable populations, and documented risk to traffic safety when combined with alcohol, and perhaps singly [62]. In the long term, as further studies demonstrate whether cannabis is effective for various indications, this should lead to development of novel modulators of the endocannabinoid system which may be prescribed and used as more traditional medicines.

CONFLICTS OF INTEREST

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REVIEW

Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects

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Tetrahydrocannabinol (THC) has been the primary focus of cannabis research since 1964, when Raphael Mechoulam isolated and synthesized it. More recently, the synergistic contributions of cannabidiol to cannabis pharmacology and analgesia have been scientifically demonstrated. Other phytocannabinoids, including tetrahydrocannabivarin, cannabigerol and cannabichromene, exert additional effects of therapeutic interest. Innovative conventional plant breeding has yielded cannabis chemotypes expressing high titres of each component for future study. This review will explore another echelon of phytotherapeutic agents, the cannabis terpenoids: limonene, myrcene, α-pinene, linalool, β-caryophyllene, caryophyllene oxide, nerolidol and phytol. Terpenoids share a precursor with phytocannabinoids, and are all flavour and fragrance components common to human diets that have been designated Generally Recognized as Safe by the US Food and Drug Administration and other regulatory agencies. Terpenoids are quite potent, and affect animal and even human behaviour when inhaled from ambient air at serum levels in the single digits ng·mL^{-1}. They display unique therapeutic effects that may contribute meaningfully to the entourage effects of cannabis-based medicinal extracts. Particular focus will be placed on phytocannabinoid-terpenoid interactions that could produce synergy with respect to treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, fungal and bacterial infections (including methicillin-resistant *Staphylococcus aureus*). Scientific evidence is presented for non-cannabinoid plant components as putative antidotes to intoxicating effects of THC that could increase its therapeutic index. Methods for investigating entourage effects in future experiments will be proposed. Phytocannabinoid-terpenoid synergy, if proven, increases the likelihood that an extensive pipeline of new therapeutic products is possible from this venerable plant.

LINKED ARTICLES

This article is part of a themed issue on Cannabinoids in Biology and Medicine. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

2-AG, 2-arachidonoylglycerol; 5-HT, 5-hydroxytryptamine (serotonin); AD, antidepressant; AEA, arachidonoylethanolamide (anandamide); AI, anti-inflammatory; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; Ca^{++}, calcium ion; CB_1/CB_2, cannabinoid receptor 1 or 2; CBC, cannabichromene; CBCA, cannabichromenic acid; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarin; CBG, cannabigerol; CBGA, cannabigerolic acid; CBGV, cannabigerivarin; CNS, central nervous system; COX, cyclo-oxygenase; DAGL, diacylglycerol lipase; ECS, endocannabinoid system; EO, essential oil; FAAH, fatty acid amidohydrolase; FDA, US Food and Drug Administration; FEMA, Food and Extract Manufacturers Association; fMRI, functional magnetic resonance imaging; GABA, gamma aminobutyric acid; GPCR, G-protein coupled receptor; GPR, G-protein coupled receptor; HEK, human embryonic kidney; IC_{50}, 50% inhibitory concentration; i.p., intraperitoneal; MAGL, monoacylglycerol lipase; MIC, minimum inhibitory concentration; MS, multiple sclerosis; NGF, nerve growth factor; NIDA, US National Institute on Drug Abuse; PG, prostaglandin; PTSD, post-traumatic stress disorder; RCT, randomized clinical trial; SPECT, single photon emission computed tomography; SSADH, succinic semialdehyde dehydrogenase; Sx, symptoms; $T_{1/2}$, half-life; TCA, tricyclic antidepressant; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; THCV, tetrahydrocannabivarin; TNF-α, tumour necrosis factor-alpha, TRPV, transient receptor potential vanilloid receptor

The roots of cannabis synergy

Cannabis has been a medicinal plant of unparalleled versatility for millennia (Mechoulam, 1986; Russo, 2007; 2008), but whose mechanisms of action were an unsolved mystery until the discovery of tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964a), the first cannabinoid receptor, CB_1 (Devane *et al.*, 1988), and the endocannabinoids, anandamide (arachidonoylethanolamide, AEA) (Devane *et al.*, 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). While a host of phytocannabinoids were discovered in the 1960s: cannabidiol (CBD) (Mechoulam and Shvo, 1963), cannabigerol (CBG) (Gaoni and Mechoulam, 1964b), cannabichromene (CBC) (Gaoni and Mechoulam, 1966), cannabidivarin (CBDV) (Vollner *et al.*, 1969) and tetrahydrocannabivarin (THCV) (Gill *et al.*, 1970), the overwhelming preponderance of research focused on psychoactive THC. Only recently has renewed interest been manifest in THC analogues, while other key components of the activity of cannabis and its extracts, the cannabis terpenoids, remain understudied (McPartland and Russo, 2001b; Russo and McPartland, 2003). The current review will reconsider essential oil (EO) agents, their peculiar pharmacology and possible therapeutic interactions with phytocannabinoids. Nomenclature follows conventions in Alexander *et al.* (2009).

Phytocannabinoids and terpenoids are synthesized in cannabis, in secretory cells inside glandular trichomes (Figure 1) that are most highly concentrated in unfertilized female flowers prior to senescence (Potter, 2004; Potter, 2009). Geranyl pyrophosphate is formed as a precursor via the deoxyxylulose pathway in cannabis (Fellermeier *et al.*, 2001), and is a parent compound to both phytocannabinoids and terpenoids (Figure 2). After coupling with either olivetolic acid or divarinic acid, pentyl or propyl cannabinoid acids are produced, respectively, via enzymes that accept either substrate (de Meijer *et al.*, 2003), a manifestation of Mechoulam's postulated 'Nature's Law of Stinginess'. Although having important biochemical properties in their own right, acid forms of phytocannabinoids are most commonly decarboxylated via heat to produce the more familiar neutral phytocannabinoids (Table 1). Alternatively, geranyl pyrophosphate may form limonene and other monoterpenoids in secretory cell plastids, or couple with isopentenyl pyrophosphate in the cytoplasm to form farnesyl pyrophosphate, parent compound to the sesquiterpenoids, that co-localizes with transient receptor potential vanilloid receptor (TRPV) 1 in human dorsal root ganglion, suggesting a role in sensory processing of noxious stimuli (Bradshaw *et al.*, 2009), and which is the most potent endogenous ligand to date on the G-protein coupled receptor (GPR) 92 (Oh *et al.*, 2008).

Figure 1

Cannabis capitate glandular (EBR by permission of Bedrocan BV, Netherlands).

An obvious question pertains to the chemical ecology of such syntheses that require obvious metabolic demands on the plant (Gershenzon, 1994), and these will be considered.

Is cannabis merely a crude vehicle for delivery of THC? Might it rather display herbal synergy (Williamson, 2001) encompassing potentiation of activity by active or inactive components, antagonism (evidenced by the ability of CBD to reduce side effects of THC; Russo and Guy, 2006), summation, pharmacokinetic and metabolic interactions? Recently, four basic mechanisms of synergy have been proposed (Wagner and Ulrich-Merzenich, 2009): (i) multi-target effects; (ii) pharmacokinetic effects such as improved solubility or bioavailability; (iii) agent interactions affecting bacterial resistance; and (iv) modulation of adverse events. Cannabis was cited as an illustration.

Could phytocannabinoids function analogously to the endocannabinoid system (ECS) with its combination of active and 'inactive' synergists, first described as an entourage (Ben-Shabat *et al.*, 1998), with subsequent refinement (Mechoulam and Ben-Shabat, 1999) and qualification (p. 136): 'This type of synergism may play a role in the widely held (but not experimentally based) view that in some cases plants are better drugs than the natural products isolated from them'. Support derives from studies in which cannabis extracts demonstrated effects two to four times greater than THC (Carlini *et al.*, 1974); unidentified THC antagonists and synergists were claimed (Fairbairn and Pickens, 1981), anticonvulsant activity was observed beyond the cannabinoid fraction (Wilkinson *et al.*, 2003), and extracts of THC and CBD modulated effects in hippocampal neurones distinctly from pure compounds (Ryan *et al.*, 2006). Older literature also presented refutations: no observed differences were noted by humans ingesting or smoking pure THC versus herbal cannabis (Wachtel *et al.*, 2002); pure THC seemed to account for all tetrad-type effects in mice (Varvel *et al.*, 2005); and smoked cannabis with varying CBD or CBC content failed to yield subjective differences combined with THC (Ilan *et al.*, 2005). Explanations include that the cannabis employed by Wachtel yielded 2.11% THC, but with only 0.3% cannabinol (CBN) and 0.05% CBD (Russo and McPartland, 2003), and Ilan's admission that CBN and CBD content might be too low to modulate THC. Another factor is apparent in that terpenoid yields from vaporization of street cannabis were 4.3–8.5 times of those from US National Institute on Drug Abuse cannabis (Bloor *et al.*, 2008). It is undisputed that the black market cannabis in the UK (Potter *et al.*, 2008), Continental Europe (King *et al.*, 2005) and the USA (Mehmedic *et al.*, 2010) has become almost exclusively a high-THC preparation to the almost total exclusion of other phytocannabinoids. If – as many consumers and experts maintain (Clarke, 2010) – there are biochemical, pharmacological and

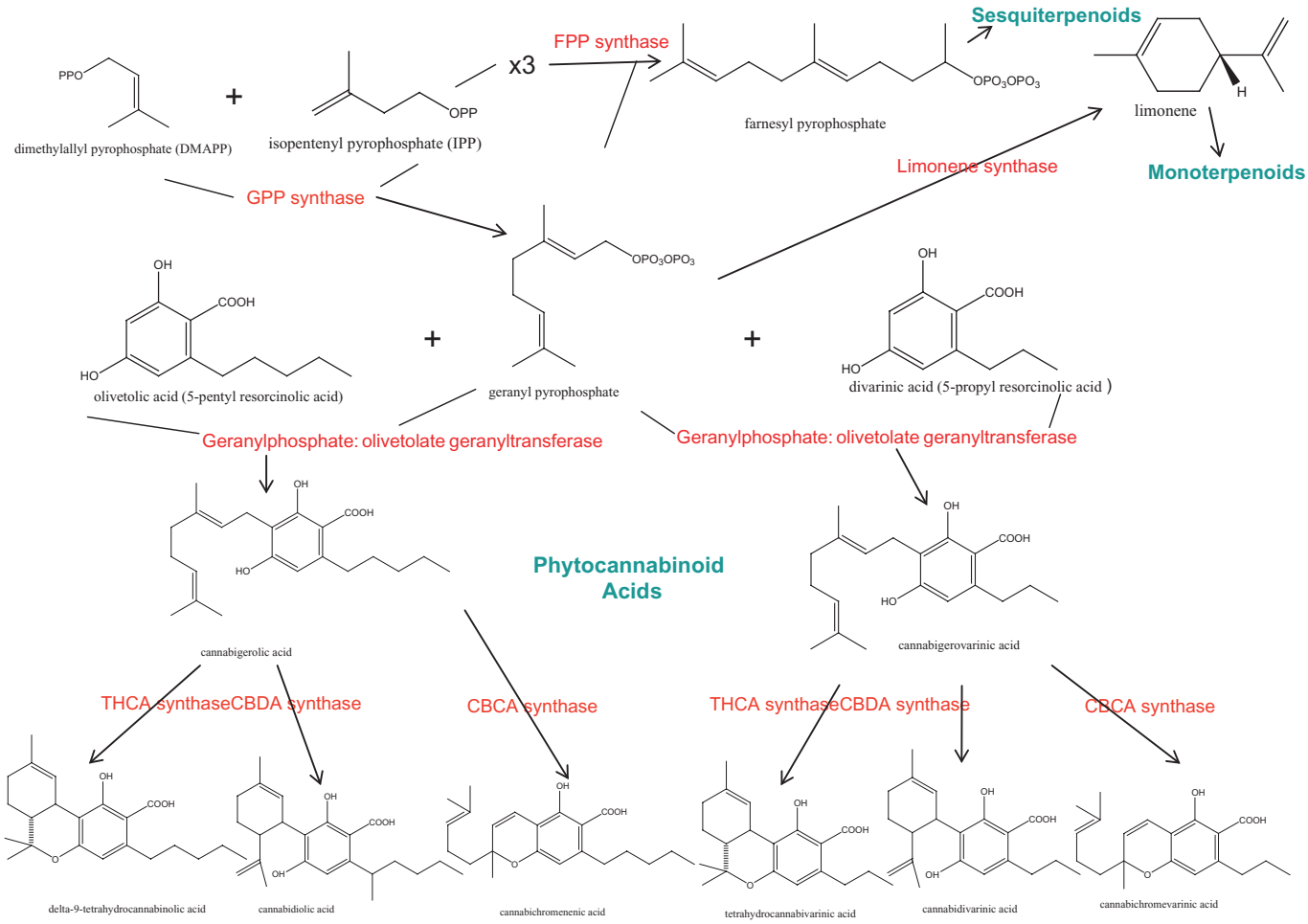


Figure 2
Phytocannabinoid and cannabis terpenoid biosynthesis.

phenomenological distinctions between available cannabis 'strains', such phenomena are most likely related to relative terpenoid contents and ratios. This treatise will assess additional evidence for putative synergistic phytocannabinoid-terpenoid effects exclusive of THC, to ascertain whether this botanical may fulfil its promise as, 'a neglected pharmacological treasure trove' (Mechoulam, 2005).

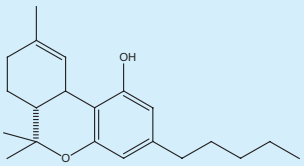
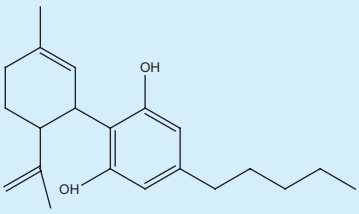
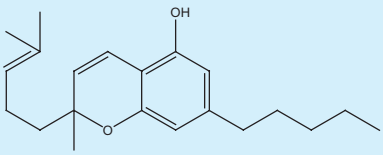
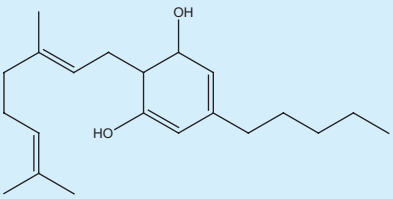
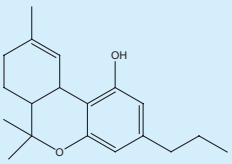
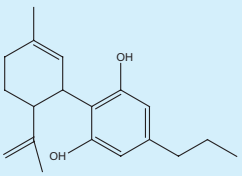
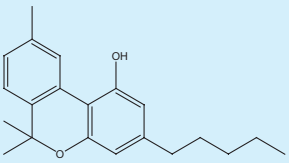
Phytocannabinoids, beyond THC: a brief survey

Phytocannabinoids are exclusively produced in cannabis (*vide infra* for exception), but their evolutionary and ecological *raisons d'être* were obscure until recently. THC production is maximized with increased light energy (Potter, 2009). It has been known for some time that CBG and CBC are mildly antifungal (ElSohly *et al.*, 1982), as are THC and CBD against a cannabis pathogen (McPartland, 1984). More pertinent, however, is the mechanical stickiness of the trichomes, capable of trapping insects with all six legs (Potter, 2009). Tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (Morimoto *et al.*, 2007), as well as cannabidiolic acid and cannabigerolic acid (CBGA; Shoyama *et al.*, 2008) produce necrosis in plant cells. Normally, the cannabinoid acids are sequestered in trichomes away from the flower tissues. Any trichome breakage at senescence may contribute to natural pruning of lower fan leaves that otherwise utilize energy that the plant preferentially diverts to the flower, in continued efforts to affect fertilization, generally in vain when subject to human horticulture for pharmaceutical production. THCA and CBGA have also proven to be insecticidal in their own right (Sirikantaramas *et al.*, 2005).

Over 100 phytocannabinoids have been identified (Brenneisen, 2007; Mehmedic *et al.*, 2010), but many are artefacts of analysis or are produced in trace quantities that have not permitted thorough investigation. The pharmacology of the more accessible phytocannabinoids has received excellent recent reviews (Pertwee *et al.*, 2007; Izzo *et al.*, 2009; De Petrocellis and Di Marzo, 2010; De Petrocellis *et al.*, 2011), and will be summarized here, with emphasis on activities with particular synergistic potential.

Table 1

Phytocannabinoid activity table

Phytocannabinoid structure	Selected pharmacology (reference)	Synergistic terpenoids
 delta-9-tetrahydrocannabinol (THC)	Analgesic via CB_1 and CB_2 (Rahn and Hohmann, 2009)	Various
	AI/antioxidant (Hampson *et al.*, 1998)	Limonene *et al.*
	Bronchodilatory (Williams *et al.*, 1976)	Pinene
	↓ Sx. Alzheimer disease (Volicer *et al.*, 1997; Eubanks *et al.*, 2006)	Limonene, pinene, linalool
	Benefit on duodenal ulcers (Douthwaite, 1947)	Caryophyllene, limonene
	Muscle relaxant (Kavia *et al.*, 2010)	Linalool?
	Antipruritic, cholestatic jaundice (Neff *et al.*, 2002)	Caryophyllene?
 cannabidiol	AI/antioxidant (Hampson *et al.*, 1998)	Limonene *et al.*
	Anti-anxiety via 5-HT_{1A} (Russo *et al.*, 2005)	Linalool, limonene
	Anticonvulsant (Jones *et al.*, 2010)	Linalool
	Cytotoxic versus breast cancer (Ligresti *et al.*, 2006)	Limonene
	↑ adenosine A_{2A} signalling (Carrier *et al.*, 2006)	Linalool
	Effective versus MRSA (Appendino *et al.*, 2008)	Pinene
	Decreases sebum/sebocytes (Biro *et al.*, 2009)	Pinene, limonene, linalool
	Treatment of addiction (see text)	Caryophyllene
 cannabichromene	Anti-inflammatory/analgesic (Davis and Hatoum, 1983)	Various
	Antifungal (ElSohly *et al.*, 1982)	Caryophyllene oxide
	AEA uptake inhibitor (De Petrocellis *et al.*, 2011)	–
	Antidepressant in rodent model (Deyo and Musty, 2003)	Limonene
 cannabigerol	TRPM8 antagonist prostate cancer (De Petrocellis *et al.*, 2011)	Cannabis terpenoids
	GABA uptake inhibitor (Banerjee *et al.*, 1975)	Phytol, linalool
	Anti-fungal (ElSohly *et al.*, 1982)	Caryophyllene oxide
	Antidepressant rodent model (Musty and Deyo, 2006); and via 5-HT_{1A} antagonism (Cascio *et al.*, 2010)	Limonene
	Analgesic, α-2 adrenergic blockade (Cascio *et al.*, 2010)	Various
	↓ keratinocytes in psoriasis (Wilkinson and Williamson, 2007)	adjunctive role?
	Effective versus MRSA (Appendino *et al.*, 2008)	Pinene
 tetrahydrocannabivarin	AI/anti-hyperalgesic (Bolognini *et al.*, 2010)	Caryophyllene *et al.* . . .
	Treatment of metabolic syndrome (Cawthorne *et al.*, 2007)	–
	Anticonvulsant (Hill *et al.*, 2010)	Linalool
 cannabidivarin	Inhibits diacylglycerol lipase (De Petrocellis *et al.*, 2011)	–
	Anticonvulsant in hippocampus (Hill *et al.*, 2010)	Linalool
 cannabinol (CBN)	Sedative (Musty *et al.*, 1976)	Nerolidol, myrcene
	Effective versus MRSA (Appendino *et al.*, 2008)	Pinene
	TRPV2 agonist for burns (Qin *et al.*, 2008)	Linalool
	↓ keratinocytes in psoriasis (Wilkinson and Williamson, 2007)	adjunctive role?
	↓ breast cancer resistance protein (Holland *et al.*, 2008)	Limonene

5-HT, 5-hydroxytryptamine (serotonin); AEA, arachidonoylethanolamide (anandamide); AI, anti-inflammatory; CB1/CB2, cannabinoid receptor 1 or 2; GABA, gamma aminobutyric acid; TRPV, transient receptor potential vanilloid receptor; MRSA, methicillin-resistant *Staphylococcus aureus*; Sx, symptoms.

THC (Table 1) is the most common phytocannabinoid in cannabis drug chemotypes, and is produced in the plant via an allele co-dominant with CBD (de Meijer *et al.*, 2003). THC is a partial agonist at CB_1 and cannabinoid receptor 2 (CB_2) analogous to AEA, and underlying many of its activities as a psychoactive agent, analgesic, muscle relaxant and antispasmodic (Pacher *et al.*, 2006). Additionally, it is a bronchodilator (Williams *et al.*, 1976), neuroprotective antioxidant (Hampson *et al.*, 1998), antipruritic agent in cholestatic jaundice (Neff *et al.*, 2002) and has 20 times the anti-inflammatory power of aspirin and twice that of hydrocortisone (Evans, 1991). THC is likely to avoid potential pitfalls of either COX-1 or COX-2 inhibition, as such activity is only noted at concentrations far above those attained therapeutically (Stott *et al.*, 2005).

CBD is the most common phytocannabinoid in fibre (hemp) plants, and second most prevalent in some drug chemotypes. It has proven extremely versatile pharmacologically (Table 1) (Pertwee, 2004; Mechoulam *et al.*, 2007), displaying the unusual ability to antagonize CB_1 at a low nM level in the presence of THC, despite having little binding affinity (Thomas *et al.*, 2007), and supporting its modulatory effect on THC-associated adverse events such as anxiety, tachycardia, hunger and sedation in rats and humans (Nicholson *et al.*, 2004; Murillo-Rodriguez *et al.*, 2006; Russo and Guy, 2006). CBD is an analgesic (Costa *et al.*, 2007), is a neuroprotective antioxidant more potent than ascorbate or tocopherol (Hampson *et al.*, 1998), without COX inhibition (Stott *et al.*, 2005), acts as a TRPV1 agonist analogous to capsaicin but without noxious effect (Bisogno *et al.*, 2001), while also inhibiting uptake of AEA and weakly inhibiting its hydrolysis. CBD is an antagonist on GPR55, and also on GPR18, possibly supporting a therapeutic role in disorders of cell migration, notably endometriosis (McHugh *et al.*, 2010). CBD is anticonvulsant (Carlini and Cunha, 1981; Jones *et al.*, 2010), anti-nausea (Parker *et al.*, 2002), cytotoxic in breast cancer (Ligresti *et al.*, 2006) and many other cell lines while being cyto-preservative for normal cells (Parolaro and Massi, 2008), antagonizes tumour necrosis factor-alpha (TNF-α) in a rodent model of rheumatoid arthritis (Malfait *et al.*, 2000), enhances adenosine receptor A_{2A} signalling via inhibition of an adenosine transporter (Carrier *et al.*, 2006), and prevents prion accumulation and neuronal toxicity (Dirikoc *et al.*, 2007). A CBD extract showed greater anti-hyperalgesia over pure compound in a rat model with decreased allodynia, improved thermal perception and nerve growth factor levels and decreased oxidative damage (Comelli *et al.*, 2009). CBD also displayed powerful activity against methicillin-resistant *Staphylococcus aureus* (MRSA), with a minimum inhibitory concentration (MIC) of 0.5–2 $\mu g \cdot mL^{-1}$ (Appendino *et al.*, 2008). In 2005, it was demonstrated that CBD has agonistic activity at 5-hydroxytryptamine $(5\text{-HT})_{1A}$ at 16 μM (Russo *et al.*, 2005), and that despite the high concentration, may underlie its anti-anxiety activity (Resstel *et al.*, 2009; Soares Vde *et al.*, 2010), reduction of stroke risk (Mishima *et al.*, 2005), anti-nausea effects (Rock *et al.*, 2009) and ability to affect improvement in cognition in a mouse model of hepatic encephalopathy (Magen *et al.*, 2009). A recent study has demonstrated that CBD 30 $mg \cdot kg^{-1}$ i.p. reduced immobility time in the forced swim test compared to imipramine ($P < 0.01$), an effect blocked by pre-treatment with the 5-HT_{1A} antagonist WAY100635 (Zanelati *et al.*, 2010), supporting a prospective role for CBD as an antidepressant. CBD also inhibits synthesis of lipids in sebocytes, and produces apoptosis at higher doses in a model of acne (*vide infra*). One example of CBD antagonism to THC would be the recent observation of lymphopenia in rats (CBD 5 $mg \cdot kg^{-1}$) mediated by possible CB_2 inverse agonism (Ignatowska-Jankowska *et al.*, 2009), an effect not reported in humans even at doses of pure CBD up to 800 mg (Crippa *et al.*, 2010), possibly due to marked interspecies differences in CB_2 sequences and signal transduction. CBD proved to be a critical factor in the ability of nabiximols oromucosal extract in successfully treating intractable cancer pain patients unresponsive to opioids (30% reduction in pain from baseline), as a high-THC extract devoid of CBD failed to distinguish from placebo (Johnson *et al.*, 2010). This may represent true synergy if the THC–CBD combination were shown to provide a larger effect than a summation of those from the compounds separately (Berenbaum, 1989).

CBC (Table 1) was inactive on adenylate cyclase inhibition (Howlett, 1987), but showed activity in the mouse cannabinoid tetrad, but only at 100 $mg \cdot kg^{-1}$, and at a fraction of THC activity, via a non-CB_1, non-CB_2 mechanism (Delong *et al.*, 2010). More pertinent are anti-inflammatory (Wirth *et al.*, 1980) and analgesic activity (Davis and Hatoum, 1983), its ability to reduce THC intoxication in mice (Hatoum *et al.*, 1981), antibiotic and antifungal effects (ElSohly *et al.*, 1982), and observed cytotoxicity in cancer cell lines (Ligresti *et al.*, 2006). A CBC-extract displayed pronounced antidepressant effect in rodent models (Deyo and Musty, 2003). Additionally, CBC was comparable to mustard oil in stimulating TRPA1-mediated Ca^{++} in human embryonic kidney 293 cells (50–60 nM) (De Petrocellis *et al.*, 2008). CBC recently proved to be a strong AEA uptake inhibitor (De Petrocellis *et al.*, 2011). CBC production is normally maximal, earlier in the plant's life cycle (de Meijer *et al.*, 2009a). An innovative technique employing cold water extraction of immature leaf matter from selectively bred cannabis chemotypes yields a high-CBC 'enriched trichome preparation' (Potter, 2009).

CBG (Table 1), the parent phytocannabinoid compound, has a relatively weak partial agonistic effect at CB_1 (K_i 440 nM) and CB_2 (K_i 337 nM) (Gauson *et al.*, 2007). Older work supports gamma aminobutyric acid (GABA) uptake inhibition greater than THC or CBD (Banerjee *et al.*, 1975) that could suggest muscle relaxant properties. Analgesic and anti-erythemic effects and the ability to block lipooxygenase were said to surpass those of THC (Evans, 1991). CBG demonstrated modest antifungal effects (ElSohly *et al.*, 1982). More recently, it proved to be an effective cytotoxic in high dosage on human epithelioid carcinoma (Baek *et al.*, 1998), is the next most effective phytocannabinoid against breast cancer after CBD (Ligresti *et al.*, 2006), is an antidepressant in the rodent tail suspension model (Musty and Deyo, 2006) and is a mildly anti-hypertensive agent (Maor *et al.*, 2006). Additionally, CBG inhibits keratinocyte proliferation suggesting utility in psoriasis (Wilkinson and Williamson, 2007), it is a relatively potent TRPM8 antagonist for possible application in prostate cancer (De Petrocellis and Di Marzo, 2010) and detrusor over-activity and bladder pain (Mukerji *et al.*, 2006). It is a strong AEA uptake inhibitor (De Petrocellis *et al.*, 2011) and a powerful agent against MRSA (Appendino *et al.*, 2008; *vide infra*). Finally, CBG behaves as a potent α-2 adrenorecep-

tor agonist, supporting analgesic effects previously noted (Formukong *et al.*, 1988), and moderate 5-HT_{1A} antagonist suggesting antidepressant properties (Cascio *et al.*, 2010). Normally, CBG appears as a relatively low concentration intermediate in the plant, but recent breeding work has yielded cannabis chemotypes lacking in downstream enzymes that express 100% of their phytocannabinoid content as CBG (de Meijer and Hammond, 2005; de Meijer *et al.*, 2009a).

THCV (Table 1) is a propyl analogue of THC, and can modulate intoxication of the latter, displaying 25% of its potency in early testing (Gill *et al.*, 1970; Hollister, 1974). A recrudescence of interest accrues to this compound, which is a CB_1 antagonist at lower doses (Thomas *et al.*, 2005), but is a CB_1 agonist at higher doses (Pertwee, 2008). THCV produces weight loss, decreased body fat and serum leptin concentrations with increased energy expenditure in obese mice (Cawthorne *et al.*, 2007; Riedel *et al.*, 2009). THCV also demonstrates prominent anticonvulsant properties in rodent cerebellum and pyriform cortex (Hill *et al.*, 2010). THCV appears as a fractional component of many southern African cannabis chemotypes, although plants highly predominant in this agent have been produced (de Meijer, 2004). THCV recently demonstrated a CB_2-based ability to suppress carageenan-induced hyperalgesia and inflammation, and both phases of formalin-induced pain behaviour via CB_1 and CB_2 in mice (Bolognini *et al.*, 2010).

CBDV (Table 1), the propyl analogue of CBD, was first isolated in 1969 (Vollner *et al.*, 1969), but formerly received little investigation. Pure CBDV inhibits diacylglycerol lipase [50% inhibitory concentration (IC_{50}) 16.6 μM] and might decrease activity of its product, the endocannabinoid, 2-AG (De Petrocellis *et al.*, 2011). It is also anticonvulsant in rodent hippocampal brain slices, comparable to phenobarbitone and felbamate (Jones *et al.*, 2010).

Finally, CBN is a non-enzymatic oxidative by-product of THC, more prominent in aged cannabis samples (Merzouki and Mesa, 2002). It has a lower affinity for CB_1 (K_i 211.2 nM) and CB_2 (K_i 126.4 nM) (Rhee *et al.*, 1997); and was judged inactive when tested alone in human volunteers, but produced greater sedation combined with THC (Musty *et al.*, 1976). CBN demonstrated anticonvulsant (Turner *et al.*, 1980), anti-inflammatory (Evans, 1991) and potent effects against MRSA (MIC 1 $\mu g \cdot mL^{-1}$). CBN is a TRPV2 (high-threshold thermosensor) agonist (EC 77.7 μM) of possible interest in treatment of burns (Qin *et al.*, 2008). Like CBG, it inhibits keratinocyte proliferation (Wilkinson and Williamson, 2007), independently of cannabinoid receptor effects. CBN stimulates the recruitment of quiescent mesenchymal stem cells in marrow (10 μM), suggesting promotion of bone formation (Scutt and Williamson, 2007) and inhibits breast cancer resistance protein, albeit at a very high concentration (IC_{50} 145 μM) (Holland *et al.*, 2008).

Cannabis terpenoids: neglected entourage compounds?

Terpenoids are EO components, previously conceived as the quintessential fifth element, 'life force' or spirit (Schmidt, 2010), and form the largest group of plant chemicals, with 15–20 000 fully characterized (Langenheim, 1994). Terpenoids, not cannabinoids, are responsible for the aroma of cannabis. Over 200 have been reported in the plant (Hendriks *et al.*, 1975; 1977; Malingre *et al.*, 1975; Davalos *et al.*, 1977; Ross and ElSohly, 1996; Mediavilla and Steinemann, 1997; Rothschild *et al.*, 2005; Brenneisen, 2007), but only a few studies have concentrated on their pharmacology (McPartland and Pruitt, 1999; McPartland and Mediavilla, 2001a; McPartland and Russo, 2001b). Their yield is less than 1% in most cannabis assays, but they may represent 10% of trichome content (Potter, 2009). Monoterpenes usually predominate (limonene, myrcene, pinene), but these headspace volatiles (Hood *et al.*, 1973), while only lost at a rate of about 5% before processing (Gershenzon, 1994), do suffer diminished yields with drying and storage (Turner *et al.*, 1980; Ross and ElSohly, 1996), resulting in a higher relative proportion of sesquiterpenoids (especially caryophyllene), as also often occurs in extracts. A 'phytochemical polymorphism' seems operative in the plant (Franz and Novak, 2010), as production favours agents such as limonene and pinene in flowers that are repellent to insects (Nerio *et al.*, 2010), while lower fan leaves express higher concentrations of bitter sesquiterpenoids that act as anti-feedants for grazing animals (Potter, 2009). Evolutionarily, terpenoids seem to occur in complex and variable mixtures with marked structural diversity to serve various ecological roles. Terpenoid composition is under genetic control (Langenheim, 1994), and some enzymes produce multiple products, again supporting Mechoulam's 'Law of Stinginess'. The particular mixture of mono- and sesquiterpenoids will determine viscosity, and in cannabis, this certainly is leveraged to practical advantage as the notable stickiness of cannabis exudations traps insects (McPartland *et al.*, 2000), and thus, combined with the insecticidal phytocannabinoid acids (Sirikantaramas *et al.*, 2005), provides a synergistic mechano-chemical defensive strategy versus predators.

As observed for cannabinoids, terpenoid production increases with light exposure, but decreases with soil fertility (Langenheim, 1994), and this is supported by the glasshouse experience that demonstrates higher yields if plants experience relative nitrogen lack just prior to harvest (Potter, 2004), favouring floral over foliar growth. EO composition is much more genetically than environmentally determined, however (Franz and Novak, 2010), and while cannabis is allogamous and normally requires repeat selective breeding for maintenance of quality, this problem may be practically circumvented by vegetative propagation of high-performance plants under controlled environmental conditions (light, heat and humidity) (Potter, 2009), and such techniques have proven to provide notable consistency to tight tolerances as Good Manufacturing Practice for any pharmaceutical would require (Fischedick *et al.*, 2010).

The *European Pharmacopoeia*, Sixth Edition (2007), lists 28 EOs (Pauli and Schilcher, 2010). Terpenoids are pharmacologically versatile: they are lipophilic, interact with cell membranes, neuronal and muscle ion channels, neurotransmitter receptors, G-protein coupled (odorant) receptors, second messenger systems and enzymes (Bowles, 2003; Buchbauer, 2010). All the terpenoids discussed herein are Generally Recognized as Safe, as attested by the US Food and Drug Admin-

istration as food additives, or by the Food and Extract Manufacturers Association and other world regulatory bodies. Germane is the observation (Adams and Taylor, 2010) (p. 193), 'With a high degree of confidence one may presume that EOs derived from food are likely to be safe'. Additionally, all the current entries are non-sensitizing to skin when fresh (Tisserand and Balacs, 1995; Adams and Taylor, 2010), but may cause allergic reactions at very low rates when oxidized (Matura *et al.*, 2005). For additional pharmacological data on other common cannabis terpenoids not discussed herein (1,8-cineole, also known as eucalyptol, pulegone, α-terpineol, terpineol-4-ol, ρ-cymene, borneol and Δ-3-carene), please see McPartland and Russo (2001b).

Are cannabis terpenoids actually relevant to the effects of cannabis? Terpenoid components in concentrations above 0.05% are considered of pharmacological interest (Adams and Taylor, 2010). Animal studies are certainly supportive (Buchbauer *et al.*, 1993). Mice exposed to terpenoid odours inhaled from ambient air for 1 h demonstrated profound effects on activity levels, suggesting a direct pharmacological effect on the brain, even at extremely low serum concentrations (examples: linalool with 73% reduction in motility at 4.22 ng·mL^{-1}, pinene 13.77% increase at trace concentration, terpineol 45% reduction at 4.7 ng·mL^{-1}). These levels are comparable to those of THC measured in humans receiving cannabis extracts yielding therapeutic effects in pain, or symptoms of multiple sclerosis in various randomized controlled trials (RCTs) (Russo, 2006; Huestis, 2007). Positive effects at undetectable serum concentrations with orange terpenes (primarily limonene, 35.25% increase in mouse activity), could be explainable on the basis of rapid redistribution and concentration in lipophilic cerebral structures. A similar rationale pertains to human studies (Komori *et al.*, 1995), subsequently discussed. Limonene is highly bioavailable with 70% human pulmonary uptake (Falk-Filipsson *et al.*, 1993), and a figure of 60% for pinene with rapid metabolism or redistribution (Falk *et al.*, 1990). Ingestion and percutaneous absorption is also well documented in humans (Jäger *et al.*, 1992): 1500 mg of lavender EO with 24.7% linalool (total 372 mg) was massaged into the skin of a 60 kg man for 10 min, resulting in a peak plasma concentration of 100 ng·mL^{-1} at 19 min, and a half-life of 13.76 min in serum (Jäger *et al.*, 1992). EO mixtures (including limonene and pinene) also increase permeation of estradiol through mouse skin (Monti *et al.*, 2002).

Government-approved cannabis supplied to patients in national programmes in the Netherlands and Canada is gamma-irradiated to sterilize coliform bacteria, but the safety of this technique for a smoked and inhaled product has never been specifically tested. Gamma-radiation significantly reduced linalool titres in fresh cilantro (Fan and Sokorai, 2002), and myrcene and linalool in orange juice (Fan and Gates, 2001).

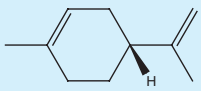
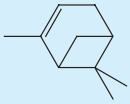
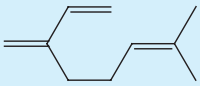
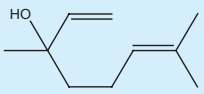
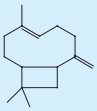
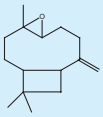
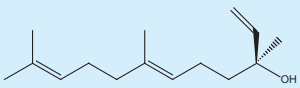
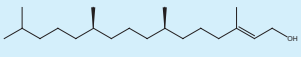
D-limonene, common to the lemon and other citrus EOs (Table 2), is the second most widely distributed terpenoid in nature (Noma and Asakawa, 2010), and is the precursor to other monoterpenoids (Figure 2) through species-specific synthetic schemes. Unfortunately, these pathways have not yet been investigated in cannabis. The ubiquity of limonene serves, perhaps, as a demonstration of convergent evolution that supports an important ecological role for this monoterpene. Studies with varying methodology and dosing in citrus oils in mice suggest it to be a powerful anxiolytic agent (Carvalho-Freitas and Costa, 2002; Pultrini Ade *et al.*, 2006), with one EO increasing serotonin in the prefrontal cortex, and dopamine (DA) in hippocampus mediated via 5-HT$_{1A}$ (Komiya *et al.*, 2006). Compelling confirmatory evidence in humans was provided in a clinical study (Komori *et al.*, 1995), in which hospitalized depressed patients were exposed to citrus fragrance in ambient air, with subsequent normalization of Hamilton Depression Scores, successful discontinuation of antidepressant medication in 9/12 patients and serum evidence of immune stimulation (CD4/8 ratio normalization). Limonene also produces apoptosis of breast cancer cells, and was employed at high doses in Phase II RCTs (Vigushin *et al.*, 1998). Subsequent investigation in cancer treatment has centred on its immediate hepatic metabolite, perillic acid, which demonstrates anti-stress effects in rat brain (Fukumoto *et al.*, 2008). A patent has been submitted, claiming that limonene effectively treats gastro-oesophageal reflux (Harris, 2010). Citrus EOs containing limonene proved effective against dermatophytes (Sanguinetti *et al.*, 2007; Singh *et al.*, 2010), and citrus EOs with terpenoid profiles resembling those in cannabis demonstrated strong radical scavenging properties (Choi *et al.*, 2000). As noted above, limonene is highly bioavailable (Falk-Filipsson *et al.*, 1993), and rapidly metabolized, but with indications of accumulation and retention in adipose tissues (e.g. brain). It is highly non-toxic (estimated human lethal dose 0.5–5 g·kg^{-1}) and non-sensitizing (Von Burg, 1995)

β-Myrcene is another common monoterpenoid in cannabis (Table 2) with myriad activities: diminishing inflammation via prostaglandin E-2 (PGE-2) (Lorenzetti *et al.*, 1991), and blocking hepatic carcinogenesis by aflatoxin (De-Oliveira *et al.*, 1997). Interestingly, myrcene is analgesic in mice, but this action can be blocked by naloxone, perhaps via the α-2 adrenoreceptor (Rao *et al.*, 1990). It is non-mutagenic in the Ames test (Gomes-Carneiro *et al.*, 2005). Myrcene is a recognized sedative as part of hops preparations (*Humulus lupulus*), employed to aid sleep in Germany (Bisset and Wichtl, 2004). Furthermore, myrcene acted as a muscle relaxant in mice, and potentiated barbiturate sleep time at high doses (do Vale *et al.*, 2002). Together, these data would support the hypothesis that myrcene is a prominent sedative terpenoid in cannabis, and combined with THC, may produce the 'couch-lock' phenomenon of certain chemotypes that is alternatively decried or appreciated by recreational cannabis consumers.

α-Pinene is a bicyclic monoterpene (Table 2), and the most widely encountered terpenoid in nature (Noma and Asakawa, 2010). It appears in conifers and innumerable plant EOs, with an insect-repellent role. It is anti-inflammatory via PGE-1 (Gil *et al.*, 1989), and is a bronchodilator in humans at low exposure levels (Falk *et al.*, 1990). Pinene is a major component of *Sideritis* spp. (Kose *et al.*, 2010) and *Salvia* spp. EOs (Ozek *et al.*, 2010), both with prominent activity against MRSA (*vide infra*). Beyond this, it seems to be a broad-spectrum antibiotic (Nissen *et al.*, 2010). α-Pinene forms the biosynthetic base for CB_2 ligands, such as HU-308 (Hanus *et al.*, 1999). Perhaps most compelling, however, is its activity as an acetylcholinesterase inhibitor aiding memory (Perry *et al.*, 2000), with an observed IC_{50} of 0.44 mM (Miyazawa

Table 2

Cannabis Terpenoid Activity Table

Terpenoid	Structure	Commonly encountered in	Pharmacological activity (Reference)	Synergistic cannabinoid
Limonene		 Lemon	Potent AD/immunostimulant via inhalation (Komori *et al.*, 1995)	CBD
			Anxiolytic (Carvalho-Freitas and Costa, 2002; Pultrini Ade *et al.*, 2006) via 5-HT_{1A} (Komiya *et al.*, 2006)	CBD
			Apoptosis of breast cancer cells (Vigushin *et al.*, 1998)	CBD, CBG
			Active against acne bacteria (Kim *et al.*, 2008)	CBD
			Dermatophytes (Sanguinetti *et al.*, 2007; Singh *et al.*, 2010)	CBG
			Gastro-oesophageal reflux (Harris, 2010)	THC
α-Pinene		 Pine	Anti-inflammatory via PGE-1 (Gil *et al.*, 1989)	CBD
			Bronchodilatory in humans (Falk *et al.*, 1990)	THC
			Acetylcholinesterase inhibitor, aiding memory (Perry *et al.*, 2000)	THC?, CBD
β-Myrcene		 Hops	Blocks inflammation via PGE-2 (Lorenzetti *et al.*, 1991)	CBD
			Analgesic, antagonized by naloxone (Rao *et al.*, 1990)	CBD, THC
			Sedating, muscle relaxant, hypnotic (do Vale *et al.*, 2002)	THC
			Blocks hepatic carcinogenesis by aflatoxin (de Oliveira *et al.*, 1997)	CBD, CBG
Linalool		 Lavender	Anti-anxiety (Russo, 2001)	CBD, CBG?
			Sedative on inhalation in mice (Buchbauer *et al.*, 1993)	THC
			Local anesthetic (Re *et al.*, 2000)	THC
			Analgesic via adenosine A_{2A} (Peana *et al.*, 2006)	CBD
			Anticonvulsant/anti-glutamate (Elisabetsky *et al.*, 1995)	CBD, THCV, CBDV
			Potent anti-leishmanial (do Socorro *et al.*, 2003)	?
β-Caryophyllene		 Pepper	AI via PGE-1 comparable phenylbutazone (Basile *et al.*, 1988)	CBD
			Gastric cytoprotective (Tambe *et al.*, 1996)	THC
			Anti-malarial (Campbell *et al.*, 1997)	?
			Selective CB_2 agonist (100 nM) (Gertsch *et al.*, 2008)	THC
			Treatment of pruritus? (Karsak *et al.*, 2007)	THC
			Treatment of addiction? (Xi *et al.*, 2010)	CBD
Caryophyllene Oxide		 Lemon balm	Decreases platelet aggregation (Lin *et al.*, 2003)	THC
			Antifungal in onychomycosis comparable to ciclopiroxolamine and sulconazole (Yang *et al.*, 1999)	CBC,CBG
			Insecticidal/anti-feedant (Bettarini *et al.*, 1993)	THCA, CBGA
Nerolidol		 Orange	Sedative (Binet *et al.*, 1972)	THC, CBN
			Skin penetrant (Cornwell and Barry, 1994)	–
			Potent antimalarial (Lopes *et al.*, 1999, Rodrigues Goulart *et al.*, 2004)	?
			Anti-leishmanial activity (Arruda *et al.*, 2005)	?
Phytol		 Green tea	Breakdown product of chlorophyll	–
			Prevents Vitamin A teratogenesis (Arnhold *et al.*, 2002)	–
			↑GABA via SSADH inhibition (Bang *et al.*, 2002)	CBG

Representative plants containing each terpenoid are displayed as examples to promote recognition, but many species contain them in varying concentrations. 5-HT, 5-hydroxytryptamine (serotonin); AD, antidepressant; AI, anti-inflammatory; CB_1/CB_2, cannabinoid receptor 1 or 2; GABA, gamma aminobutyric acid; PGE-1/PGE-2, prostaglandin E-1/prostaglandin E-2; SSADH, succinic semialdehyde dehydrogenase.

and Yamafuji, 2005). This feature could counteract short-term memory deficits induced by THC intoxication (*vide infra*).

D-Linalool is a monoterpenoid alcohol (Table 2), common to lavender (*Lavandula angustifolia*), whose psychotropic anxiolytic activity has been reviewed in detail (Russo, 2001). Interestingly, linalyl acetate, the other primary terpenoid in lavender, hydrolyses to linalool in gastric secretions (Bickers *et al.*, 2003). Linalool proved sedating to mouse activity on inhalation (Buchbauer *et al.*, 1991; Jirovetz *et al.*, 1992). In traditional aromatherapy, linalool is the likely suspect in the remarkable therapeutic capabilities of lavender EO to alleviate skin burns without scarring (Gattefosse, 1993). Pertinent to this, the local anaesthetic effects of linalool (Re *et al.*, 2000) are equal to those of procaine and menthol (Ghelardini *et al.*, 1999). Another explanation would be its ability to produce hot-plate analgesia in mice ($P < 0.001$) that was reduced by administration of an adenosine A_{2A} antagonist (Peana *et al.*, 2006). It is also anti-nociceptive at high doses in mice via ionotropic glutamate receptors (Batista *et al.*, 2008). Linalool demonstrated anticonvulsant and antiglutamatergic activity (Elisabetsky *et al.*, 1995), and reduced seizures as part of *Ocimum basilicum* EO after exposure to pentylenetetrazole, picrotoxin and strychnine (Ismail, 2006). Furthermore, linalool decreased K^+-stimulated glutamate release and uptake in mouse synaptosomes (Silva Brum *et al.*, 2001). These effects were summarized (Nunes *et al.*, 2010, p. 303): 'Overall, it seems reasonable to argue that the modulation of glutamate and GABA neurotransmitter systems are likely to be the critical mechanism responsible for the sedative, anxiolytic and anticonvulsant properties of linalool and EOs containing linalool in significant proportions'. Linalool also proved to be a powerful anti-leishmanial agent (do Socorro *et al.*, 2003), and as a presumed lavender EO component, decreased morphine opioid usage after inhalation versus placebo ($P = 0.04$) in gastric banding in morbidly obese surgical patients (Kim *et al.*, 2007).

β-Caryophyllene (Table 2) is generally the most common sesquiterpenoid encountered in cannabis (Mediavilla and Steinemann, 1997), wherein its evolutionary function may be due to its ability to attract insect predatory green lacewings, while simultaneously inhibiting insect herbivory (Langenheim, 1994). It is frequently the predominant terpenoid overall in cannabis extracts, particularly if they have been processed under heat for decarboxylation (Guy and Stott, 2005). Caryophyllene is common to black pepper (*Piper nigrum*) and Copaiba balsam (*Copaifera officinalis*) (Lawless, 1995). It is anti-inflammatory via PGE-1, comparable in potency to the toxic phenylbutazone (Basile *et al.*, 1988), and an EO containing it was on par with etodolac and indomethacin (Ozturk and Ozbek, 2005). In contrast to the latter agents, however, caryophyllene was a gastric cytoprotective (Tambe *et al.*, 1996), much as had been claimed in the past in treating duodenal ulcers in the UK with cannabis extract (Douthwaite, 1947). Caryophyllene may have contributed to antimalarial effects as an EO component (Campbell *et al.*, 1997). Perhaps the greatest revelation regarding caryophyllene has been its demonstration as a selective full agonist at CB_2 (100 nM), the first proven phytocannabinoid beyond the cannabis genus (Gertsch *et al.*, 2008). Subsequent work has demonstrated that this dietary component produced anti-inflammatory analgesic activity at the lowest dose of 5 $mg \cdot kg^{-1}$ in wild-type, but not CB_2 knockout mice (Gertsch, 2008). Given the lack of attributed psychoactivity of CB_2 agonists, caryophyllene offers great promise as a therapeutic compound, whether systemically, or in dermatological applications such as contact dermatitis (Karsak *et al.*, 2007). Sensitization reactions are quite rare, and probably due to oxidized product (Skold *et al.*, 2006).

Nerolidol is a sesquiterpene alcohol with sedative properties (Binet *et al.*, 1972), present as a low-level component in orange and other citrus peels (Table 2). It diminished experimentally induced formation of colon adenomas in rats (Wattenberg, 1991). It was an effective agent for enhancing skin penetration of 5-fluorouracil (Cornwell and Barry, 1994). This could be a helpful property in treating fungal growth, where it is also an inhibitor (Langenheim, 1994). It seems to have anti-protozoal parasite control benefits, as a potent antimalarial (Lopes *et al.*, 1999; Rodrigues Goulart *et al.*, 2004) and anti-leishmanial agent (Arruda *et al.*, 2005). Nerolidol is non-toxic and non-sensitizing (Lapczynski *et al.*, 2008).

Caryophyllene oxide (Table 2) is a sesquiterpenoid oxide common to lemon balm (*Melissa officinalis*), and to the eucalyptus, *Melaleuca stypheloides*, whose EO contains 43.8% (Farag *et al.*, 2004). In the plant, it serves as an insecticidal/anti-feedant (Bettarini *et al.*, 1993) and as broad-spectrum antifungal in plant defence (Langenheim, 1994). Analogously, the latter properties may prove therapeutic, as caryophyllene oxide demonstrated antifungal efficacy in a model of clinical onychomycosis comparable to ciclopiroxalamine and sulconazole, with an 8% concentration affecting eradication in 15 days (Yang *et al.*, 1999). Caryophyllene oxide is non-toxic and non-sensitizing (Opdyke, 1983). This agent also demonstrates anti-platelet aggregation properties *in vitro* (Lin *et al.*, 2003). Caryophyllene oxide has the distinction of being the component responsible for cannabis identification by drug-sniffing dogs (Stahl and Kunde, 1973).

Phytol (Table 2) is a diterpene (McGinty *et al.*, 2010), present in cannabis extracts, as a breakdown product of chlorophyll and tocopherol. Phytol prevented vitamin A-induced teratogenesis by inhibiting conversion of retinol to a harmful metabolite, all-*trans*-retinoic acid (Arnhold *et al.*, 2002). Phytol increased GABA expression via inhibition of succinic semialdehyde dehydrogenase, one of its degradative enzymes (Bang *et al.*, 2002). Thus, the presence of phytol could account for the alleged relaxing effect of wild lettuce (*Lactuca sativa*), or green tea (*Camellia sinensis*), despite the latter's caffeine content.

Selected possibilities for phytocannabinoid-terpenoid synergy

Cannabis and acne

AEA simulates lipid production in human sebocytes of sebaceous glands at low concentrations, but induces apoptosis at higher levels, suggesting that this system is under ECS control (Dobrosi *et al.*, 2008). CBD 10–20 μM did not affect basal lipid synthesis in SZ95 sebocytes, but did block such stimulation by AEA and arachidonate (Biro *et al.*, 2009). Higher doses of CBD (30–50 μM) induced sebocyte apoptosis, which was augmented in the presence of AEA. The effect of CBD to increase

Ca^{++} was blocked by ruthenium red, a TRP-inhibitor. RNA-mediated silencing of TRPV1 and TRPV3 failed to attenuate CBD effects, but experiments did support the aetiological role of TRPV4, a putative regulator of systemic osmotic pressure (T. Bíró, 2010, pers. comm.). Given the observed ability of CBD to be absorbed transcutaneously, it offers great promise to attenuate the increased sebum production at the pathological root of acne.

Cannabis terpenoids could offer complementary activity. Two citrus EOs primarily composed of limonene inhibited *Propionibacterium acnes*, the key pathogen in acne (MIC 0.31 $\mu L \cdot mL^{-1}$), more potently than triclosan (Kim *et al.*, 2008). Linalool alone demonstrated an MIC of 0.625 $\mu L \cdot mL^{-1}$. Both EOs inhibited *P. acnes*-induced TNF-α production, suggesting an adjunctive anti-inflammatory effect. In a similar manner, pinene was the most potent component of a tea-tree eucalyptus EO in suppression of *P. acnes* and *Staph* spp. in another report (Raman *et al.*, 1995).

Considering the known minimal toxicities of CBD and these terpenoids and the above findings, new acne therapies utilizing whole CBD-predominant extracts, via multi-targeting (Wagner and Ulrich-Merzenich, 2009), may present a novel and promising therapeutic approach that poses minimal risks in comparison to isotretinoin.

MRSA

MRSA accounted for 10% of cases of septicaemia and 18 650 deaths in the USA in 2005, a number greater than that attributable to human immunodeficiency virus/acquired immunodeficiency syndrome (Bancroft, 2007). Pure CBD and CBG powerfully inhibit MRSA (MIC 0.5–2 $\mu g \cdot mL^{-1}$) (Appendino *et al.*, 2008).

Amongst terpenoids, pinene was a major component of *Sideritis erythrantha* EO that was as effective against MRSA and other antibiotic-resistant bacterial strains as vancomycin and other agents (Kose *et al.*, 2010). A *Salvia rosifolia* EO with 34.8% pinene was also effective against MRSA (MIC 125 $\mu g \cdot mL^{-1}$). The ability of monoterpenoids to enhance skin permeability and entry of other drugs may further enhance antibiotic benefits (Wagner and Ulrich-Merzenich, 2009).

Given that CBG can be produced in selected cannabis chemotypes (de Meijer and Hammond, 2005; de Meijer *et al.*, 2009a), with no residual THC as a possible drug abuse liability risk, a whole plant extract of a CBG-chemotype also expressing pinene would seem to offer an excellent, safe new antiseptic agent.

Psychopharmacological applications: depression, anxiety, insomnia, dementia and addiction

Scientific investigation of the therapeutic application of terpenoids in psychiatry has been hampered by methodological concerns, subjective variability of results and a genuine dearth of appropriate randomized controlled studies of high quality (Russo, 2001; Bowles, 2003; Lis-Balchin, 2010). The same is true of phytocannabinoids (Fride and Russo, 2006). Abundant evidence supports the key role of the ECS in mediating depression (Hill and Gorzalka, 2005a,b), as well as anxiety, whether induced by aversive stimuli, such as post-traumatic stress disorder (Marsicano *et al.*, 2002) or pain (Hohmann *et al.*, 2005), and psychosis (Giuffrida *et al.*, 2004). With respect to the latter risk, the presence of CBD in smoked cannabis based on hair analysis seems to be a mitigating factor reducing its observed incidence (Morgan and Curran, 2008). A thorough review of cannabis and psychiatry is beyond the scope of this article, but several suggestions are offered with respect to possible therapeutic synergies operative with phytocannabinoids-terpenoid combinations. While the possible benefits of THC on depression remain controversial (Denson and Earleywine, 2006), much less worrisome would be CBD- or CBG-predominant preparations. Certainly the results obtained in human depression solely with a citrus scent (Komori *et al.*, 1995), strongly suggest the possibility of synergistic benefit of a phytocannabinoid-terpenoid preparation. Enriched odour exposure in adult mice induced olfactory system neurogenesis (Rochefort *et al.*, 2002), an intriguing result that could hypothetically support plasticity mechanisms in depression (Delgado and Moreno, 1999), and similar hypotheses with respect to the ECS in addiction treatment (Gerdeman and Lovinger, 2003). Phytocannabinoid-terpenoid synergy might theoretically apply.

The myriad effects of CBD on 5-HT_{1A} activity provide a strong rationale for this and other phytocannabinoids as base compounds for treatment of anxiety. Newer findings, particularly imaging studies of CBD in normal individuals in anxiety models (Fusar-Poli *et al.*, 2009; 2010; Crippa *et al.*, 2010) support this hypothesis. Even more compelling is a recent randomized control trial of pure CBD in patients with social anxiety disorder with highly statistical improvements over placebo in anxiety and cognitive impairment (Crippa *et al.*, 2011). Addition of anxiolytic limonene and linalool could contribute to the clinical efficacy of a CBD extract.

THC was demonstrated effective in a small crossover clinical trial versus placebo in 11 agitated dementia patients with Alzheimer's disease (Volicer *et al.*, 1997). THC was also observed to be an acetylcholinesterase inhibitor in its own right, as well as preventing amyloid β-peptide aggregation in that disorder (Eubanks *et al.*, 2006). Certainly, the anti-anxiety and anti-psychotic effects of CBD may be of additional benefit (Zuardi *et al.*, 1991; 2006; Zuardi and Guimaraes, 1997). A recent study supports the concept that CBD, when present in significant proportion to THC, is capable of eliminating induced cognitive and memory deficits in normal subjects smoking cannabis (Morgan *et al.*, 2010b). Furthermore, CBD may also have primary benefits on reduction of β-amyloid in Alzheimer's disease (Iuvone *et al.*, 2004; Esposito *et al.*, 2006a,b). Psychopharmacological effects of limonene, pinene and linalool could putatively extend benefits in mood in such patients.

The effects of cannabis on sleep have been reviewed (Russo *et al.*, 2007), and highlight the benefits that can accrue in this regard, particularly with respect to symptom reduction permitting better sleep, as opposed to a mere hypnotic effect. Certainly, terpenoids with pain-relieving, anti-anxiety or sedative effects may supplement such activity, notably, caryophyllene, linalool and myrcene.

The issue of cannabis addiction remains controversial. Some benefit of oral THC has been noted in cannabis withdrawal (Hart *et al.*, 2002; Haney *et al.*, 2004). More intriguing, perhaps, are claims of improvement on other substance dependencies, particularly cocaine (Labigalini *et al.*, 1999; Dreher, 2002). The situation with CBD is yet more promising. CBD and THC at doses of 4 $mg \cdot kg^{-1}$ i.p. potentiated extinction of cocaine- and amphetamine-induced conditioned place preference in rats, and CBD produced no hedonic effects of its own (Parker *et al.*, 2004). CBD 5 $mg \cdot kg^{-1} \cdot d^{-1}$ in rats attenuated heroin-seeking behaviour by conditioned stimuli, even after a lapse of 2 weeks (Ren *et al.*, 2009). A suggested mechanism of CBD relates to its ability to reverse changes in α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate glutamate and CB_1 receptor expression in the nucleus accumbens induced by heroin. The authors proposed CBD as a treatment for heroin craving and addiction relapse. A recent study demonstrated the fascinating result that patients with damage to the insula due to cerebrovascular accident were able to quit tobacco smoking without relapse or urges (Naqvi *et al.*, 2007), highlighting this structure as a critical neural centre mediating addiction to nicotine. Further study has confirmed the role of the insula in cocaine, alcohol and heroin addiction (Naqvi and Bechara, 2009; Naqvi and Bechara, 2010). In a provocative parallel, CBD 600 mg p.o. was demonstrated to deactivate functional magnetic resonance imaging (fMRI) activity in human volunteers in the left insula versus placebo ($P < 0.01$) without accompanying sedation or psychoactive changes (Borgwardt *et al.*, 2008), suggesting the possibility that CBD could act as a pharmaceutical surrogate for insular damage in exerting an anti-addiction therapeutic benefit. Human studies have recently demonstrated that human volunteers smoking cannabis with higher CBD content reduced their liking for drug-related stimuli, including food (Morgan *et al.*, 2010a). The authors posited that CBD can modulate reinforcing properties of drugs of abuse, and help in training to reduce relapse to alcoholism. A single case report of a successful withdrawal from cannabis dependency utilizing pure CBD treatment was recently published (Crippa *et al.*, 2010).

Perhaps terpenoids can provide adjunctive support. In a clinical trial, 48 cigarette smokers inhaling vapour from an EO of black pepper (*Piper nigrum*), a mint-menthol mixture or placebo (Rose and Behm, 1994). Black pepper EO reduced nicotine craving significantly ($P < 0.01$), an effect attributed to irritation of the bronchial tree, simulating the act of cigarette smoking, but without nicotine or actual burning of material. Rather, might not the effect have been pharmacological? The terpenoid profile of black pepper suggests possible candidates: myrcene via sedation, pinene via increased alertness, or especially caryophyllene via CB_2 agonism and a newly discovered putative mechanism of action in addiction treatment.

CB_2 is expressed in dopaminergic neurones in the ventral tegmental area and nucleus accumbens, areas mediating addictive phenomena (Xi *et al.*, 2010). Activation of CB_2 by the synthetic agonist JWH144 administered systemically, intranasally, or by microinjection into the nucleus accumbens in rats inhibited DA release and cocaine self-administration. Caryophyllene, as a high-potency selective CB_2 agonist (Gertsch *et al.*, 2008), would likely produce similar effects, and have the advantage of being a non-toxic dietary component. All factors considered, CBD, with caryophyllene, and possibly other adjunctive terpenoids in the extract, offers significant promise in future addiction treatment.

Taming THC: cannabis entourage compounds as antidotes to intoxication

Various sources highlight the limited therapeutic index of pure THC, when given intravenously (D'Souza *et al.*, 2004) or orally (Favrat *et al.*, 2005), especially in people previously naïve to its effects. Acute overdose incidents involving THC or THC-predominant cannabis usually consist of self-limited panic reactions or toxic psychoses, for which no pharmacological intervention is generally necessary, and supportive counselling (reassurance or 'talking down') is sufficient to allow resolution without sequelae. CBD modulates the psychoactivity of THC and reduces its adverse event profile (Russo and Guy, 2006), highlighted by recent results above described. Could it be, however, that other cannabis components offer additional attenuation of the less undesirable effects of THC? History provides some clues.

In 10th century Persia, Al-Razi offered a prescription in his *Manafi al-agdhiya wa-daf madarri-ha* (p. 248), rendered (Lozano, 1993, p. 124; translation EBR) ' – and to avoid these harms {from ingestion of cannabis seeds or hashish}, one should drink fresh water and ice or eat any acid fruits'. This concept was repeated in various forms by various authorities through the ages, including ibn Sina (ibn Sina (Avicenna), 1294), and Ibn al-Baytar (ibn al-Baytar, 1291), until O'Shaughnessy brought Indian hemp to Britain in 1843 (O'Shaughnessy, 1843). Robert Christison subsequently cited lemon (Figure 3A) as an antidote to acute intoxication in numerous cases (Christison, 1851) and this excerpt regarding morning-after residua (Christison, 1848) (p. 973):

> Next morning there was an ordinary appetite, much torpidity, great defect and shortness of memory, extreme apparent protraction of time, but no peculiarity of articulation or other effect; and these symptoms lasted until 2 P.M., when they ceased entirely in a few minutes after taking lemonade.

Literary icons on both sides of the Atlantic espoused similar support for the citrus cure in the 19th century, notably Bayard Taylor after travels in Syria (Taylor, 1855), and Fitzhugh Ludlow after his voluntary experiments with ever higher cannabis extract doses in the USA (Ludlow, 1857). The sentiment was repeated by Calkins (1871), who noted the suggestion of a friend in Tunis that lemon retained the confidence of cure of overdoses by cannabis users in that region. This is supported by the observation that lemon juice, which normally contains small terpenoid titres, is traditionally enhanced in North Africa by the inclusion in drinks of the limonene-rich rind, as evidenced by the recipe for *Agua Limón* from modern Morocco (Morse and Mamane, 2001). In his comprehensive review of cannabis in the first half of the 20th century, Walton once more supported its prescription (Walton, 1938).

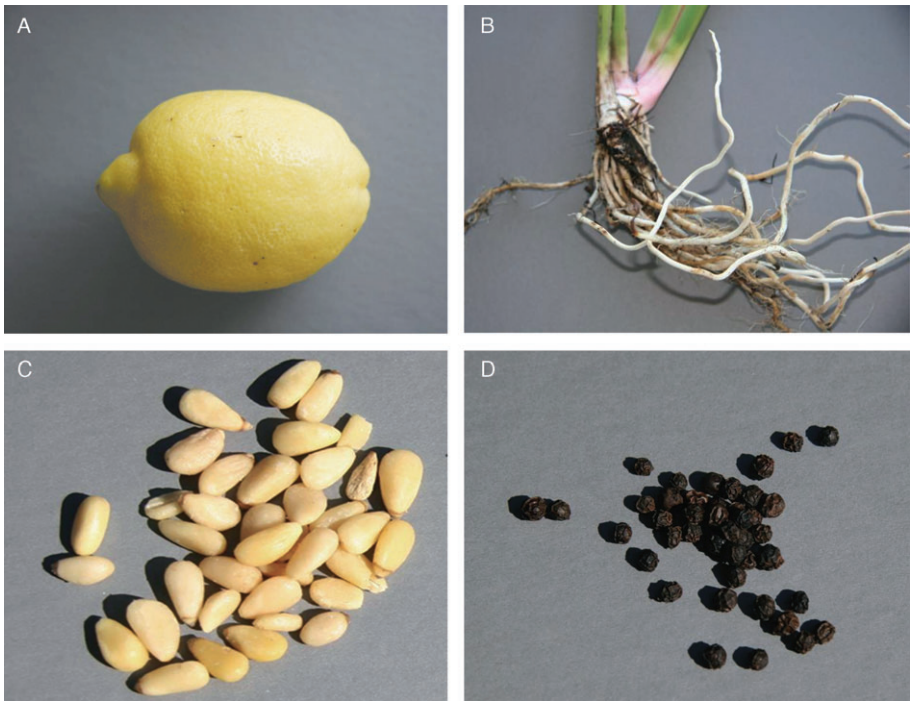


Figure 3

Ancient cannabis antidotes. (A) Lemon (*Citrus limon*). (B) Calamus plant roots (*Acorus calamus*). (C) Pine nuts (*Pinus* spp.). (D) Black pepper (*Piper nigrum*).

Another traditional antidote to cannabis employing *Acorus calamus* (Figure 3B) is evident from the Ayurvedic tradition of India (Lad, 1990, p. 131):

> Calamus root is the best antidote for the ill effects of marijuana. . . . if one smokes a pinch of calamus root powder with the marijuana, this herb will completely neutralize the toxic side effects of the drug.

This claim has gained credence, not only through force of anecdotal accounts that abound on the Internet, but with formal scientific case reports and scientific analysis (McPartland *et al.*, 2008) documenting clearer thinking and improved memory with the cannabis–calamus combination, and with provision of a scientific rationale: calamus contains beta-asarone, an acetylcholinesterase inhibitor with 10% of the potency of physotigmine (Mukherjee *et al.*, 2007). Interestingly, the cannabis terpenoid, α-pinene, also has been characterized as a potent inhibitor of that enzyme (Miyazawa and Yamafuji, 2005), bolstering the hypothesis of a second antidote to THC contained in cannabis itself. Historical precedents also support pinene in this pharmacological role.

In the firstt century, Pliny wrote of cannabis in his *Natural History, Book XXIV* (Pliny, 1980, p. 164):

> The gelotophyllis ['leaves of laughter' = cannabis] grows in Bactria and along the Borysthenes. If this be taken in myrrh and wine all kinds of phantoms beset the mind, causing laughter which persists until the kernels of pine-nuts are taken with pepper and honey in palm wine.

Of the components, palm wine is perhaps the most mysterious. Ethanol does not reduce cannabis intoxication (Mello and Mendelson, 1978). However, ancient wines were stored in clay pots or goatskins, and required preservation, usually with addition of pine tar or terebinth resin (from *Pistacia* spp.; McGovern *et al.*, 2009). Pine tar is rich in pinene, as is terebinth resin (from *Pistacia terebinthus*; Tsokou *et al.*, 2007), while the latter also contains limonene (Duru *et al.*, 2003). Likewise, the pine nuts (Figure 3C) prescribed by Pliny the Elder harbour pinene, along with additional limonene (Salvadeo *et al.*, 2007). Al-Ukbari also suggested pistachio nuts as a cannabis antidote in the 13th century (Lozano, 1993), and the ripe fruits of *Pistacia terebinthus* similarly contain pinene (Couladis *et al.*, 2003). The black pepper (Figure 3D), might offer the mental clarity afforded by pinene, sedation via myrcene and helpful contributions by β-caryophyllene. The historical suggestions for cannabis antidotes are thus supported by modern scientific rationales for the claims, and if proven experimentally would provide additional evidence of synergy (Berenbaum, 1989; Wagner and Ulrich-Merzenich, 2009).

Conclusions and suggestions for future study

Considered ensemble, the preceding body of information supports the concept that selective breeding of cannabis chemotypes rich in ameliorative phytocannabinoid and terpenoid content offer complementary pharmacological activities that may strengthen and broaden clinical applications and improve the therapeutic index of cannabis extracts containing THC, or other base phytocannabinoids. Psychopharmacological and dermatological indications show the greatest promise.

One important remaining order of business is the elucidation of mono- and sesquiterpenoid biosynthetic pathways in cannabis, as has been achieved previously in other species of plants (Croteau, 1987; Gershenzon and Croteau, 1993; Bohlmann *et al*., 1998; Turner *et al*., 1999; Trapp and Croteau, 2001).

Various cannabis component combinations or cannabis extracts should be examined via high throughput pharmacological screening where not previously accomplished. Another goal is the investigation of the biochemical targets of the cannabis terpenoids, along with their mechanisms of action, particularly in the central nervous system. Possible techniques for such research include radio-labelling of select agents in animals with subsequent necropsy. On a molecular level, investigation of terpenoid changes to phytocannabinoid signal transduction and trafficking may prove illuminating. While it is known that terpenoids bind to odorant receptors in the nasal mucosa (Friedrich, 2004) and proximal olfactory structures (Barnea *et al*., 2004), it would be essential to ascertain if direct effects in limbic or other cerebral structures are operative. Given that farnesyl pyrophosphate is a sesquiterpenoid precursor and the most potent endogenous agonist yet discovered for GPR92 (McHugh *et al*., 2010), *in silico* studies attempting to match minor cannabinoids and terpenoids to orphan GPCRs may prove fruitful. Behavioural assays of agents in animal models may also provide clues. Simple combinations of phytocannabinoids and terpenoids may demonstrate synergy as antibiotics if MICs are appreciable lowered (Wagner and Ulrich-Merzenich, 2009). Ultimately, fMRI and single photon emission computed tomography studies in humans, with simultaneous drug reaction questionnaires and psychometric testing employing individual agents and phytocannabinoid-terpenoid pairings via vaporization or oromucosal application, would likely offer safe and effective methods to investigate possible interactions and synergy.

Should positive outcomes result from such studies, phytopharmaceutical development may follow. The development of zero-cannabinoid cannabis chemotypes (de Meijer *et al*., 2009b) has provided extracts that will facilitate discernment of the pharmacological effects and contributions of different fractions. Breeding work has already resulted in chemotypes that produce 97% of monoterpenoid content as myrcene, or 77% as limonene (E. de Meijer, pers. comm.). Selective cross-breeding of high-terpenoid- and high-phytocannabinoid-specific chemotypes has thus become a rational target that may lead to novel approaches to such disorders as treatment-resistant depression, anxiety, drug dependency, dementia and a panoply of dermatological disorders, as well as industrial applications as safer pesticides and antiseptics. A better future via cannabis phytochemistry may be an achievable goal through further research of the entourage effect in this versatile plant that may help it fulfil its promise as a pharmacological treasure trove.

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Conflict of Interest

The author is a Senior Medical Advisor to GW Pharmaceuticals and serves as a consultant.

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