The Clinical Endocannabinoid System Consortium (CESC) Responds to:

Request for Information: Increasing the Varieties of Marijuana and Marijuana products for Research

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INTRODUCTION

NIDA is interested in gathering information on whether other specific marijuana varieties or marijuana-derived products are of interest to the research community. Based on this Request For Information (RFI) The Clinical Endocannabinoid System Consortium (The CESC) has prepared this response. The CESC is a non-profit organization with a mission to align Cannabis science, clinical practice, and public policy to ensure best outcomes. Our initial observation of the marijuana varieties used in states with medical cannabis laws noted higher levels of THC and CBD than the varieties available in the marijuana plant material of the NIDA drug supply program. Furthermore, we noted that levels of terpenoids or flavonoids were not addressed. In this RFI response we specifically address NIDA's 3 questions as follows:

1) The specific marijuana varieties, strains, or constituent chemotypes that are of research interest;

We present observations on marijuana varieties analyzed by a commercial medical cannabis testing lab that are distinct from the marijuana varieties available in NIDA's drug supply program. Included is a discussion and recommendations in the section on CANNABIS CHEMOTYPES. This discussion extends beyond the principal cannabinoids (THC and CBD) that are already understood to be of importance by NIDA – to include additional cannabinoids, as well as terpenoids and flavonoids. The marijuana varieties discussed are being used with anecdotal reports of medical efficacy. Our recommendations include a proposal for observational study of dosing and clinical efficacy; The Dosing Project.

2) The marijuana constituents, products and/or preparations that are of research interest;

We discuss the use of the extracted cannabis oil commonly known as "Phoenix Tears" or "RSO". This preparation may have specific use in patients that have been diagnosed with Glioblastoma and other cancer types. Our discussion includes the ethical need to determine whether the extracted cannabis oil has therapeutic value in Glioblastoma.

3) The particular research questions that could or would be addressed with such products.

The likely occurrence of marijuana varieties distinct from the NIDA drug supply program commonly being used for medical purposes highlights a fundamental question; "how much?". How much cannabis is necessary to produce statistically significant clinical efficacy, if any? The Dosing Project addresses this question by first observing what varieties or extracts are being used and rapidly identifying potential for formal clinical trial.

Finally, the observed distinction between marijuana varieties commonly used and those supplied by the NIDA drug supply program exposes the problem of producing varieties that have consistent chemotypes and are pathogen free. By determining the genetics, growing method and exposure to pathogens a nursery stock can be produced that is consistent and viable for producing medical cannabis products.

BACKGROUND & RATIONALE FOR THE DOSING PROJECT

For the past several decades, the standard of medicine has championed evidence-based treatment as the optimum choice of action when confronted with an illness. Using treatment without evidence can lead to unexpected and unwanted outcome. However, the growing interest in complementary and alternative medicine presents the physician and patient with a conundrum. Should the clinician negate the utility of alternative treatment if it doesn't have the appropriate evidence? What if the treatment appears to be working? Such is the case with Cannabis. Clinical trials are methodical, time-consuming endeavors. With little documented observation, it is anyone's guess as to the appropriate dose or mode of administration of cannabis. A previous study surveyed medical cannabis users in order to identify a popular distinction between the symptom relief of Cannabis Sativa and Cannabis Indica [1]. In their discussion, the authors identify "anecdote" and "unreliable opinion" as confounding variables; calling for improved methodology. The work of Hillig and Mahlberg[2] identified differences in THC and CBD ratios that may be useful in distinguishing different cannabis strains. Medical cannabis dispensaries and laboratories now routinely identify chemotype by percentage weight of THC and CBD for their clients. As a solution, the CESC proposes a unique project that solicits knowledge of chemotype and uses current methods of cannabis medication to direct targeted clinical trials.

Study Rationale

The Dosing Project is a Clinical Study designed to evaluate trends in Cannabis efficacy. Our initial approach is observational, not prescriptive. We do not assign subjects (informants) to pre-defined treatment groups. The closest example in terms of a "traditional" FDA- approved clinical trial might be a Phase IV post-approval study with emphasis on surveillance. We record what subjects voluntarily reveal about cannabis efficacy. We anticipate that trends will emerge from this analytical approach. Thus, the Dosing Project serves as the foundation for our next CESC project; to design fully compliant, IND-enabled, prescriptive clinical trials.

The launch of The Dosing Project involves the successful completion of multiple phases. The initial phase is described as the Proof of Concept (POC) phase. The overarching goal of this phase includes determining weight-based dosing efficacy for at least 1 out of 9 major Cannabis chemotype groups (defined below) for symptom relief of pain, and disordered sleep.

The POC Phase includes several sub-phases, the first of which is the Initial Roll-Out. During this sub-phase we intend to accomplish the following:

1) We will establish self-reporting of the indication (either pain or disordered sleep), subject height and weight;
2) We will also establish methodology for self-reporting of Cannabinoid chemotype group (see below: High CBD, Equivalent CBD:THC, or High THC), as well as Terpenoid chemotype groups based on aroma: "Floral", "Fuel", or "Earth"),; and
3) We will establish the Self-reporting of symptom relief on a 4 part categorical scale.

Finally, we would note that this initial Roll-Out is limited to Modes of Administration (MOA) of Cannabis that include smoking or vaporizing only. The main questions we intend to answer during the initial Roll-Out include:
1) How well does the mobile app work?
2) How robust is recruitment?
3) How precisely can a statistically significant dose-response model be obtained for any of the Cannabis chemotype groups at this early stage?

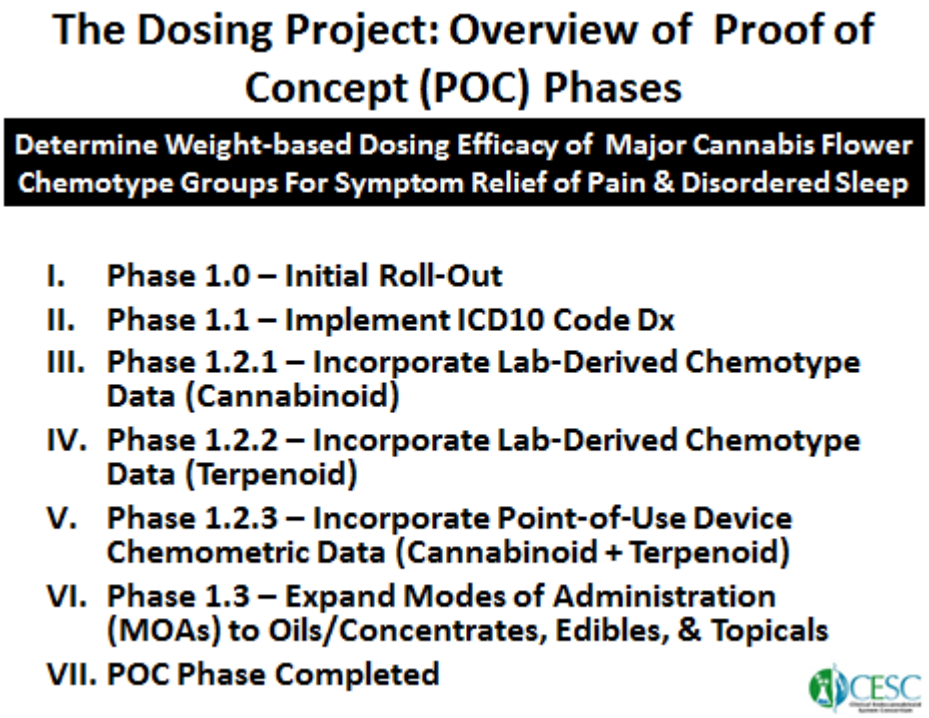


Figure 1 The Dosing Project: Overview of Proof of Concept (POC) Phases.
Additional factors are added in each subsequent phase. Phase 1.3 completion signals the end of POC; future program development will then center on expanding the scope of the response variables.

In subsequent sub-phases of The Dosing Project POC we next incorporate provider-assigned diagnostic codes (ICD10) for their specific indications contained within the broad categories of Pain and Disordered Sleep. By adding this parameter to the patient response, we achieve the ability to further stratify the dose-response model based on specific ICD10 diagnoses. Furthermore, in conjunction with diagnosis, we will incorporate the patient's Provider ID code as a credentialing parameter at login. This provides the ability to move parts of the data structure behind a HIPAA wall. It allows linkage with other patient chart data that may become valuable for future analyses. In particular, we note that at that stage, in addition to the "subject height and weight", the information regarding subject sex and age will be extremely valuable. We note that new NIH guidelines regarding both preclinical and clinical studies now require the inclusion or consideration of sex as a biological variable

Next, we will add the analysis results produced by certified testing labs on the cannabis medicine being reported. For this to occur, we anticipate developing relationships with verified dispensaries and testing labs with access to their analytical data. At that point, we can phase out the self-reporting of chemotype and replace it with the actual lab-derived cannabinoid and terpenoid content data.

Finally, our data structure permits expansion of the study into additional MOAs. This last POC sub-phase will include expansion of medicine presentations to include: concentrates / oils, edibles (including capsules and juices), and topical products.

Our study is unique and innovative in its approach to evidence based conclusions for complementary and alternative treatments. Cannabis is only one of many plants that are popularly being used. In addition to herbalism, acupuncture, chiropractic and many complementary and alternative treatments have or may become popular. Our observational study addresses that need for modern scientific proof and eventually leads to directed evidence based trial. We believe that in our approach trends will emerge quickly and evidence more speedily provided to patients and the community at large.

CANNABIS CHEMOTYPES

Cannabinoid

The initial assignment of chemotype for an individual Cannabis plant can be based on its THC/CBD ratio and assigned to a discrete chemical phenotype. Since 1973 [3,4], 3 main chemotype groupings have been recognized: Group I plants have a high THC/CBD ratio (>> 1) – most typical "drug" type plants fall into this category; Group II plants have an intermediate ratio (close to 1); and include such varietals as "Harlequin" . Group III plants have a low THC/CBD ratio (<< 1) – and would include varietals such as: "Cannatonic", "AC/DC", and "Charlotte's Web", as well as the bulk of hemp (fiber) varietals. A preliminary genetic model involving one

locus, B, with two alleles, B_D (High CBD producing) and B_T, (High THC producing) has been proposed, with the two alleles being codominant [5]. This genetic model, however, may require further refinement, especially given the major sequence differences that have been described between the THCA and CBDA synthases. Hillig & Mahlberg [2] used Gas chromatography to quantify THC and CBD cannabinoid levels in 96 Cannabis plant accessions, and demonstrated the presence of these 3 main cannabinoid chemotypes in a scatterplot of %THC vs %CBD (with linear scaled axes.

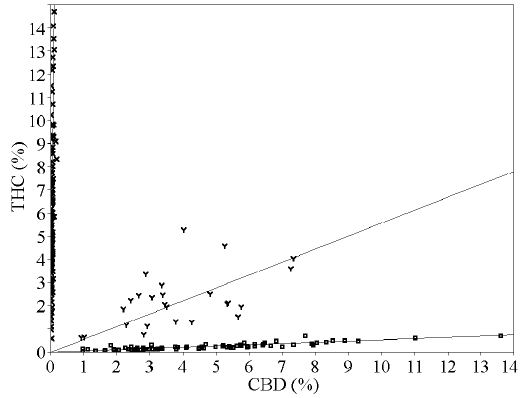


Figure 2 Plot of Δ^9-tetrahydrocannabinol (THC) % vs. cannabidiol (CBD)% for 253 *Cannabis* plants. Chemotype I, II, and III plants are marked with an X, Y, and square, respectively. Linear regression lines (forced through the origin) are drawn for each chemotype. [From Hillig and Mahlberg[2]]

In 2014 we analyzed THC and CBD quantity data from over 680 Cannabis flower samples that had been submitted to a commercial Cannabis testing lab. We independently reproduced and verified the 3 major cannabinoid chemotypes described above. Furthermore, by presenting the % CBD vs % THC scatterplot data on log10 scaled axes, we were able to display these 3 chemotypes as clusters (Figure 1). We have assigned the average % THC and % CBD for each of these chemotype groups by determining the center of each cluster. These center values provide the basis for the initial %THC and % CBD quantities assigned to each of the 3 chemotype groups during the early, initial roll-out phase of The Dosing Project.

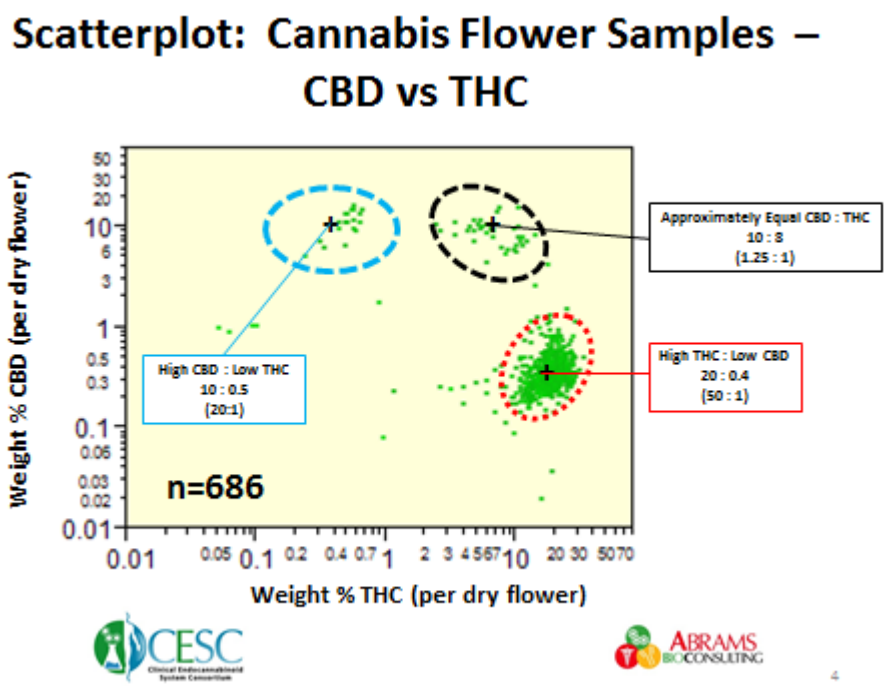


Figure 3 Plot of Cannabidiol (CBD)% vs. Δ^9-tetrahydrocannabinol (THC) % on log scaled axes.
This Scatter plot of 686 distinct plant-derived flower samples reveals that Cannabis strains fall into three major groups; a high THC, low CBD group, a roughly equal THC:CBD group and a low THC, high CBD group. THC and CBD concentrations in methanol extracts of dried flower samples were analyzed using Gas Chromatography, a method that converts all plant-derived cannabinoids to their decarboxylated forms from their acidic precursors. This analysis was based on a dataset kindly provided by SD PharmLabs LLC (San Diego, CA).

As a point of comparison, we include in Figure 4 the observed % THC and % CBD chemotype groups overlaid with the current NIDA chemotype groupings for bulk marijuana. There is discrepancy between the NIDA groupings and our observations. In fact, some assigned NIDA groups do not appear to include natural Cannabis cultivars. Below in Figure 10 and Table 2 we address this with specific recommendations.

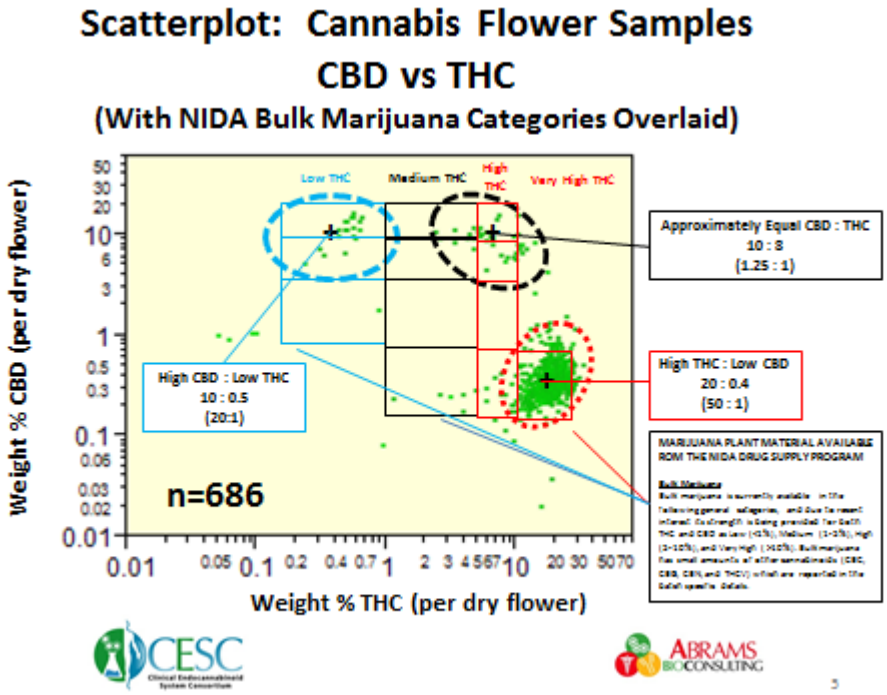


Figure 4 Cannabis Flower Samples CBD vs THC, with NIDA Bulk Marijuana Categories Overlaid
The dataset of Figure 3 is compared with NIDA proposed medical marijuana potency categories. It can be observed that several of the NIDA categories fall outside the ranges of what is observed for currently available Cannabis flower samples

We believe that values for cannabinoid content (such as % THC and % CBD) in an individual flower sample are best described by indicating what range they fall into. We do not believe that a single potency determination for a single flower sample is indicative of what is actually in the batch or lot that it came from. Instead, we advocate that multiple samples from the batch or lot of origin be analyzed to determine the potency range for a given cannabinoid. We suspect there is just too much variation within a given plant, let alone the entire harvest lot, to permit reliance on a single sample determination. We are currently carrying out potency variance analysis both across an individual plant as well as within entire plots. We anticipate that the Power Analyses applied to these datasets will provide guidance for optimal sampling strategies going forward.

Beyond THC and CBD Cannabinoids we support the cultivation of strains that permit the evaluation of additional Cannabinoids like: CBG(A), THCV(A) and CBN, this later is not a natural product, but arises from THC oxidation. CBG(A) has been reported to demonstrate anti-inflammatory activity in a mouse model of IBD [6]. CBG(A) is a precursor of Cannabinoids like THC(A), CBD(A), and CBC(A) and would likely be present transiently unless its conversion to product were synchronized and either delayed or halted. CBN has been suggested as a sleep aid. This interesting cannabinoid oxidation product (which may serve as a stability parameter

for dried flower preparations) would need to be produced under controlled conditions in order to furnish qualified material for research. Finally, the recent positive results in a Type II Diabetes Phase II trial with THCV (and CBD) are very encouraging and argue for inclusion of South African or equatorial origin strains such as Durban Poison which has been reported to produce THCV(A) at elevated levels.

Terpenoid:

Using the phytocannabinoids THC and CBD levels as criteria, Cannabis strains can be separated into as few as 3 principal categories. While these categories have shown utility in guiding patients in the right general direction, the widely varying effects among strains within a category is the basis for the "entourage effect"- the synergistic effect of cannabinoids with other phytochemicals which either act directly on the CB1 or CB2 receptor or indirectly by inhibiting enzymes responsible for the synthesis or degradation of endogenous cannabinoids (endocannabinoids). This complex interplay of phytocannabinoids and terpenoids is of high interest to the few laboratories fortunate enough to have the permission and funding to conduct investigations. Thus, a rigorous classification system is needed to de-convolute the entourage effect and thereby facilitate both accurate prescriptions by medical professionals and recommendations by point-of-sale dispensary employees.

In contrast to cannabinoids, terpenoids (and flavonoids) are very ubiquitous among land plants. Terpenoids, in a plant, contribute to its aroma. We believe that by grouping cannabis based on aroma, patients can empirically segregate cannabis into useful categories determined by the content of its principal terpenes. This principal terpene class includes those with putative activity within the endocannabinoid system. Our goal is to analyze the clinical efficacy of mixtures of cannabinoids and terpenes (both naturally occurring and processed formulations). In the Initial Roll-Out, we will observe what patients know about the cannabinoid and terpenoid content of the medicine they are using. Later POC phases will incorporate efficacy studies based on actual content data derived from laboratory analyses. We believe that somewhere in the range of 6 to 9 Cannabis Chemotypes (3 cannabinoid groups X 3 terpenoid groups) are sufficient to group cannabis for meaningful initial clinical efficacy studies.

We have based our initial terpenoid chemotyping analysis on profiles from a set of contestant submitted flower samples at a recent Cannabis cup competition. The 2015 Golden Tarp Awards (GTA) were unique in requiring that contestants identify which of 4 aroma categories: "Earth", "Floral", "Fruity", or "Fuel" their flower product belonged to. We carried out multivariate analysis techniques on the terpenoid content data matrix, and examined how well clusters corresponded to the aroma categories. Through that work, we identified 3 of the 4 categories which were well correlated with specific terpenoid content. These included the "Earth", "Floral", and "Fuel" categories. Principal Component Analysis showed alpha-pinene, myrcene, and beta-caryophyllene were principal loading factors. In the ANOVA in Figure 5 below, we see

that each of the 3 terpenoids provide good model significance (all show p<<0.05) for classifying aroma category.

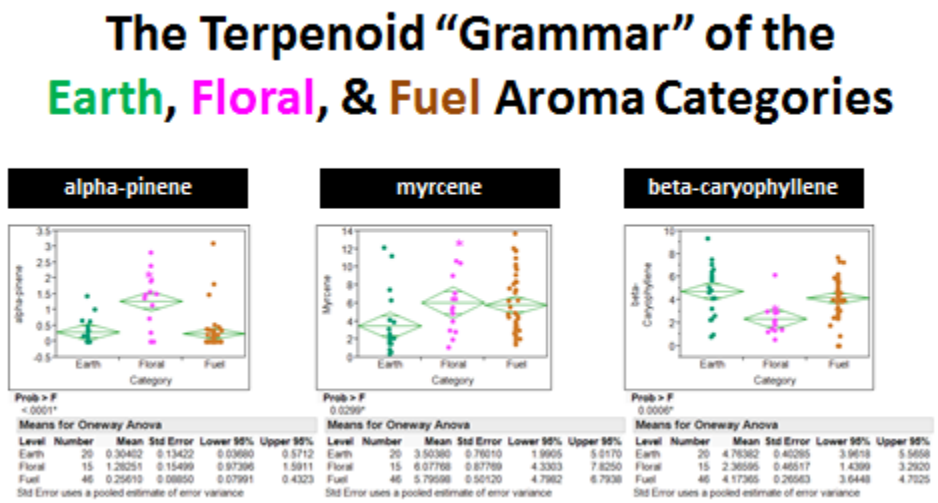


Figure 5 The Terpenoid "Grammar" of the Earth, Floral, & Fuel Aroma Categories

We therefore have derived the following "grammar" to describe the terpenoid content underlying the 3 aroma categories: These are:

Table 1 Principal Terpenoid Factors & Levels Underlying Aroma Categories

Aroma Category	alpha-pinene	myrcene	Beta-caryophyllene
Earth	**Low**	**Low**	**High**
Floral	**High**	**High**	**Low**
Fuel	**Low**	**High**	**High**

Studies have shown that the terpenoids responsible for the olfactory classification of strains into "fuel", "floral", or "earth" are also responsible for modulating the endocannabinoid system to produce the varying strain-dependent effects. Indeed, the principal Cannabis terpene β-caryophyllene has been shown to have direct activity on the CB_2 receptor in mouse models of neuropathic pain [7], and in doing so earns the alias "phytocannabinoid" along with THC and CBD. alpha-pinene has been implicated as an acetylcholine esterase inhibitor [8], thereby believed to promote memory and cognition- two hallmarks of the patient-reported "sativa effect". Cannabis produces a large number of terpenoids, many of which are used as aromatherapuetics to relieve stress and anxiety while others are used topically to treat skin conditions. In order to accurately identify and quantify the various terpenoids in a cannabis

product, analytical method validation is imperative. Due to the similarities in the physical properties of terpenoids, a major challenge in Cannabis analytics is the accurate identification of terpenoids in a complex matrix such as Cannabis flower.

An individual terpene synthase can produce multiple products from the same type of substrate. While surprising, that is the most likely reason underlying the tight correlation between β-caryophyllene (BCP) and α-humulene (AHum) levels in Cannabis varietals. We have observed a 3:1 ratio (BCP:AHum) when a valid methodology is used in gas chromatography. The closely related Cannabaceae family member, *Humulus lupulus* (Hops) expresses a homologous terpene synthase enzyme (H1STS1). It has been previously reported that Hops also produces both of these sesquiterpenes products,[9] but at the reciprocal of the Cannabis ratio, (1:3 BCP:AHum)/ Available protein sequence data supports the hypothesis that single amino acid substitutions in the active site are responsible for these catalytic rate differences. The stability of this biochemical parameter permits us to recommend this ratio as a quality control parameter. We suggest that the 3:1 BCP:AHum ratio can be adapted as a quality control parameter for terpene analyses as well as an identification parameter for the Cannabis species.

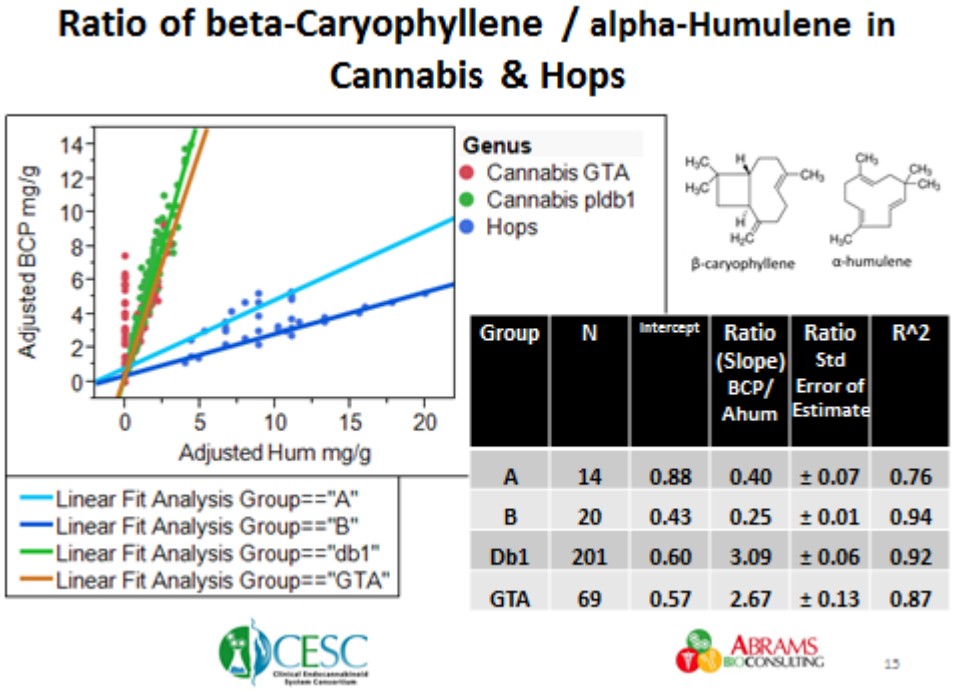


Figure 6 Ratio of beta-Caryophyllene / alpha-Humulene in Cannabis & Hops

Flavonoid

Chemotaxonomic support for a two-species hypothesis is provided by an analysis of flavonoid variation that detected luteolin C-glycuronide in 30 of 31 plants assignable to C. sativa, but not in 21 of 22 plants assignable to C. indica [10] More investigative work is required.

GENERATION OF PATHOGEN-FREE NURSERY STOCK

The current propagation model largely begins with diseased plants. Most of the popular strains of cannabis are from long-existing lines of cloned plants containing some elements of disease carried over from mother plants before it. Pathogens include not only well-known fungal and bacterial pathogens, but largely undocumented putative plant viruses. It is important that farmers grow plants that are free from pests and diseases to ensure the medicine they produce isn't contaminated. The future of propagation in the cannabis industry will rely largely upon advanced techniques in tissue culturing to eliminate pests and pathogens in breeding stock, pre-nursery. Sourcing clean nursery stock is a prerequisite for successful research programs. A CESC-affiliated organization, Humboldt DNA, is developing biotechnology to control the Cannabis virome.

GLIOBLASTOMA & THE ENDOCANNABINOID SYSTEM

Recent work [11,12] has documented the efficacy of cannabinoids such as THC and CBD in various in vitro and animal models of glioblastoma (GBM). Various biochemical mechanisms are being explored to uncover potential beneficial effects of cannabinoids in treating this disease with no known cure. A recent in silico meta-analysis undertaken by The CESC suggests that there is a statistically significant decrease of survival rate in the cohort of patients with GBM expressing upregulated cannabinoid receptors (CNR1, CNR2, or TRPV2).

Genetic alterations in CNR1, CNR2 and TRPV2 genes in GBM patients:

In order to understand the role of the cannabinoid signaling in glioblastoma, we surveyed the expression and genetic status of cannabinoid receptor genes in a large cohort of GBM patients in The Cancer Genome Atlas (TCGA). We found

1. A subset of GBM patients were found to have genetic alterations in cannabinoid receptors (CNR1, NCR2 and TRPV2).
2. The vast majority of these alterations are upregulation in mRNA expression, and this upregulation tends to be mutually exclusive in the three cannabinoid receptor genes.

We further investigated the potential correlation between the expression levels of cannabinoid receptor genes and clinical outcome of GBM patients. Strikingly enough, our analyses showed that the upregulation of CNR1, CNR2 and TRPV2 genes significantly correlated with a worse

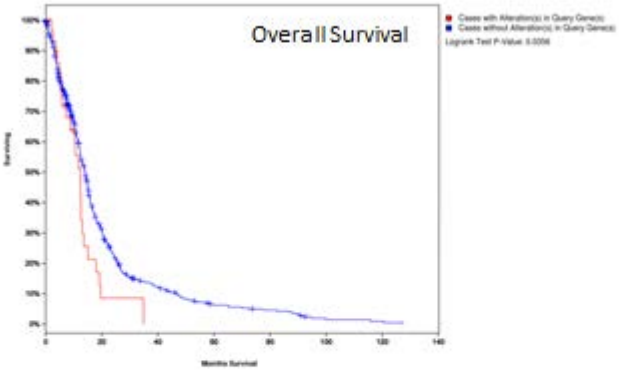
treatment outcome in both Overall Survival (Logrank Test, *p = 0.03, n = 607) and Disease Free Survival in a large cohort of GBM patients (Logrank Test, *p = 0.01, n = 607). These analyses strongly indicate that the cannabinoid receptors may serve as an independent set of biomarkers to predict the clinical outcome of GBM patients. In the meanwhile, our analyses raised a very important question regarding the role of cannabinoid receptors in GBM oncogenesis, and their action in response to therapy. These questions need to be experimentally addressed to help clinicians fully understand the beneficial effects of cannabinoids in GBM treatment

Distribution of CNR1, CNR2 and TRPV2 Alterations in all 136 complete tumors (TCGA, provisional dataset): we can see the upreguation of the 3 receptors tends to be mutually Exclusive!

Mutual exclusivity Co-occurrence Significant pairs

CNR1	**TRPV2**	0.724	<-3	Tendency towards mutual exclusivity
CNR2	**TRPV2**	0.765	<-3	Tendency towards mutual exclusivity
CNR1	**CNR2**	0.795	<-3	Tendency towards mutual exclusivity

Figure 7 Distribution of CNR1, CNR2 and TRPV2 in Glioblastoma.
The upregulation in all 136 complete tumors (TCGA, provisional dataset): of the 3 receptors tends to be mutually exclusive.



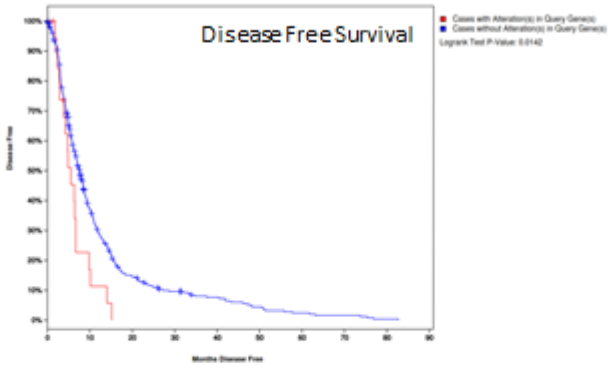


Figure 8 CNR1/2 and TRPV2 as potential biomarkers for GBM outcome
Survival Analyses (607 patients, {TCGA provisional dataset). Patients with upregulation of the CNR1/2 and TRPV2 levels showed a statistically significant worse outcome, and they have a significantly shorter overall survival time as well as a much faster tumor relapse.

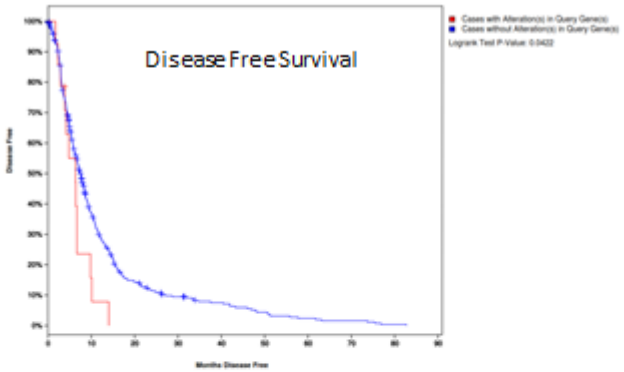


Figure 9 Survival Analyses (607 patients, {TCGA provisional dataset) stratified for GBM patients with upregulation of the CNR1/2 receptors

Interestingly, using CNR1/2 alone, we can still clearly see that the upreguated CNR1/2 levels are correlated with a shorter disease free survival time (p = 0.04, Logrank test)

Recommendations for further clinical studies with Cannabis products

In view of the promising in vitro and animal model studies with cannabinoids, we feel it is urgent to explore therapeutic administration of cannabinoids, especially in the subset of GBM patients expressing upregulated cannabinoid receptors. It is our view that ethically, we need to rule this in or out as quickly as possible as a therapeutic approach. A popular method used by patients to treat cancer is by ingestion of the extracted oil from the cannabis flower. The extracted oil commonly known as “Phoenix Tears” or “RSO” was suggested as a cure for cancer by Rick Simpson, a Canadian farmer, and became popular as a cancer treatment option. In view of the long development times needed to produce a well characterized pharmaceutical agent, we recommend starting initial studies observing ingestion of extracted oil from plant pathogen-free and pesticide-free, well characterized dried herbal preparations of natural cannabis from each of the 3 cannabinoid chemotypes described above. In conjunction with knowledge that cannabis is also ingested as a palliative agent in the oncology setting, we may have enough data to suggest a cannabinoid and terpenoid chemotype most efficacious for GBM. Once such data

becomes available, a subsequent prospective clinical trial exploring the most promising extract should be launched.

SPECIFIC RECOMMENDATIONS FOR NIDA

<u>1) The specific marijuana varieties, strains, or constituent chemotypes that are of research interest;</u>

1) **We recommend that the % THC and % CBD ranges for the NIDA Bulk Marijuana Categories be revised as depicted below in Figure 10 and Table 2. This will harmonize NIDA categories with what is currently observed in the field.**
    i) **Furthermore, we recommend that such material be limited to flower and therefore not include vegetating plants before they flower. Cannabinoid content in vegetating plants will likely be lower, much more variable, and difficult to control and standardize.**

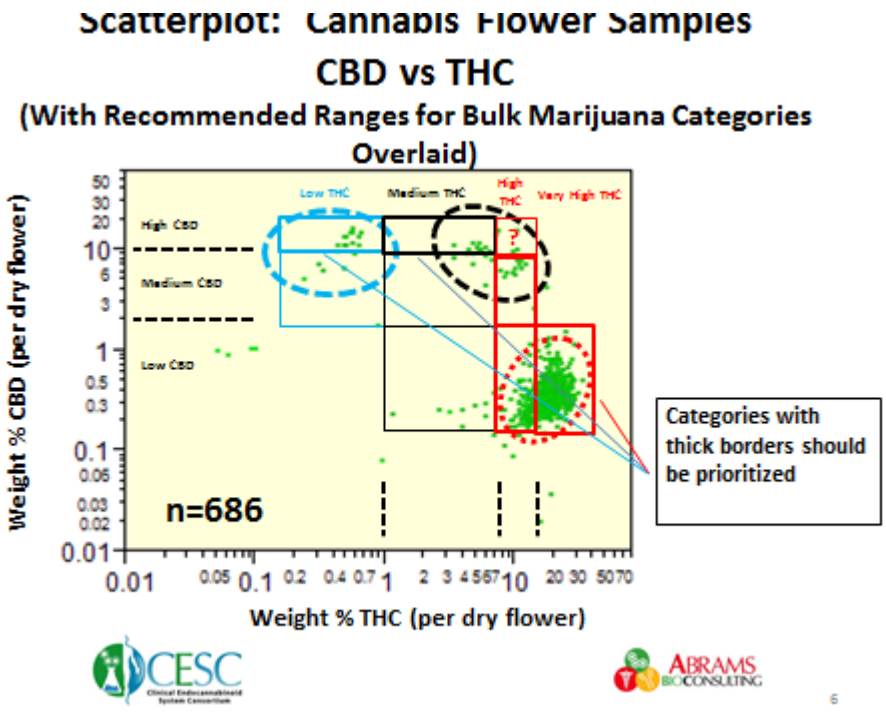


Figure 10 Cannabis Flower Samples : CBD vs THC with recommended ranges for bulk marijuana categories overlaid.

Table 2 Recommended Revised Ranges for Bulk Marijuana Categories

		THC			
		< 1 %	Between 1 - 8 %	Between 8 – 15 %	> 15 %
CBD	< 2 %		Medium THC; Low CBD	High THC; Low CBD	Very High THC; Low CBD
	Between 2 – 10 %	Low THC; Medium CBD	Medium THC; Medium CBD	High THC; Medium CBD	
	> 10 %	Low THC; High CBD	Medium THC; High CBD	High THC; High CBD (may not exist)	

Shaded categories should be prioritized

2) **We recommend the cultivation of strains that permit the evaluation of additional Cannabinoids like: CBG(A), THCV(A).**
3) **We recommend that Cannabis strains representing the different aroma categories of "Floral", "Fuel", and "Earth" be identified and cultivated. We define these aroma categories based on the levels of the principal terpenes: alpha-pinene, myrcene, and beta-caryophyllene (as described above in Table 1). Furthermore, we recommend that each THC /CBD Bulk Marijuana Category (described above in Table 2) include representatives from each aroma category. This will ensure that a broad collection of cannabinoid and terpenoid profiles are available for research.**
4) **We propose that the tight relationship between BCP and AHum be used as a Quality Assurance (QA) parameter when analyzing terpenoid profiles in Cannabis flower samples.**
5) **We recommend that further work be carried out to better characterize Cannabis flavonoid chemotype profiles.**
6) **We recommend that NIDA source clean, pathogen-free nursery stock as a prerequisite for providing material for research programs.**

<u>2) The marijuana constituents, products and/or preparations that are of research interest;</u>

1) **We recommend that CBN, the natural oxidation product of THC, be studied. We recommend that standardized protocols be developed to control for the conversion of THC to CBN.**
2) **We recommend inclusion of aqueous extracts (juicing of raw plant material). Such preparations would contain the Cannabinoid acid forms suitable for ingestion.**
   a) **In addition, flavonoids would likely be present and will doubtless be of interest as their content in Cannabis is better described.**

3) **We recommend inclusion of (ethanol) extracted oil, commonly known as "Phoenix Tears" or "RSO". This process, which was suggested for cancer therapy by Rick Simpson, a Canadian farmer, has become popular as a cancer treatment option.**
   a) **We advocate exploring the profile of Cannabis products isolated using both hot as well as cold ethanol extraction. The resulting volatile terpenoid profiles will likely differ as a function of ethanol extraction temperature.**

<u>3) The particular research questions that could or would be addressed with such products.</u>

1. **We recommend establishing dosing guidelines for Cannabis used for medical purposes:**
   a. **We recommend characterizing marijuana varieties based on data from observational studies of cannabis used by patients and tested by certified analytical labs. Included in the observation should be a report on dosage so weight based dosing guidance (milligrams per kilogram used for reported symptom relief) can be established.**
   b. **Our recommendations include an observational study of dosing and self-reported clinical efficacy which would include NIDA Bulk Marijuana varieties along with other available Cannabis materials (in those US states with permitted medical cannabis). The Dosing Project is the recently launched flagship program of The CESC, and is designed to address this question.**
2. **We recognize there is an ethical need to determine whether any extracted cannabis oil has therapeutic value in Glioblastoma, in view of Endocannabinoid System receptor upregulation in subsets of patients with GBM**

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