The Dosing Project:

BACKGROUND

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. As a solution, the CESC proposes a unique project that gathers data on current methods of cannabis medication and uses the information 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?

The Dosing Project: Overview of Proof of Concept (POC) Phases

Determine Weight-based Dosing Efficacy of Major Cannabis Flower Chemotype Groups For Symptom Relief of Pain & Disordered Sleep

I. Phase 1.0 – Initial Roll-Out
II. Phase 1.1 – Implement ICD10 Code Dx
III. Phase 1.2.1 – Incorporate Lab-Derived Chemotype Data (Cannabinoid)
IV. Phase 1.2.2 – Incorporate Lab-Derived Chemotype Data (Terpenoid)
V. Phase 1.2.3 – Incorporate Point-of-Use Device Chemometric Data (Cannabinoid + Terpenoid)
VI. Phase 1.3 – Expand Modes of Administration (MOAs) to Oils/Concentrates, Edibles, & Topicals
VII. POC Phase Completed

In subsequent sub-phases of The Dosing Project POC we will 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 this, 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 so-called HIPAA wall. It also allows linkage with other patient chart data that may become very valuable for future analyses.

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

Finally, we have already developed the requisite data structure to permit expansion of the study into additional MOAs. This last POC sub-phase will include expansion of medicine presentations to include: concentrates, oils, capsules, juices, and topicals.

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 (ref 1,2), three 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 (Ref 4). 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 (Ref 3) used Gas chromatography to quantify THC and CBD cannabinoid levels in 96 Cannabis plant accessions, and demonstrated the presence of these three main cannabinoid chemotypes in a scatterplot of %THC vs %CBD (with linear scaled axes.

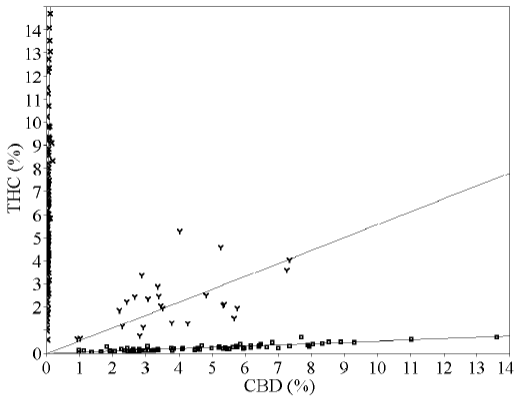
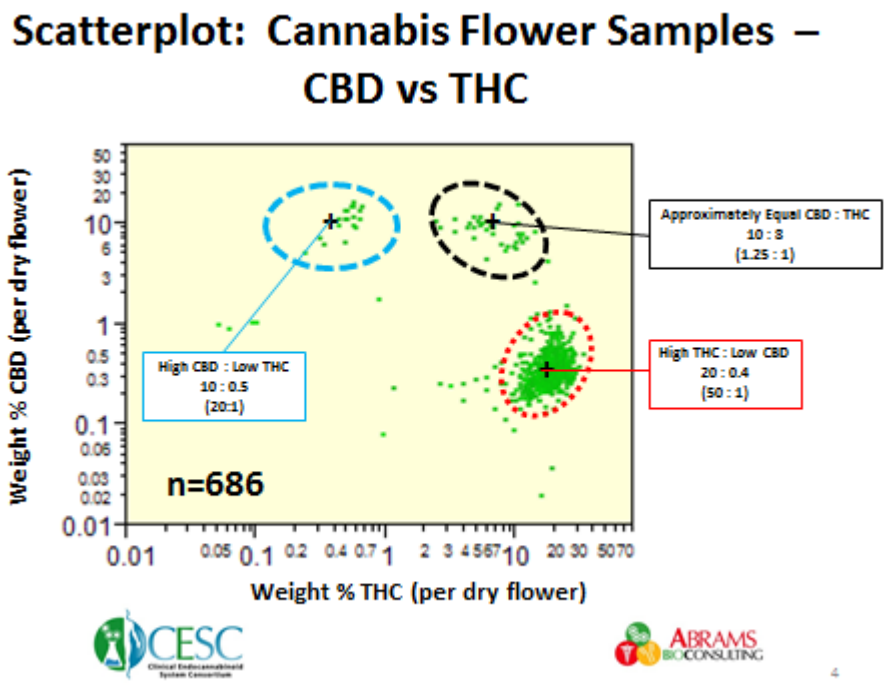


Fig. 4. Plot of Δ⁹-tetrahydrocannabinol (THC) % vs. cannabidiol (CBD) % for 253 *Cannabis* plants. Chemotype I, II, and III plants are marked with an X, Y, and square, respectively. Linear regression lines (forced through the origin) are drawn for each chemotype.

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 three 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 three chemotype grtoup during the early, initial roll-out phase of The Dosing Project.



Terpenoid

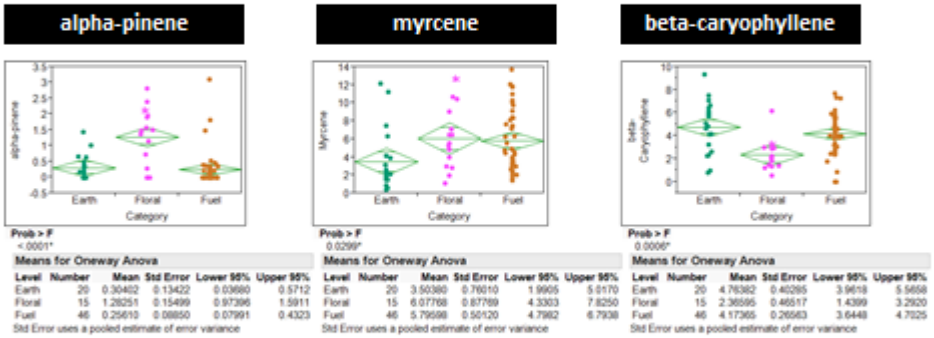
Using the phytocannabinoids THC and CBD levels as criteria, Cannabis strains can be separated in to as few as three to as many as 11 categories (https://www.drugabuse.gov/researchers/research-resources/nida-drug-supply-program-dsp/marijuana-plant-material-available-nida-drug-supply-program). While these 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 more rigorous classification system is needed to deconvolute

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, one 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 six to nine Cannabis chemotypes (three cannabinoid groups X three 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 3 below, we see that each of the three terpenoids provide good model significance (all show $p<<0.05$) for classifying aroma category.

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:

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 (ref), and in doing so earns the alias "phytocannabinoid" along with THC and CBD. α-pinene, has been implicated as an acetylcholine esterase inhibitor, thereby promoting memory and cognition- two hallmarks of the "sativa effect". Cannabis produces over 100 terpenoids, many of which are used as aroma therapeutics 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.

By virtue of terpene synthesis enzymes ability to produce multiple products from a single substrate, two principal terpenes in Cannabis were found to have highly correlated levels in Cannabis varietals. β-caryophyllene (BCP) and α-humulene (AHum) show a 3:1 ratio (BCP:AHum) when a valid methodology is used in gas chromatography. In the closely related *Humulus lupulus* (Hops) which expresses a homologous terpene synthase enzyme (H1STS1), an inverse correlation (1:3 BCP:AHum) has been shown confirming that the corresponding ratio in Cannabis is not an artifact, with the reciprocity in ratios readily explained by differences in the enzyme active sites. Thus, a quality control parameter (3:1 BCP:AHum) can be implemented for terpene analyses as well as an identification parameter to differentiate Cannabis from other similar plant materials encountered by law enforcement.

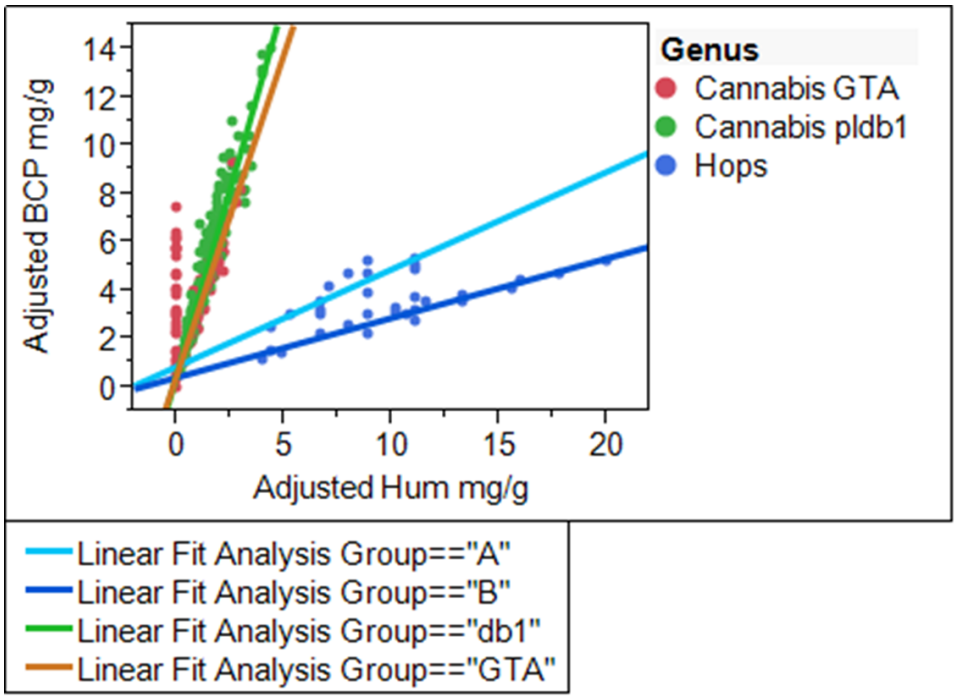


Figure 4

Flavonoids

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 (Clark and Bohm, 1979)….. more discussion to come.

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.

Refs:

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