CBD-induced biomarkers of inflammation reduction in people living with HIV at the single cell level

NCT05209867

07/29/2022

This is the cover page for the approved protocol document.
The protocol document follows this page.

1. **Title: CBD-induced biomarkers of inflammation reduction in people living with HIV at the single cell level**

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3. Abstract: With modern therapies, people living with HIV (PLWH) show a life expectancy approaching that of the general population. However, PLWH are affected by non-HIV-related comorbidities, including myocardial infarction and cancer, which typically occur earlier than in otherwise healthy individuals. These comorbidities appear to be strongly related to chronic inflammation and pain, conditions that often characterize PLWH. Phyto-cannabinoids such as cannabidiol (CBD), available on-line and in many grocery stores in the US, are known to have anti-inflammatory and analgesic properties. The intra-cellular mechanisms and pathways that cannabinoids use to alter inflammation and pain, however, are yet to be fully understood. Our objective will be to conduct a highly innovative pilot clinical study that investigates the therapeutic effects of a CBD-rich oil administration under the tongue on mechanisms and processes that underlie chronic inflammation and pain associated with PLWH. Our preliminary results show (a) different sub-types of pro-inflammatory monocytes emerging from single-cell RNA sequencing (scRNAseq) data in an inflammation model, and (b) an enrichment in MAPK-signaling pathways in microarray transcriptional profiling from PLWH using cannabis. More specifically, to investigate the presence of pro-inflammatory cell populations, we utilized the data from our previous work, a mouse model based on trauma [0], and considered monocytes both at baseline (uninjured, day 0) and inflammation peak (burn/tenotomy, day 3). We found the emergence of a specific pro-inflammatory monocyte population (Figure 1). The trauma model generates a post-wound inflammatory peak at day 3. Monocytes were identified by unsupervised clustering and marker analysis, validated by automated labelling of state-of-the-art algorithm SingleR10. We found six monocyte clusters (labeled 0 to 5, Figure 1, B and C). For each cell, we calculated an inflammatory score based on the fraction of the counts per cell (over its total counts) associated with the Gene Ontology term “regulation of inflammatory response” (GO:0050727), including 556 mouse genes. Different clusters (Figure 1, A) have a different inflammatory profile, with 0 and 4 showing a low inflammatory profile; clusters 1, 2, and 3 a high inflammatory profile; and cluster 5 very high inflammatory profile, mostly composed by monocytes on day 3. We compared high and very high inflammatory transcriptome profiles versus low

ones, and enriched the differentially expressed genes with iPathway11, finding differentially expressed pathways in interleukins (IL-10 Signaling, IL-17A Signaling in Fibroblasts, LPS/IL-1 Mediated Inhibition of RXR Function, IL-6 Signaling, IL-12 Signaling and Production in Macrophages); glucocorticoid receptor signaling, and Toll-like Receptor Signaling, and Neuroinflammation Signaling Pathway.

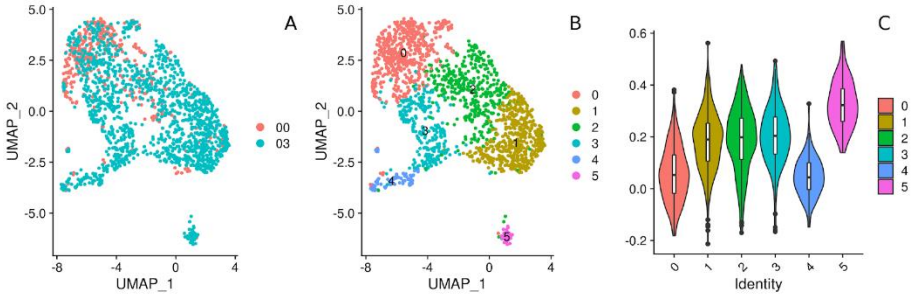


Figure 1 (**PI Marini, unpublished)**. Monocyte sub-populations in a mouse inflammatory model. (A, B) Uniform Manifold Approximation and Projection (UMAP) plot of monocytes from the tenotomy area. Each dot represents a monocyte. Spatial distance reflects phenotypic distance (A) Monocytes are labeled as baseline (day 0) and peak of inflammation (day 3). (B) Monocytes are clustered into sub-populations. (C) inflammation score (Y axis) is calculated

This proposal aims to study the effects of CBD on chronic pain and inflammation in PLWH by investigating the molecular role of different immune cells (such as monocytes) in this process. The hypothesis was shaped by our findings relating the effect of cannabis in altering the role of monocyte phenotypes. The aims of this project will be the identification of inflammation reduction biomarkers linked to MAPK-dependent alterations after CBD administration, as well as other systemic effect cell pathways. We will use scRNAseq, a top-notch technology that has brought tremendous advancements in medicine and molecular biology, to isolate CBD-specific cellular phenotypes. Investigating the role of cell-specific biomarkers that describe the molecular response to CBD may open additional therapeutic strategies that can help reduce the burden of chronic pain and inflammatory-related comorbidities in PLWH.

4. Background

Overview and Rationale for Study. Inflammation is a primary HIV-related complication. HIV patients experience non-AIDS-related complications that are typical of older patients, such as presence of neurocognitive disorders, metabolic syndrome, bone abnormality, non-HIV-associated cancers, and neurocognitive disorders [1]. Chronic inflammation in PLWH is a significant problem that impacts the quality of life in PLWH. The effects of cannabinoids in reducing inflammation are renowned; however, the intra-cellular mechanisms and pathways that cannabinoids use to alter inflammation are yet to be discovered. Our central hypothesis is that CBD is an extremely beneficial therapeutic drug for PLWH, because it reduces inflammation by hindering pro-inflammatory monocytes via MAPK-dependent pathways. This hypothesis targets two fundamental unanswered problems in the field of HIV/AIDS, that are high HIV/AIDS NIH priorities: (i) identifying the mechanism regulating PLWH persistent inflammation, which in turn plays a pivotal role in

all the main causes of death in PLWH; and (ii) investigating the role of cell-specific candidate biomarkers describing the molecular response to CBD, which may open for new cure strategies.

By mapping the effects of a controlled trial of CBD-administration at the single-cell level, we will pave the way for new candidate therapies, from CBD- and other cannabis-based therapies to the identification of novel protein targets for drug repurposing or discovery. Understanding how CBD reverses inflammation (potentially at the systemic level), will help overcome a significant challenge for HIV care and treatment, particularly for infected young adults who have a lifetime in combined antiretroviral therapy (cART). Filling this gap is particularly relevant because our findings will provide a framework for the evolving policies for clinical use of FDA-approved drugs for HIV cure strategies. As such, it has an enormous repercussion as THC is not legalized for its psychoactive properties, while CDB can be found in any grocery store in the US. Therefore, understanding CBD influence on HIV-induced inflammation is also extremely important to inform the medical community and the public answering which compound has a therapeutic validity. Our findings will potentially open a new therapeutic window for personalized and precision medicine.

Although cART suppresses the viral load, it does not eradicate HIV infection, which remains associated with relatively high levels of inflammation that may increase the risk for a range of health problems. Our preliminary data indicates that PLWH who use cannabis on a recreational level show a decrease in chronic inflammation, possibly MAPK-signaling mediated. We hypothesize that CBD is able to reduce inflammation via MAPK-dependent alterations of the circulating monocyte population.

Cannabis sativa (marijuana) and its cannabinoid derivatives, Δ9-tetrahydrocannabinol (THC) and CBD, are largely used by people living with HIV (PLWH) to stimulate appetite, prevent weight loss (FDA approved Marinol or Dronabinol), or manage chronic pain. CBD is sold in grocery stores in the US. Although beneficial effects of cannabis on inflammation are well known, [2] and despite its widespread use, the differential impact of cannabinoids on health outcomes in PLWH remains understudied.[3] As a consequence, the cannabinoids molecular chain leading to inflammation reduction remains to be unveiled.

Cannabinoids bind to endocannabinoid system receptors CB1R, which is mostly found in the central nervous system, and CB2R, mostly found in your peripheral nervous system, especially immune cells. THC binds to CB1R and CB2R, found on the surface of immune cells and tissues, including T-cells, macrophages/monocytes, and microglia. It is known that monocyte cells treated during differentiation with THC displayed reduced expression of CD14, CD16, and CD163, therefore suggesting a fundamental alteration in phenotype [4]. Our preliminary microarray transcriptome study evidenced that peripheral blood mononuclear cells (PBMCs), obtained from patients using marijuana recreationally, show MAPK-signaling pathway alterations [4]. Based on these results, the long-term goals of this research are to determine whether it is possible to attenuate and possibly resolve chronic inflammation in PLWH with CBD. As a first step, this proposed research will determine cell-type specific CBD mechanisms altering HIV-1 infected cell state at the RNA level, and how these gene expression pattern shifts influence HIV-associated

persistent inflammation reduction. In other words, we will obtain cell-type specific biomarkers that are associated with inflammation reduction in PLWH after CBD long-term assumption. We hypothesize that CBD reduces inflammation by causing cell-type specific MAPK-dependent alterations of monocyte phenotype, reducing their overall migratory capacity. We will take advantage of the established technique of single-cell RNA sequencing (scRNAseq), that has already been used to quantify the plasticity of immune cell expression profiles [5], including showing how SIV (simian immunodeficiency virus) induces senescence in macrophages [6]. We will investigate cellular type/state alteration using cross-sectional PBMC samples obtained from PLWH that do not use CBD or marijuana and who consent to use CBD for two months to address unanswered questions regarding CBD effects of chronic inflammation: In which cell population is the MAPK-signaling pathway deregulated? How is CBD responsible for immunomodulation? Which CBD mechanism alters the inflammation process? Our team proposes to leverage our existing expertise in HIV genomics/transcriptomics at the single cell level, UF infrastructures, and collaborations to test our hypothesis via the following specific aims.

5. Specific Aims:

The main objective of this project will be to identify inflammatory reduction biomarkers linked to MAPK-dependent alterations after CBD administration, as well as other systemic effects on cell signaling pathways. Investigating the role of cell-specific biomarkers describing the molecular response to CBD-administration may open avenues for additional therapeutic strategies that can help reduce the burden of chronic musculoskeletal pain and other pain-related symptoms and comorbidities in people living with HIV (PLWH). The team proposes to study the effects of CBD on inflammation and chronic pain in PLWH by enrolling five persons with HIV who will provide blood samples before and after taking CBD for two months. In the process, the goal will be to establish the molecular role(s) of different immune cells using single cell RNA-sequencing. Preliminary data from this study are expected to pave the way for further investigations in larger clinical cohorts. The primary aim of this project will be to identify key inflammatory reduction biomarkers linked to MAPK-dependent alterations after CBD administration. Secondary outcomes include pain relief and reductions in pain-related symptoms. We will use a prospective pilot study design where patients will self-administer the investigational product (CBD-extract) for 60-days (2 months).

Specific Aim #1 – Determine the effects of CBD on chronic on quality of life in PLWH. We will compareQuality of Life (QoL) measures between treatment conditions, including pain and pain-related symptoms, if present.

Specific Aim #2 – Determine key biobehavioral mechanisms contributing to anti-inflammatory and analgesic effects of CBD administration in PLWH. We hypothesize that change in molecular (alterations in cell signaling and reduction in inflammatory cytokines), psychological (reduction in pain-related fear and pain catastrophizing), and pain sensitivity regulation measures will be associated with pain relief, inflammation

reduction, and reservoir shrinkage. We will quantify the shift in anti-inflammatory macrophage/monocyte population caused by CDB administration by examining gene expression at the single-cell level from the PBMC samples. This will allow us to accurately pinpoint gene under- or over-expression patterns at the RNA level (biomarkers), that are both induced by CBD administration in PLWH, and specific to different cells (e.g., monocytes, T cells, B cells, or other immune cells).

6. Research Plan

Study Population. Our clinical population will be men and women (N=5 to 7) 21-60 years old who are currently living with HIV (under antiretroviral therapy for 5+ years with a suppressed viral load).

Recruitment strategy. Co-I Cook has extensive experience in HIV patient recruitment and will oversee the recruitment team, during the recruitment phase, as well as the safety monitoring. Five PLWH (men and women) will be primarily recruited from The SHARC Registry (IRB# 201400073), a UF-IRB-approved registry of over 600 HIV patients who have agreed to be contacted about future research. Written informed consent will be obtained, and blood will be collected at the UF CTRB clinical research center. Inclusion criteria are: ability to read English and provide written consent; willingness to take CBD and to participate in follow up for two months; older than 21 and younger than 60; under antiretroviral therapy for 5+ years and suppressed viral load (CD4 count <350cells/ml); patients must agree to practice acceptable methods of birth control, namely sterilization (female tubal ligation or occlusion, male vasectomy), long-acting reversible contraceptives (intrauterine devices, hormonal implants), short-acting hormonal methods (pill, mini pills, patch, shot, vaginal ring), and condoms.

The main exclusion criterion will be conditions/medications that may impair the immune response (e.g. rheumatoid arthritis, cancer, diabetes chronic infections, CAD, cellulitis, autoimmune diseases such as lupus, sarcoidosis, and all medications they may affect inflammation such as aspirin, steroids, statins);), pregnancy, and current marijuana or CBD use (urine drug screen). Further exclusion criteria will be: excluding subjects taking anti-seizure medicines, with any history of seizure disorder or head trauma, any history of hepatic/liver disease or renal disease, any history of cardiovascular diseases; women who are lactating (in addition to existing exclusion criteria for pregnancy). Co-I Dr Cook (a physician) will review conditions/medications after the initial interview before the patient is enrolled to exclude risks of drug interactions or hepatotoxicity. UF/Shands staff and students will not be considered for enrollment. Presence of chronic pain will not be considered as a necessary condition for enrollment.

Motivation to study single cells. The molecular and cellular changes underlying the inflammation mitigation caused by CBD-administration are largely unknown, and stand as a major challenge both for understanding its basic biology (e.g., pathways involved, molecular mechanisms) and for the urgent unmet need of reducing the long-term harm chronic inflammation causes in PLWH. Our hypothesis is that CBD alters monocyte migration capacity with MAPK-dependent alterations. We expect this alteration to have

chain-effects based on cellular cross-talk, i.e., that different PBMC cell types (such as T-cells and B-cells) might express specific CBD-induced phenotypes. We hypothesize CBD-alterations to reverberate along the whole immune-modulation system through cellular cross-talk. Given the complexity of the immune response, we deem traditional (bulk) RNA sequencing as insufficient for the task. Population-averaged genomic profiling cannot adequately unravel the inter-cellular regulation in situ. This study will leverage the efficiency of newly available single-cell technologies to create a powerful dataset unveiling the functional properties of thousands of individual cells.

Sample collection and processing. We plan to collect samples from five to seven patients over three time points: baseline (no CBD), and after one and two months of CBD-administration with 5,000 cells sequenced per sample. Five to seven patients (15 to 21 samples) will provide the transcriptomes of 75,000-105,000 single cells, a large dataset for scRNAseq. Immediately after collection, samples will be collected by TBD Lab technician to be processed for scRNAseq at UF ICBR (PBMC scRNAseq experiments are routinely run at ICBR).

Data analysis. During the post-processing period, single-cell reads (count matrices) will be filtered for quality control. Unsupervised clustering will isolate statistically robust populations of similar cells. Clusters will be visualized using Principal Component Analysis (PCA), t-Distributed Stochastic Neighbor Embedding (tSNE), and Uniform Manifold Approximation and Projection (UMAP). The most informative gene expression markers will be identified by a host of non-parametric tests (e.g., the Kolmogorov–Smirnov test), comparing a given cluster against either a nearby cluster or all other clusters. Clusters of cells will be annotated by using their marker genes/pathways, and prior biological knowledge to characterize each sample's cell composition.

CBD biomarker extraction. We will contrast clusters of the same cell type at baseline (no CBD) and their counterparts after CBD administration. The difference in gene expression, measured in fold change, p-value, and ratio of cell fraction expressing the marker gene, will determine the phenotypic effect of CBD. For each cell type, we expect to extract one or more sets of CBD-specific candidate biomarkers that together will determine a phenotypic CBD per cluster (cell type) signature.

Investigational Product & Dosing Schedule. Dr. Borsa (Co-I) will oversee CBD dosage and administration.We will source the investigational product from the same batch of our hemp-extract CBD product that is being used in the original protocol under IND 147985, 'Efficacy of a controlled short-term trial of Cannabidiol (CBD) ingestion on reducing symptomatic response and facilitating recovery after induced muscle injury' (Source: SunFlora, Inc, 411 19th Street South, St. Petersburg, FL 33712)". SunFlora is a third-party tested hemp-derived product manufacturing company. SunFlora is dedicated to producing high quality hemp-derived products formulated to be safely used by the public and are manufactured in accordance with the Food and Drug Administration (FDA) Current Good Manufacturing Practices (cGMP) for Dietary Supplements, 21 CFR Part 111. Their hemp derived products are assessed multiple times at production process control points throughout the manufacturing process. Dietary ingredients used in the

manufacturing process are accompanied by certificates of analyses (CoAs) confirming their identity and specifications. All finished products are tested to ensure they meet specifications prior to being released for distribution. The label and all labeling satisfy FDA requirements found under 21 CFR 101.

Each participant will be provided two 30mL bottles (2000mg CBD per bottle) with a syringe dropper during their initial (baseline) visit with instructions/demonstration to administer the solution under the tongue (0.5 cc ~1/2 dropper) twice per day (BID) 8-12 hours apart (morning and evening) at the prescribed daily dosage (63mg/day). The sublingual route of administration allows the product to be absorbed directly into venous circulation, and thus bypasses the liver and eliminates the consequences of first pass metabolism; ultimately increasing its bio-availability. The bioavailability of CBD administered orally (including sublingual) is estimated to be 13-19% and its half-life estimated to be around 18-32 hours.

Physical Discomforts and Intervention Risks:

In previous clinical trials using hemp-derived products administered orally (liquid or capsule) and swallowed, a small percentage of patients experienced tiredness, change in appetite and gastro-intestinal discomfort following ingestion. We will be using daily doses of hemp extract that are considerably lower than the daily doses used in the clinical trials. Therefore, the chance of our participants experiencing these side effects is minimal.

Occasionally one or more of the following potential side effects of taking blood samples may occur: pain, bruising, slight bleeding, light-headedness, fainting and (rarely) an infection. A trained technician will be drawing the blood.

Safety Monitoring and Stopping Criteria: Safety monitoring will occur during the initial baseline visit as well as throughout the participant's duration in the study, including a 1-week follow-up contact after study termination. As noted below, the baseline assessment conducted during the first visit will include a brief physical exam (including vital signs) and a thorough medical and psychiatric history, including a review of past medical history, concomitant medications, inclusion/exclusion criteria, and potential contraindications. Safety monitoring will include active and passive surveillance procedures using pre-existing safety monitoring guidelines. Participants will be assessed actively at each laboratory visit by study personnel using the side effects/adverse events checklist. Each participant will be read each side effect/adverse event from the checklist and instructed to self-report as yes (go) or no (no go). If a participant experiences any of the side effects/adverse events from the checklist provided at the baseline visit, study personnel will then contact the study physician (Dr. Cook) for consultation. The side effects/adverse events will be triaged as mild, moderate or severe, and a determination will be made by the study physician as to whether the side effect/adverse event is due to the investigational test article or some other circumstance (e.g. food poisoning). If the reaction or event is determined to be due to the investigational test article and graded moderate to severe by the study physician, the participant will then be instructed to stop participation and advised to seek medical care by their primary physician or hospital. (Refer to stopping

criteria below). Any serious adverse reaction or event will be followed until full resolution. All adverse reactions and events will be reported to the UF IRB within 24-48-hrs and documented in the participant's file (redcap). The PI/physician decides that the participant should be withdrawn for **safety reasons** (presence of active lesions in the oral cavity/sublingual region or suffering side effects or adverse events associated with the use of the investigational product). List of side effects and adverse reactions are listed below*

Stopping Criteria:

1. Participant is unwilling to continue in the study.
2. Lack of compliance with protocol.
3. Investigator or study sponsor stops the study for any reason.
4. Participant is enrolled in error or lost to follow-up (lost to follow-up will be defined as a subject failing to attend planned study visits or failure to respond to contacts by study personnel after documented 2 attempts).

*Potential Side Effects and/or Adverse Events (AEs):

1. Feelings of anxiety (nervousness, restlessness or being tense, feelings of danger, panic, or dread), paranoia, or negative mood (depression or suicidal thoughts), or paranoia.
2. Suicidal ideation
3. Poor quality of sleep (persistent insomnia).
4. Feelings of extreme fatigue, sleepiness or drowsiness.
5. Feelings of nausea (upset stomach, vomiting or gastro-intestinal (GI) distress (e.g. diarrhea).
6. Change in or loss of appetite.
7. Feelings of light-headedness or dizziness.
8. Visual disturbances such as blurry/double vision.
9. Hypotension, hypertension, tachycardia, heart palpitations, syncope or dyspnea.
10. Development of a skin rash with redness and itching.

Each participant will be provided a detailed safety monitoring checklist of side effects and/or adverse reactions that they may potentially experience from ingesting the investigational product. Suicidal thoughts are not expected to be common, and there is no evidence that we know of about any association of CBD to suicide. In the unlikely event that a research participant would indicate suicidal thoughts or ideation during the visits or phone calls, they will be immediately interviewed for a formal assessment by Dr. Cook or Research Coordinator Taylor LeCorgne (both are trained to perform such an assessment). If the assessment finds out they are not seriously suicidal, they can continue in the study. Reversely, if we the assessment finds the patient is serious about their intentions, we will either call 911 or have them agree to go to an ER for evaluation. The patient will be asked to to stop assuming CBD. In that case, we will continue to follow them if we do refer them somewhere. At the end of the study, we will try to assess whether there is any possibility that the CBD product itself is associated with the suicidal thinking or behavior.

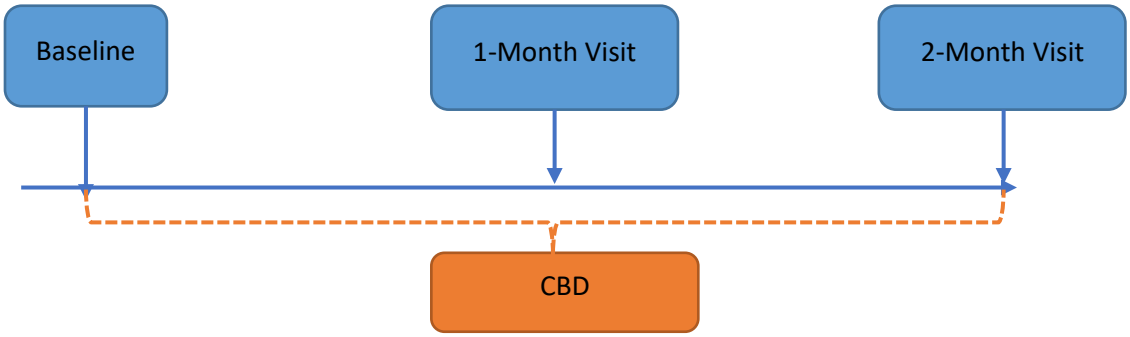
Participants will then be instructed on how to perform their own passive surveillance for identifying these side effects/adverse events and what to do if they experience any of them between study visits while they are ingesting the investigational product. Each participant will be provided a daily log chart to record their daily CBD doses for compliance and monitoring purposes. During the passive surveillance period participants will be instructed to contact study personnel if they experience any of the side effects/adverse events from the checklist provided at the baseline visit. Study personnel will then contact the study physician (Dr. Cook) for consultation. The side effects/adverse reactions will be triaged as mild, moderate or severe, and a determination will be made as to whether the side effect/adverse reaction is due to the investigational product or some other circumstance (e.g. food poisoning). If the event is determined to be due to the investigational product and graded moderate to severe by the study physician, the participant will then be instructed to stop participation and seek medical care by their primary physician or hospital. Any participant that reports a serious adverse event will be followed to full resolution.

Patients will be contacted weekly and asked about missing doses. We will take note of any missed dose. Approximately at days 15 and 45, patients will be asked to provide a picture, sent via text to the study phone, with a close up of their CDB bottles, so that we can verify the liquid level. Patients will be instructed to not include anything identifiable in the background. Patients will be also instructed to bring their CBD bottles at every visit after the first. In case of non compliance (e.g., the patient forgets to bring the bottle or does not send the picture), the PI will consider whether to withdraw the patient for the study.

Study personnel will perform a follow-up phone call at 1 week after completion from the study for continued safety monitoring. Study personnel will refer to the checklist to determine if the participant has experienced or is experiencing any side effects or adverse events using the safety monitoring guidelines. If the participant reports experiencing any of the side effects/adverse events from the checklist the study physician will be consulted immediately for guidance and the participant's condition will be followed until full resolution.

Experimental Procedures:

Screening: Study coordinator will screen candidate to determine if they meet the inclusion criteria for the study. The baseline visit activity will be described to the patient. The patient will sign the consent form. Instruction/demonstration about CBD and dropper use will be provided.



Visit 1: Baseline Evaluation before CBD administration

Activities during visit 1:

- Patient reads and signs the consent form.
- Patient completes a brief form providing information (e.g. age, address, phone number, etc., health history).
- Measure of patient's height, weight, and vital signs (HR, blood pressure, body temperature).
- Patient completes a urine drug test
- Patient completes a urine pregnancy test is they have the ability to become pregnant
- Patient fills questionnaires: PASS (Pain Anxiety Symptom Scale; four dimensions of pain measured: cognitive anxiety, escape/avoidance, fearful appraisal, physiological anxiety), VAS (Pain Experience Visual Analogue Scales; anxiety, depression, anger), PSQI (Pittsburg Sleep Quality Index; self-report used to assess a subject's usual quality of sleep)
- A trained nurse or technician draws a blood sample
- The patient is given 2 30mL bottles with a syringe dropper with instructions/demonstration to take the solution under the tongue (0.5 cc ~1/2 dropper) twice per day (BID), 12 hours apart (morning and evening), at the prescribed daily dosage (62.5mg/day).
- The patient schedules the next test visits to the laboratory.
- The patient receives $100 for completing this visit.

Visit 2: Follow-up after a month period of CBD administration

Activities during visit 2 (day 26-34):

- Measure vital signs of the patient as in visit 1.
- Questionnaire filling as in visit 1.
- Blood draw as in visit 1.
- The patient schedules the next test visits to the laboratory.
- The patient receives $100 for completing this visit.

Visit 3: Follow-up after a two-month period of CBD administration

Activities during visit 3 (day 56-64):

- Measure vital signs of the patient as in visit 1.
- Questionnaire filling as in visit 1.
- Blood draw as in visit 1.
- The patient schedules the next test visits to the laboratory.
- The patient receives $100 for completing this visit.

Primary Outcomes: This study takes a single-cell perspective, i.e., we will collect a large number of cells from a small number of patients, and we will measure RNA expression per single cell (not per patient). Note that from the single cell point of view, the number of patients (5-7) is sufficient to derive statistically viable results, as we will collect 5,000 cells from each patient at each time point (total 75,000-105,000 cells). Each cell will be sequence individually to read the RNA expression from a panel of approximately 20,000 genes. Cells will be pooled from all patients, and clustered via unsupervised algorithms into clusters of different cell types (e.g. T cells, B cells, monocytes). Our primary outcomes will be the extraction of gene expression patterns (RNA) from specific immune cell types found in PBMC (e.g., B cells, T cells, monocytes). We will regress these gene markers against the variation in baseline inflammation (C-reactive protein, erythrocyte sedimentation rate, and procalcitonin). After CBD administration to obtain CBD-related biomarkers for inflammation reduction. The difference in gene expression will be measured in fold change, p-value, and ratio of cell fraction expressing the marker gene. These biomarker gene will be unique for each cell type. We expect to extract specific biomarkers for each cell type and understand how CBD alters gene expressions of different immune cells in a different way—overall, we expect the genes of the MAPK signaling pathway (MAPK families play an important role in complex cellular programs like proliferation, differentiation, development, transformation, and apoptosis) to be strongly altered.

Secondary Outcomes. Self-report pain ratings will be used to determine peak pain intensity and pain persistence. The brief pain inventory (BPI) will be used to self-report pain perception. The inventory consists of rating pain on a visual analog scale (VAS). A 10cm line is drawn with 0 (no pain) on the left pole and 10 (worst pain imaginable) on the right pole. The inventory asks participants to rate their level of pain by placing a slash on the line that best represents their current level of pain as well as pain at its worst, best and average over the past 24 hours. The highest worst pain intensity reported during the intervention will be recorded as the peak pain intensity.

Participants will complete self-report questionnaires to identify their level of pain-related anxiety and sleep quality during the course of the intervention. The **Pain Anxiety Symptom Scale** (PASS-20) is a 20 item, 5-point rating scale that assesses 4 theoretically distinct components of pain-related anxiety including cognitive anxiety, fear of pain, escape/avoidance behavior, and physiological anxiety [7]. The **Pittsburgh Sleep Quality Index** (PSQI) is a self-report questionnaire that assesses sleep quality over a 1-month time interval. The measure consists of 19 individual items, creating 7 components that

produce one global score, and takes 5–10 minutes to complete.[8] The SF-12 is one of three generic measures (along with the SF-20 and SF-36) from the Medical Outcomes Study (MOS). It consists of 12 items in 8 domains: physical functioning, role-physical, role-emotional, bodily pain, general health, vitality, social functioning and mental health, allowing for the generation of physical and mental health summary scores. During the visits, we will also administer a standard demographic questionnaire.

A **Data and Safety Monitoring Plan** will consist of two components: (1) overseeing participant safety and data monitoring, and (2) performance of monitoring. Since this project is viewed as low potential risk, all key personnel will perform all data and safety monitoring on an as needed basis. The principal investigator will meet quarterly with all members of the research team to discuss data and safety monitoring issues and any issues that arise will be handled in a manner that is consistent with the University of Florida's policies. Annually, data and safety monitoring information will be reported to and reviewed by the University of Florida IRB.

7. Possible Benefits

There is no known direct benefit from participating in this study. All study participants will be paid for their time performing research related duties that may or may not be considered a benefit. In this study, participants will receive $300 for completion of 3 in-person sessions. There are no other obvious benefits to the study participant.

The benefit to society is that completion of this investigation may increase scientific knowledge related to benefit of short-term sublingual administration of either a low-dose or high-dose CBD extract for relief of muscle pain and pain-related fear/anxiety symptoms. Another benefit from this study is that the data generate may be used to plan a larger clinical trial.

8. Conflict of Interest

No conflicts of interest exist for any study investigators or members.

9. References

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