Mass General Brigham

Institutional Review Board
Intervention/Interaction Detailed Protocol

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Project Title:	Evaluation of Cannabidiol (CBD) for Reduction of Brain Neuroinflammation
Version Date:	3/16/2022

For Intervention/Interaction studies, submit a Detailed Protocol that includes the following sections. If information in a particular section is not applicable, omit and include the other relevant information.

1. Background and Significance

Millions of individuals suffer from chronic pain
Chronic pain is defined as pain without apparent biological value that has persisted beyond the normal tissue healing time (usually taken to be 3 or 6 months[1,2]). Chronic pain is a widespread public health issue[3], and its prevalence is enormous. The weighted mean prevalence of chronic pain in the general population has been estimated by some at 35.5%, or 105 million, in the United States[4]. Not only does chronic pain affect both physical and mental functioning, thus compromising quality of life; it is also associated with astronomical costs. In addition to the direct costs of treating pain—including health care for diagnosis and treatment, drugs, therapies, and other medical expenses—chronic pain results in lost work time and reduced productivity[5,6]. Past estimates of the annual cost of chronic pain in the United States, including healthcare expenses, lost income and productivity, were close to $100 billion[7].

Treatment for chronic pain is unsatisfactory
Despite the enormity of the phenomenon, clinical needs for chronic pain are largely unmet. The treatment of choice for the largest majority (as many as 90%[8]) of patients seeking chronic pain management is based on opioid analgesics. However, the evidence supporting long-term effectiveness of opioid drugs in relieving pain and improving functional status is weak[9]. For instance, despite the widespread use of opioids for palliative care, more than half of all hospitalized patients experience pain in the last days of their lives, and 50-75% of cancer patients die in moderate to severe pain[10].

The current opioid-based pharmacological approaches to treat chronic pain are not only ineffective, but they generally have multiple unpleasant side effects, including constipation, pruritus, respiratory depression, nausea, vomiting, hyperalgesia, dizziness, sedation[9], as well as abuse and dependence[8,11,12]. Taken together, the unsatisfactory treatment efficacy and the occurrence of significant side effects, clearly stress the importance of achieving a deeper

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understanding of the pathophysiological mechanisms underlying chronic pain, in order to eventually identify viable treatment options alternative to ones currently available.

Microglia and pain

One of the reasons for the poor efficacy of the treatment options currently available for chronic pain might be that these are primarily aimed at suppressing neuronal activity within nociceptive pathways of the nervous system. However, it is now increasingly clear that neurons are far from being the only players that drive the establishment and/or maintenance of clinical pain symptoms. Rather, evidence from animal studies now suggests a central role of glial cells in the nervous system, including microglia[13,14].

Microglia are a subpopulation of macrophages that rapidly activate in response to a variety of pathological conditions[15], including persistent pain[16–23]. Microglial activation (MA) is characterized by a stereotypic pattern of cellular responses, including specific morphological changes, proliferation, increased or de-novo expression of cell surface markers or receptors, and migration to the site of injury[24]. MA generally represents an adaptive homeostatic defense response which enables the destruction of invading micro-organisms, the removal of potentially deleterious debris as well the promotion of tissue repair. However, animal studies have now showed that the uncontrolled activation of microglial cells under pathological pain conditions induces the release of substances that can sensitize pain pathways, such as proinflammatory cytokines, complement components, and others[25]. While evidence of pain-related MA was originally observed in the spinal cord, more recently it was also discovered at the level of the brain, including in the rostral ventromedial medulla [20,26], the trigeminal nuclear complex[19,27], and the ventral posterolateral nucleus of the thalamus [28,29].

While most of the evidence on the occurrence of pain-related glial responses in the central nervous system comes from animal studies, a few important observations indicate that similar phenomena should occur also in humans[13]. First, immunohistochemical markers of microglial and astroglial activation have been detected in the spinal cord of a patient with chronic regional pain syndrome in a postmortem study[30]. Furthermore, an increase in the concentration of the glial marker s-100β was reported in the cerebrospinal fluid of patients with lumbar disc herniation and in the serum of children with recurrent headaches[31,32]. Finally, a positron emission tomography (PET) study has revealed that human subjects with neuropathic pain secondary to peripheral nerve damage express increased thalamic binding for [^{11}C](R)-PK11195[33], an in vivo marker of microglial cell activation[34,35].

Recently, Co-PI Dr. Marco Loggia has also shown that patients with chronic low back pain (cLBP) have increased brain levels of the 18kDa translocator protein (TSPO), a marker of glial activation[36]. In addition, preliminary data collected from a different cohort of patients with cLBP and sciatica suggest an increase in spinal cord TSPO levels. Together, these results suggest that human chronic pain conditions are likely to be associated with a glial reaction, both in the spinal cord, as well as in the brain.

Cannabidiol and pain

There is a growing body of evidence to suggest that cannabinoids are beneficial for a range of clinical conditions, including pain, inflammation, epilepsy, and sleep disorders[37]. A large body of

preclinical and clinical research indicates that the cannabinoid system modulates a broad range of physiological processes and behaviors including, but not limited to, pain, mood, appetite, neuronal activity, memory, immunity, and cell development. The endocannabinoid system's contribution to the regulation of such a variety of processes makes phytocannabinoid pharmacological modulation a promising therapeutic strategy[38].

The primary cannabinoids found in the cannabis plant include delta-9 -tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), with THC being the primary psychoactive compound. The second most abundant compound in the plant is CBD, which is minimally psychoactive[39]. Cannabinoid receptor type 1 (CB1) and type 2 (CB2) belong to a family of seven transmembrane Guanosine Binding Protein-Coupled Receptors, and are widely expressed and distinguished by their specific functions, localization and signaling mechanisms. The psychotropic effects of cannabis are principally mediated by CB1, which is widely distributed throughout the brain, while CB2 is considered the peripheral cannabinoid receptor, found mainly in immune cells, as well as in chondrocytes, osteocytes and fibroblasts. Agonists targeting CB2 receptors have been proposed as therapies for the treatment or management of a range of painful conditions, including acute pain, chronic inflammatory pain, and neuropathic pain[40]. In a preclinical model, researchers showed that stimulation of CB2 suppresses microglial activation[41].

In the current study, we will test whether CBD is a glial inhibitor in patients with chronic lower back pain (cLBP) with and without mild-to-moderate depression. CBD was recently FDA-approved as a liquid formulation (see EPIDIOLEX package insert) for epilepsy for children ages 2 and up as well as adults, demonstrating significant reductions in total seizure frequency with minimal side effects. It is unclear whether cannabidiol reduces glial activation in humans. We will study 80 patients diagnosed with chronic low back pain (pain duration > 6 months) longitudinally before and after 4 weeks of treatment with cannabidiol or placebo. Endpoints will be pain scores as well as brain levels of the 18kDa translocator protein (TSPO), a marker of glial activation[36].

2. Specific Aims and Objectives

<u>Primary Aim:</u> Assess whether CBD compared to placebo reduces pain-related neuroinflammation in patients with cLBP.

> **Hypothesis 1:** Patients in the CBD arm will demonstrate significantly larger treatment-related reductions in thalamic [^{11}C]PBR28 PET signal, compared to patients in the placebo arm.
>
> **Hypothesis 2:** In the CBD arm, reductions in thalamic [^{11}C]PBR28 PET signal will be directly proportional to reductions in clinical pain ratings.

<u>Aim 2:</u> Assess whether CBD compared to placebo reduces depression-related neuroinflammation in cLBP patients.

> **Hypothesis 1:** Patients in the CBD arm will demonstrate significantly larger treatment-related reductions in limbic (pgACC, aMCC) [^{11}C]PBR28 PET signal, compared to

patients in the placebo arm.

Hypothesis 2: In the CBD arm, reductions in pgACC/aMCC [^{11}C]PBR28 PET signal will be directly proportional to reductions in depressive symptoms, as measured using the Beck Depression Inventory-II (BDI-II).

Aim 3 (Exploratory): Assess the effect of CBD on functional reward brain circuitry.

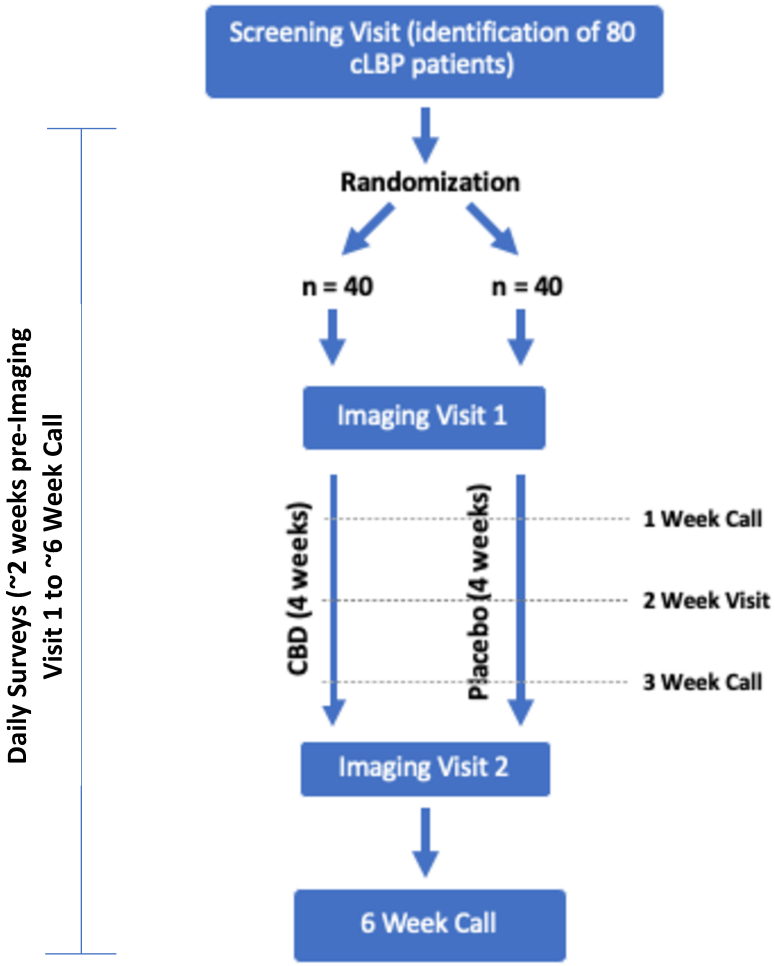
Hypothesis 1: Patients in the CBD arm will demonstrate significantly larger treatment-related increases in striatal responses to the anticipation and consumption of rewards/losses in the Monetary Incentive Delay task, compared to patients in the placebo arm. This will be indicative of a possible normalization of striatal function[42], which we have previously found to be dampened in cLBP (and other pain conditions).[43]

Hypothesis 2: In the CBD arm, increases in striatal activation will be proportional to increases in behavioral facilitation (i.e., slowing of reaction times during loss or rewards trials, indicative of an increase in sensitivity to incentives) and to reductions in depressive symptoms.

3. General Description of Study Design

We will conduct a 4-week randomized, double-blind, 2-arm mechanistic trial that assesses the effects of CBD vs. placebo in 80 patients with cLBP, using PET/MRI scans. Subjects will be randomized to receive either CBD (n = 40) or placebo (n = 40). Following randomization, subjects will participate in their first imaging visit, during which they will undergo a simultaneous PET/MRI scan and fill out questionnaires assessing their pain and other psychological constructs. At this visit, subjects will receive CBD or placebo, which they will be instructed to take daily for the 4 weeks prior to the date of their second scan. After 2 weeks of taking CBD or placebo, participants will undergo a follow-up appointment with a study clinician. We will also call participants at the end of the first and third weeks of taking CBD or placebo. Then, as soon as possible after the end of the 4-week drug trial period, all subjects will be scanned again and will complete several questionnaires (including some or all of those administered on the first imaging visit) to determine if any changes occurred since they entered the trial. Additionally, from about 2 weeks prior to the first scan and for 2 weeks after discontinuation of CBD or placebo, subjects will be sent a daily survey to assess the effect of the medication on their pain, mental health, sleep quality, fatigue, and other measures. Finally, we will conduct a follow-up call 2 weeks after the discontinuation of CBD or placebo.

Study Schema:



4. Subject Selection

We plan to identify 80 patients with chronic low back pain (cLBP; i.e., with a pain duration longer than 6 months), who will complete the study. In order to achieve the final sample size of 80 study completers, we will consent up to a total of 150 participants, in order to account for screen fails and attrition. As millions of people in the United States live with chronic low back pain[4], we believe that our recruitment goal will be attainable.

We are not planning to enroll subjects from at-risk populations (e.g., children and minors, cognitively impaired persons, prisoners). Written informed consent form will be obtained in all cases.

Inclusion Criteria:

1. Age ≥ 18 and ≤ 75;

2. The ability to give written, informed consent;
3. Fluency in English;
4. Average worst daily pain of at least 4 on a 0-10 scale of pain intensity, during a typical day. Pain needs to be present for at least 50% of days during a typical week;
5. On a stable pain treatment (pharmacological or otherwise) for the previous four weeks;
6. Diagnosis of chronic low back pain, ongoing for at least 6 months prior to enrollment.

Exclusion Criteria:

1. Outpatient surgery within 2 weeks and inpatient surgery within 1 month of the time of scanning (this timeframe may be extended if they are not fully recovered from the surgery);
2. Elevated baseline transaminase (ALT and AST) levels above 3 times the Upper Limit of Normal (ULN), accompanied by elevations in bilirubin above 2 times the ULN;
3. Any interventional pain procedures within 6 weeks prior to scanning procedure or at any point during study enrollment;
4. Surgical intervention or introduction/change in opioid regimen at any point during study enrollment;
5. Contraindications to fMRI scanning and PET scanning (including presence of a cardiac pacemaker or pacemaker wires, metallic particles in the body, vascular clips in the head or previous neurosurgery, prosthetic heart valves, claustrophobia);
6. Implanted spinal cord stimulator (SCS) for pain treatment;
7. Any history of neurological illness or major medical illness, unless clearly resolved without long-term consequences;
8. Current or past history of major psychiatric illness (PTSD, depression, and anxiety are exclusion criteria only if the conditions were so severe as to require hospitalization in the past year);
9. Harmful alcohol drinking as indicated by an AUDIT score ≥ 16;
10. Pregnancy or breast feeding;
11. History of head trauma requiring hospitalization;
12. Major cardiac event within the past 10 years;
13. Regular use of recreational drugs in the past 3 months;
14. Any marijuana use, medical or recreational, in the past 2 weeks;
15. An abnormal physical exam (e.g., peripheral edema);
16. Use of immunosuppressive medications, such as prednisone, TNF medications within 2 weeks of the visit;
17. Current bacterial or viral infection likely affecting the central nervous system;
18. Epilepsy or any prescription of an anti-epileptic drug;
19. Use of the medications valproate and clobazam, which may increase risk of hepatic AEs;
20. Safety concerns related to use of any of the following medications will be discussed on an individualized basis with a physician:
    - Strong and moderate CYP3A4 inhibitors including boceprevir, cobicistat, conivaptan, danoprevir, elvitegravir, ritonavir, indinavir, itraconazole, ketoconazole, lopinavir, paritaprevir and ombitasvir and/or dasabuvir, posaconazole, saquinavir and telaprevir, tipranavir, clarithromycin, diltiazem, idelalisib, nefazodone, nelfinavir, troleandomycin, voriconazole, aprepitant, cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, dronedarone,

erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, disulfiram, and verapamil;
- Strong and moderate inhibitors of CYP2C19 including fluoxetine and ticlopidine;
- Sensitive and moderately sensitive substrates of CYP2C19 including clobazam, lansoprazole, omeprazole, S-mephenytoin, and rabeprazole;
- Sensitive and moderately sensitive substrates of CYP1A2 including alosetron, duloxetine, ramelteon, tasimelteon, theophylline, tizanidine, pirfenidone, and ramosetron;
- Sensitive and moderately sensitive substrates of CYP2B6 including bupropion and efavirenz;
- Sensitive and moderately sensitive substrates of CYP2C8 including repaglinide, montelukast, pioglitazone, and rosiglitazone;
- Sensitive and moderately sensitive substrates of CYP2C9 including tolbutamide, celecoxib, glimepiride, and warfarin;
- Sensitive and moderately sensitive substrates of UGT1A9 including diflunisal, propofol, and fenofibrate;
- Sensitive and moderately sensitive substrates of UGT2B7 including, gemfibrozil, lamotrigine, and morphine;

21. CNS depressants including all antipsychotics, benzodiazepines (except for alprazolam, clonazepam, and lorazepam, which have low binding affinity to TSPO[44–48]), and non-benzodiazepine sleep aids that have a known unsafe reaction with CBD;
22. Use of opioids ≥ 30 mg morphine equivalents on average per month;
23. Actively suicidal, history of suicide attempt or an aborted attempt within the last 5 years, or engagement in non-suicidal self-injurious behavior within the last year;
24. Allergy to sesame oil, and any other ingredients of EPIDIOLEX;
25. Any other contraindications to CBD administration noted by the study physician**;**
26. Any significant change in drug use and pain treatment from screening visit;
27. In the opinion of the investigators, unable to safely participate in this study and/or provide reliable data (e.g., unable to reliably rate pain; unlikely to remain still during the imaging procedures, etc).

Local Recruitment Procedures:
Subjects will be recruited on an ongoing basis by trained study staff. We will identify potential subjects through advertising by flyers and printed announcements posted within as well as outside of our Partners community. In addition, email, web, and bulletin board announcements posted in the community will be used. To recruit subjects, we will also use multiple research databases such as the Partners' RSVP for Health system, Partners Clinical Trials, Rally, EPIC, RPDR, and ResearchMatch, a database of research volunteers developed by Vanderbilt University and approved for use by the PHRC. We will run queries on EPIC and RPDR through MGB to find subjects with chronic low back pain, meeting the eligibility criteria for this research study. Subjects identified through these mechanisms will receive a recruitment letter via Patient Gateway or in the mail from study staff. The letter will not be sent to those who have opted out of receiving research invitations. Other methods that advertise the study to the greater community will be used, including social media posts, posting flyers on community billboards in the Greater Boston area, emails to physicians and family medicine centers, and advertisements in newspapers. All advertisements will briefly describe the study and invite subjects to call if they

are interested. Newspaper advertisements in particular have been shown to be an effective strategy for recruiting minority populations[49]. Additionally, participants will be offered parking vouchers for each on-site study visit in order to ease financial burden of attendance.

5. Subject Enrollment

Telephone Pre-Screening:
All subjects will undergo a telephone pre-screening that will distinguish the majority of potentially eligible subjects from those not meeting eligibility criteria. This will consist of a brief discussion of the research study, as well as confirming a potential participant's understanding of the basic study procedures and interest in participation. To determine whether he/she may meet eligibility criteria, we will ask for information including current medications, gender, age, pregnancy status, substance use, and history of psychiatric conditions. Those who are likely to be eligible will be scheduled for an in-person screening visit. Note that in addition to using office phones, any calls made to participants for phone-screening or other reasons throughout the entire study may also be placed using Doximity Dialer, an MGB-approved platform. Also note that participants who express interest in the study may be asked to complete a REDCap survey containing questions from the phone screen, instead of completing the screen via phone call.

Procedures for Obtaining Informed Consent:
During the in-person screening visit, potential participants will be fully informed of the purpose and activities involved in the research study. Written informed consent will be obtained prior to initiating any of the study procedures. One copy of the signed consent form will be given to the patient and one will be kept in the study files for documentation. No time limits will be imposed on the informed consent process. Participants will be permitted to take as much time as they desire to engage in the informed consent process; any and all of their questions will be answered. It is anticipated that obtaining written informed consent will take approximately 15-25 minutes, on average. Comprehension of the consent information will be assessed via solicitation of answers to questions throughout the process. If comprehension appears to be limited, participants will be actively queried to determine whether they need further explanation.

In order to comply with public health efforts to address COVID-19, virtual visits may be conducted as necessary. Virtual visits will be conducted via MGB approved platforms (i.e., video calls over Zoom and phone conferences via Cisco Jabber) and will mirror in-person visits with the identical personnel present on the call. All questionnaires typically collected during the in-person screening visit may be collected during the remote screening visit, as they are largely already completed on secure online platforms (i.e., REDCap). All screening visit study procedures may be performed during the remote screening visit, with the exception of the urine drug test, blood draw, and physical exam, and any other assessments that cannot be performed remotely, which will be performed at the first in-person visit (i.e., first imaging visit) or at an extra, separate visit prior to the first imaging visit.

At the start of the virtual screening visit, informed consent will be obtained remotely. This will be done via electronic consent (e.g., Partners REDCap e-consent, Adobe Sign), or a remote consent process where the participant will be asked to sign the consent form and return it by email or mail. In either case, the consent discussion will occur identically to an in-person visit,

but instead held over phone call or video conference. Following the informed consent process, a copy of the signed consent document will be provided to the patient (electronically if e-consent was used). In the case of e-consent, consent will be documented on Partners REDCap or Adobe Sign. These are equivalent to written consent and are FDA compliant. As is with in-person consent, we will obtain and document informed consent before the participant is enrolled and any study procedures begin. Note that we may also use e-consent even for in-person screening visits.

Either a physician investigator or non-physician investigator will obtain informed consent in all cases. Note that the only non-physician investigators who will be allowed to obtain informed consent are Dr. Jodi Gilman (the IND holder) and nurse practitioner study staff members.

Treatment Assignment and Randomization:
Following the screening visit, participants who meet inclusion criteria, pass exclusion criteria, and provide their signed consent will be randomized in a 1:1 ratio to receive either CBD (n = 40) or placebo (n = 40). Randomization will be performed by stratifying subjects by age (>50 vs. ≤50) and sex (male vs. female). Each subject will be assigned a randomization number via a computerized random number generator. The Clinical Trials Pharmacy will maintain the specific subjects' treatment assignments (CBD or placebo) for later identification. Patients and study staff will be blinded to CBD or placebo assignment.

6. Study Procedures

Individuals who express interest in participating in the study will undergo a telephone screening to assess eligibility. If they are likely to be eligible, they will be scheduled for an in-person screening visit, during which a consent procedure will be conducted and a baseline assessment of questionnaires, interviews, and laboratory assessments will be conducted. Those who meet all eligibility criteria will be randomized to the CBD or placebo group. Then, subjects will be scheduled for their first imaging visit, during which they will undergo a simultaneous PET/MRI scan. At this visit, subjects will receive CBD or placebo, which they will be instructed to take daily for the 4 weeks prior to the date of their second scan. Note that, in cases where the screening visit and the first imaging visit are more than three months apart, eligibility criteria will be re-assessed.

Table of Study Procedures:

Domain	Measure	Source	Screen	Scan I (Pre-Treatment)	1 Week Call	2 Week Visit	3 Week Call	Scan II (Post-Treatment)	6 Week Call
Demographics	Custom (PhenX-based)	Participant	x						
General Health	Physical Examination	Physician/ NP/Nurse	x						
	Medical History	Physician/ NP/Nurse	x						
	Family History	CRC	x						
	Psychological Interview (MINI)	CRC	x						
	Characterization of Pain	Physician/ NP/Nurse	x						

	Concomitant Medications	Physician/ NP/Nurse	x	x					
	Weekly Medication Questions	CRC			x	x	x	x	x
Genetics	Oragene (CGR-500)	CRC	x						
Adverse Events	Adverse Event Record	CRC, reviewed by Physician		x	x	x	x	x	x
Depression, Psychological Functioning, Sleep	BDI-II	Participant	x	x				x	x
	BAI [a]	Participant	x						
	PROMIS-29	Participant		x				x	
	C-SSRS	Clinician	x	x		x		x	x
	CHRT [a]	Participant	x						
	MID	Done in PET/MRI scanner		x				x	
	PSQI	Participant	x	x				x	
Pain	BPI-SF	Participant	x	x				x	x
	ACR FM survey	Participant		x		x		x	
	PCS	Participant		x		x		x	x
	PainDETECT	Participant		x				x	
	Oswestry	Participant		x				x	
	Symptom-Mapper	Participant		x				x	
	Daily Survey	Participant	*Daily from ~2 Weeks before Scan I until 6-Week Call*						
Substance Use	TLFB (MJ, EtOH, nicotine, other substances)	CRC	x						
	AUDIT [a]	Participant	x						
	CUDIT-R [a]	Participant	x						
	FTND [a]	Participant	x						
	ECDI [a]	Participant	x						
Expectancy	Next Visit/Call	Participant		x	x	x	x		
	Final Visit	Participant		x					
Quality of Life	Patient Global Impression of Change (PGIC)	Participant						x	
IQ	WRAT5	CRC	x						
Delay Discounting	MCQ [a]	Participant	x						
Impulsivity	UPPS-P [a]	Participant	x						
ADHD	ASRS [a]	Participant	x						
COVID-19	EPII [a]	Participant	x						
	COVID-19 History [a]	Participant	x						
	Vaccination Questionnaire	Participant		x					
Urine Test	Urine Drug Test	CRC	x	x				x	
	Urine Pregnancy Test	CRC	x						
Blood Test	Liver function tests	Nurse / Physician / trained study staff	x					x	
	Whole Blood DNA (Genotype	Nurse / Physician /	x	x				x	

	& PBMC & iPSC)	trained study staff							
	Serum Pregnancy Test (if applicable)	PET Tech/Nurse		x				x	
	Serum extraction (Cytokine panel)	Nurse / Physician		x				x	
	Arterial line (optional)	Anesthesia-trained clinician		x				x	
	COVID antibody	Nurse / Physician		x				x	
	CBD/THC Metabolites in plasma	CRC		x				x	

[a] Indicates a measure that may be completed by the participant at home, following the screening visit.

Study Drug:
Epidiolex, an agent within the anti-epileptic drug class, will be used. Epidiolex, Greenwich Biosciences Inc.'s CBD formulation, is a 100 mg/mL purified oral solution, dissolved in the excipients sesame oil and anhydrous ethanol with added sweetener (sucralose) and strawberry flavoring. The drug is formulated from extracts prepared from Cannabis sativa L. plants that have a defined chemical profile and contain consistent levels of CBD as the principal phytocannabinoid. Extracts from these plants are processed to yield pure (>95%) CBD that typically contains less than 0.5% (w/w) THC. Cannabidiol is the active ingredient in Epidiolex; inactive ingredients include dehydrated alcohol, sesame seed oil, strawberry flavor, and sucralose. Of note, CBD has no psychoactive properties. The empirical formula of Epidiolex is $C_{21}H_{30}O_2$ and its molecular weight is 314.46. The structure of CBD is provided in the figure below.

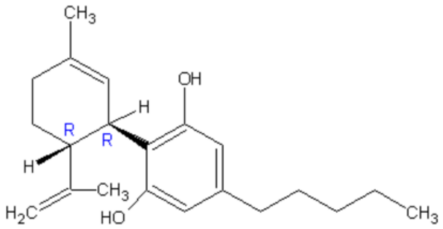


Figure 1. Cannabidiol Structure

Dose and Exposure:
Either EPIDIOLEX® or placebo will be dispensed by the Research Pharmacy at Massachusetts General Hospital. The recommended starting dosage is 2.5mg/kg taken twice daily. Participants will follow a titration schedule, with 2.5mg/kg taken orally twice daily in week 1, 5mg/kg twice daily in week 2, 7.5mg/kg twice daily in week 3, and 10mg/kg twice daily in week 4. Subjects will increase to 10mg/kg twice daily on the first day of the final week of the study (week 4) and take Epidiolex at this dose for the remainder of this final week. If participants report AEs (tiredness, dizziness, not tolerating the medication well) during the second, third, or fourth week of taking the study drug, the physician will decrease the dose to the previous week's dose. Participants will be treated for 4 weeks in total.

The 4-week duration of CBD or placebo administration is proposed because in the current study, we are investigating an endophenotype of pain- neuroinflammation- which may be detectable before verbal reports of pain reduction, which is notoriously noisy and susceptible to placebo effects. This study will inform us of whether 4 weeks is enough to detect changes in TSPO binding that may precede reports of pain reduction.

Screening Visit:
Subjects eligible to participate will be recruited to participate in an approximately 3-hour characterization session. In this session, we will obtain a signed consent form from the subjects, explain the procedures involved in the experiment, and administer some or all of the following validated assessments. We will also collect detailed contact information (address, social security number, medical record number) and demographics, collect a saliva sample for genetic testing, assess medical and family history, and perform a physical examination, and assess concomitant medications. Finally, we will collect a blood sample and a urine sample. Computer-based rating scales and questionnaires will be completed on a laptop. Assessments will be performed by fully trained study staff members such as post-doctoral research fellows and Clinical Research Coordinators, under the supervision of and periodic monitoring by the Principal Investigator (PI).

During the informed consent procedure, participants will be informed about other treatment alternatives for chronic low back pain they can pursue (e.g., medications, transcutaneous nerve stimulation, physical exercise and stretching) in lieu of participation in this clinical trial.

Many of these assessments are already in use in one or more IRB approved protocols (e.g., 2011P002311). Note that participants may complete some of the following questionnaires at home after the screening visit if time does not allow for their completion during the screening visit.

History and physical examination: An MD, NP, or nurse will also collect medical history and perform a formal physical examination, including the recording of vital signs (heart rate, blood pressure, and body temperature). If these assessments are done by a nurse or NP, a physician will review them prior to prescribing the study drug.

Beck Depression Inventory-II (BDI-II)[50]*:* The 21-item BDI-II has shown good reliability and validity for assessing depression in chronic pain patients[51].

Brief Pain Inventory – Short Form (BPI-SF): The BPI is a 15-item questionnaire assessing pain location, and 0–10 ratings of pain intensity, relief, quality, pain-related quality of life, and function. It has been validated in cancer and noncancer pain conditions[52].

Timeline Followback (TLFB)[53]*:* The TLFB uses memory aids to trigger recall of substance use. It will be used to measure participants' use of cannabis, tobacco, alcohol, and other substances in the previous 90 days.

Mini International Neuropsychiatric Interview (MINI)[54]*:* The MINI 7.0.2 is a structured diagnostic interview used to assess DSM-5 psychiatric disorders. It will be administered by trained study staff.

Wide Range Achievement Test, 5th Edition (WRAT5), Word Reading[55]*:* The word reading subset of the WRAT5 will be used to assess speech and dictation.

Monetary Choice Questionnaire (MCQ)[56]*:* The MCQ presents participants with 27 questions, each of which asks them to choose between smaller, immediate rewards, and larger, delayed rewards. Participants' patterns of answers are able to provide an estimate of their delay discounting rate.

Short UPPS-P Impulsive Behavior Scale[57]*:* The 20-item Short UPPS-P assesses five components of impulsivity, including sensation seeking, lack of premeditation, lack of perseverance, negative urgency, and positive urgency. Scores on many of these factors have been shown to relate to risky behaviors.

Beck Anxiety Inventory (BAI)[58]*:* The 21-item BAI assess the frequency of anxiety symptoms, including both cognitive and somatic symptoms.

Alcohol Use Disorders Identification Test (AUDIT)[59]: The AUDIT is a 10-item questionnaire used to screen for harmful alcohol consumption. It assesses drinking frequency and problems related to alcohol use. The scale ranges from 0 – 40; a score of 8 or higher is an indicator of harmful alcohol consumption.

Cannabis Use Disorders Identification Test – Revised (CUDIT-R)[60]*:* The CUDIT-R is an 8-item questionnaire that screens for problematic cannabis use in the past six months. It assesses problems related to cannabis use, dependence, and use frequency. The scale ranges from 0 – 32; a score of 13 or higher is indicative of possible cannabis use disorder.

Fagerstrom Test for Nicotine Dependence (FTND)[61]: The 6-item FTND assesses nicotine dependence. It measures amount of cigarette use, dependence on cigarettes, and compulsion to use. The scale ranges from 0 – 10, with a higher score indicating greater dependence.

Electronic Cigarette Dependence Index (ECDI)[62]*:* The 10-item ECDI assesses dependence on electronic cigarettes. The scale ranges from 0 – 20, with scores 13 and higher indicating high dependence.

ADHD Self-Report Scale (ASRS)[63]*:* The 6-item screener scale of the ASRS will be used to assess participants' ADHD symptoms, including both inattentive symptoms and hyperactive-impulsive symptoms, during the past 6 months.

Concise Health Risk Tracking Self-Report form (CHRT-SR)[64]: The 12-item CHRT-SR assesses active suicidal ideation and behavior, perceived lack of social support, and hopelessness. The scale ranges from 0 – 48, with a higher score indicating greater suicidal thoughts and propensity[65].

Pittsburgh Sleep Quality Index (PSQI)[66]*:* The PSQI is a 19-item questionnaire that assesses sleep quality and patterns during the previous month. The scale ranges from 0 - 21, with a higher score

indicating less healthy sleep quality.

Epidemic-Pandemic Impacts Inventory (EPII)[67]*:* The EPII will be used to assess how the COVID-19 pandemic has impacted participants' lives, including impacts on work, home, and social life, as well as impact on emotional and physical health. It includes 92 items.

Suicidal Ideation: The Columbia-Suicide Severity Rating Scale (C-SSRS)[68] will be used for prospective suicidality assessment. C-SSRS is a tool used to assess the lifetime suicidality of a participant and to track suicidal events through the treatment. The structured interview prompts recollection of suicidal ideation, including the intensity of the ideation, behavior and attempts with actual/potential lethality. The scale will be administered by study staff at the screening visit, baseline, two-week visit, four-week visit, and six-week call. The C-SSRS "Screening/Baseline" will be collected at Screening and Baseline and the C-SSRS "since last visit" will be collected at subsequent visits. Participants who answer "yes" to any suicidal behavior questions or to suicidal ideation questions 4 or 5 on the C-SSRS during the study should be referred for appropriate psychiatric care. If there is more than a 30% increase in symptoms of anxiety or depression, this will be immediately reported to the PI, who will consult with study clinicians. Clinicians will then determine, with the participant, whether it is in their best interest to continue the medication. The decision to discontinue the participant from the study will be made by the PI in conjunction with clinical Co-Investigators.

Safety monitoring: As participant suicidality and depression is monitored throughout the study with the C-SSRS and BDI-II questionnaires, any new or worsening expression of suicidal ideation or Answers of "Yes" to questions 4 or 5 on C-SSRS throughout the study will be evaluation by a licensed clinician member of study staff. The Standard Operation Procedure will be reviewed. To summarize, if any risk for self-harm or suicidality is identified at any visit, the research coordinators will immediately report this to study clinicians, who will determine whether a safety assessment is needed. If a clinician is needed to perform a safety assessment, study staff will record the date, clinician initials, and comments related to the suicidality assessment in the REDCap C-SSRS module. Following the initial suspicion or identification of self-harm and/or suicidality, a study clinician will follow up with the participant on the current nature of their situation, querying about any new ideation, intent, and/or plan since the last visit. These clinicians, along with the PI will then determine whether a participant can safely continue the study. If the clinicians determine that the participant cannot safely continue the study, the participant will be discontinued, and will be provided with a list of resources for follow-up care.

Demographics: Demographic information, including age, sex, gender, sexual orientation, education level, income, race, height, language, employment status, marital status, and residence, as well as information about the participant's caregivers during childhood, will be collected.

DNA Collection, Saliva (optional): DNA samples will be collected using Oragene (OGR-500) saliva kits. Participants may be asked to provide a second sample if a re-collect is recommended after DNA extraction (i.e., there is very little DNA in the sample). Participants are not required to provide another sample if they do not wish to do so. Once extracted samples will be transferred to long term storage until genotyping. Samples will be stored with a unique participant ID.

Family history: The family history subsection of the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS)[69] will be used to assess family history of psychiatric treatment, including treatment for depression, mania, anxiety, ADHD, schizophrenia, and substance use, as well as history of suicide.

Concomitant medications: Any prescription medications or over-the-counter drugs being taken by the participant at the time of the screening visit will be assessed, and dose and frequency will be recorded.

Blood tests: a trained member of the study staff will draw venous blood (up to 10 ml) from all subjects in order to have them genotyped for the Ala147Thr TSPO polymorphism in the *TSPO* gene (rs6971) (unless this genotype information is already available), and to check liver enzyme values. Additionally, during screening for eligibility, we will conduct routine chemistry and LFT/GGT. We will obtain serum transaminases (ALT and AST) and total bilirubin levels in all patients prior to starting treatment with CBD.

While [^{11}C]PBR28 has the advantage of binding to the TSPO protein with a higher ratio of specific-to-nonspecific binding than [^{11}C](R)PK11195[70], it also presents a potential limitation, in that about 10% of human subjects show no binding to PBR28[71] (whereas [^{11}C](R)-PK11195 has never been associated with non-binding[72]). A recent study has demonstrated that the rs6971 polymorphism predicts PBR28 binding affinity in human platelets[73] . Since the low-affinity binder phenotype is consistent across all tissues within the same subject[72] , testing for the Ala147Thr polymorphism has been suggested to predict low affinity for [^{11}C]PBR28 in all organs, including the brain. High or Mixed affinity binders (Ala/Ala or Ala/Thr) will be considered eligible, whereas the Low affinity binders (Thr/Thr) will be considered ineligible to continue in the research study. The MGH lab responsible for genotyping typically runs the genotyping assay only twice per month, requiring that we normally schedule screening and scanning visits approximately two weeks apart.

We may also use saliva instead of blood to genotype subjects for the Ala147Thr TSPO polymorphism. Saliva genotyping may be done prior to the in-person screening visit to eliminate low affinity binders, thus saving subjects the burden of having to travel to and attend the screening visit only to find they are not eligible for the study. Verbal consent will be obtained and documented if patient-subjects agree to this saliva collection, and subjects will be asked if they wish to receive a copy of the Privacy Notice and documentation linking them to the research study. Both forms will be provided upon request. Saliva collection kits with a pre-assigned study ID and a pre-paid return mailing supply will be mailed out to applicable subjects. Eligible subjects will be scheduled for an in-person visit. If subjects are ineligible for the study based on the saliva genotyping results, they will be compensated for providing the saliva sample.

An additional 10 mL of venous blood will be collected and stored for future investigations on the roles of genetic, molecular, and cellular factors in pain disorders. This will include the future possibility to generate induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells[74–76] to assess in-vitro alterations in patient-derived neural or glial cells[77,78].

Urine drug test: We will also perform a urine test to screen for use of opioids and illicit drugs

(including amphetamine, barbiturates, cocaine, marijuana, etc.). The urine drug screen will be performed during the screening visit. A rapid urine drug screening that utilizes monoclonal antibodies to detect elevated levels of specific drugs in urine, will be used for this purpose. Results will be read five minutes after the test was started.

Urine pregnancy test: Urine will be collected at screening for a pregnancy test in female participants of childbearing age.

COVID-19 History: Participants will complete a short questionnaire that asks about COVID-19, including whether they have ever been exposed, experienced symptoms, and/or tested positive. Those reporting a positive test will be asked about how severe the course of illness was, including whether they were hospitalized. All participants will be asked about vaccination status.

Note that we may also do a brief MRI test scan with participants on the day of their screening visit, or on a different day, to ensure that they are a suitable candidate for scanning.

PET/MRI Visits:
Participants eligible to continue into the study based on the screening visit and genotype analyses will be asked to participate in a first PET/MRI visit.

Prior to each scan session, subjects will complete screening checklists for MRI and PET. These checklists will ask the patients whether they have any contraindications for MRI or PET scanning. Female participants of childbearing age will be asked to have ~3mL of their blood drawn in order to perform a serum pregnancy test on the day of the scan (blood will be sent to the core lab for super stat testing). They will also be asked about the date of their last menstrual cycle. In addition, a urine drug test will be repeated on the day of each PET/MRI visit.

At the beginning of the scan session, an intravenous catheter will be placed in the participant's antecubital vein of the left or right arm, prior to going to the scanning area. Up to 15mL of blood will be drawn to assess the levels of various substances in the blood, such as the proinflammatory cytokines IL-6 and TNF-alpha. Blood will be collected in various vials (e.g., purple top K3EDTA).

Blood may also be drawn for SARS-CoV-2 antibody serology testing, and the presence of antibodies will be used to explore the possible effects of prior exposure to the coronavirus on neuroinflammation, in exploratory analyses. Up to 10 ml of blood will be drawn for this purpose. This testing will be performed through a third party vendor or through the MGH core lab.

Following procedures identical to those adopted in other PET studies (including using [^{11}C]PBR28) from our center (e.g., 2015P001594, 2013P001297, 2011P001546, 2011P002311, 2016P001009, etc), an arterial line will be placed in a radial artery with local anesthesia (20 or 18 gauge cannula, 2-5 ml of lidocaine 1% intradermal and subcutaneous) using sterile techniques, if the participant has consented to this procedure. The placement of an arterial line will be presented as optional to the participants, and we will ask for the participants' consent each time. The arterial line will not be placed if the participant has any contraindications to arterial line placement, such as Raynaud syndrome, bleeding disorder, or use of anticoagulants

such as Coumadin, Plavix or Lovenox. The arterial line will be placed in the arm contralateral to the intravenous line that is used for the [^{11}C]PBR28 radiotracer injection. The arterial line will enable blood sampling (1mL to 12mL) at various times during the imaging study for at most 160 mL of blood. The collected arterial blood will be used to compute metabolite-corrected arterial input function for kinetic modeling analyses (see Data Acquisition and Analyses). The catheter will be placed by an individual with anesthesia training (i.e., board-certified anesthesiologist, fully licensed anesthesia resident, or a certified registered nurse anesthetist), monitored throughout and accessed by an experienced research nurse. The catheter will be discontinued at the end of the study by a physician, a nurse practitioner, certified registered nurse anesthetist, or registered nurse. We will have all RNs who do this sign a form attesting that discontinuing the catheter and associated post-procedure monitoring is within the scope of their work and clinical privileges.

Subjects will be instructed to remain still, with eyes open, for the total duration of the scans, except when prompted to engage in various tasks (e.g., rate pain, perform the Monetary Incentive Delay task, etc.).

During the scan visit, subjects will be asked to complete the *BPI-SF, BDI-II, C-SSRS,* and *PSQI,* which were also assessed at the screening visit. We will again assess concomitant medications. Additionally, subjects will complete some or all of the following validated assessments. Any questionnaire may be sent home with participants to complete if there is not time to complete them during the scan visit.

Patient Reported Outcomes Measurement Information System (PROMIS-29) questionnaire[79]*:* The PROMIS-29 is a 29-item self-report measure assessing physical, mental, and social health.

PainDETECT[80]: The PainDETECT is a screening questionnaire used to estimate the likelihood of a neuropathic component in chronic pain.

The Pain Catastrophizing Scale (PCS)[81]*:* It is a 13-item self-report scale which measures pain-related Rumination, Magnification and Helplessness.

Oswestry Disability Index (ODI)[82]*:* The ODI is an extensively used 10-item scale to describe the level of disability in patients with chronic low back pain and will be used to characterize the study population.

SymptomMapper: The SymptomMapper app is a digital tablet-based application used to localize areas where patients are experiencing pain. In the app, the patient picks a pain descriptor, i.e., burning or shooting, notes the severity of that descriptor, and then marks where on the body that descriptor is felt. All data are stored de-identified and securely onto local drives.

Fibromyalgia Survey[83]*:* The American College of Rheumatology's fibromyalgia survey will be used to assess widespread pain and fibromyalgia symptom severity. The widespread pain subscale ranges from 0 - 19, with a higher score indicating more widespread pain. The fibromyalgia symptom severity subscale ranges from 0 - 12, with a higher score indicating more severe symptoms.

Treatment Expectancy: Expectancy of symptom improvement will be assessed using three statements that ask about how participants expect to feel at the end of treatment (at their last visit), as well as 3 statements about how they expect to feel at their next study visit or call. These statements will assess expected pain intensity, pain bothersomeness, and depression, and will each be scored on a 0 – 10 scale, with a higher score indicating worse symptoms.

Adverse events: Any untoward or unfavorable medical occurrence participants have experienced will be assessed, whether or not the occurrences are considered related to their participation in the research.

Vaccination Questionnaire: The date of administration and manufacturer of each COVID-19 vaccine dose the participants have received will be assessed using a brief questionnaire. Additionally, participants will be asked whether they have received any other vaccinations in the past 14 days to capture a potential acute immune response.

During the scan, participants will be asked to complete the *Monetary Incentive Delay (MID) task*. The MID task features balanced incentive delivery and analytic strategies designed to identify activity specific to anticipation or consumption of incentives. In the reward condition, successful trials are associated with monetary gains whereas unsuccessful trials lead to no change. In the loss condition, successful trials are associated with no change whereas unsuccessful trials are associated with monetary penalties.

At the end of the scan, for those participants who received an a-line, an experienced nurse or MD will remove the catheter. These subjects will be kept under observation for a minimum of 30 minutes.

The total duration of each scanning visit will be approximately 4 hours (and up to 6 hours) (~45min for preparation, ~30min for a-line placement, if applicable, ~120 min for scanning procedures, ~20-30min optional spinal scan after completion of the brain scan, and ~60min for filling out questionnaires and observation after removal of arterial line, plus an additional ~90min to perform pregnancy test in women of childbearing age). In case of equipment failure (e.g., failure in radiosynthesis) delays of > 2 hours may be possible. In this case, we will ask the participant if he or she feels comfortable with staying longer than anticipated, or will prefer reschedule to another date.

Depending on the patients' level of discomfort and time constraints, we may occasionally shorten and simplify the scan visits. For instance, if the participant would feel too uncomfortable to lay down in the scanner for the full ~2:00 hours of scanning, we may administer the radioligand in the injection room and then scan the participant between ~45 and 90 minutes post-injection. If the participant cannot remain for the full 6-hour scan visit, it will be acceptable to forego the arterial line placement. Eliminating this procedure will save the time needed for the placement and the ~30 minutes of observation needed after the removal of the a-line (from these scans we will derive metrics that do not depend on arterial sampling, such as standardized uptake value ratio (SUVR)).

The imaging visit, including all the procedures described above, will be repeated a second time after a 4-week trial of CBD or placebo. Blood drawn at the second imaging visit will also be tested to check liver enzyme values. Also, up to 4 additional mL of blood will be drawn, and the blood plasma samples will be sent to collaborators at Clinical Research and Development facilities Department of Anesthesiology, at the University of Colorado Anschutz Medical Campus. These blood plasma samples will be analyzed for quantitative levels of cannabinoids, including CBD and THC. All analyses will be carried out following standard operation procedures, which are based on all applicable CAP, CLSI, ICH, OECD and FDA guidelines. All samples will be fully de-identified, and will not contain any identifiers that could be used to link the specimens or data to individual subjects.

Parts of the PET/MRI visits may be conducted virtually, as necessary.

Follow-up Visits/Calls:
1-Week Call. Patients will have a phone call 1 week after their first scan with a study team member. In this call, adverse events, treatment expectancy, and medication use will be assessed and participants will be reminded to increase medication dose.

2-Week Visit. Patients will undergo a follow-up appointment at Week 2 with a trained study staff member, where health, other medication use, and adverse events will be assessed, and patients will complete questionnaires and will be reminded to increase medication dose. Treatment expectancy will also be assessed. Participant may receive a ride to and from the study visit if requested. Some or all of this visit may also be conducted virtually as necessary. If there are any adverse events or issues reported during this visit, we will refer the participant to a study clinician who will get back to them.

3-Week Call. Patients will have a phone call during Week 3 with a study team member. In this call, adverse events, treatment expectancy, and medication use will be assessed and participants will be reminded to increase medication dose.

4-Week Visit. Patients will undergo a second follow-up appointment immediately after the four-week treatment period with a study clinician, where we will assess back pain, general health, adverse events, and medication use. Portions of this visit may be conducted virtually, if necessary. On the same day or as close as possible depending on scheduling, patients will be re-scanned, using identical protocols, to evaluate the hypothesis that CBD reduces glial activation. We will also repeat the questionnaires administered during the first imaging visit to assess any changes in subjective pain. We will take a small sample of blood for a follow-up liver function test. Participant may receive a ride to and from the study visit if requested.

6-Week Call. We will conduct a follow-up call 2 weeks after the discontinuation of the study medication. In this call, we will assess back pain, general health, adverse events, and medication use.

Daily Surveys:
From about 2 weeks before Scan 1 to the 6-week call, we will ask participants to complete brief daily surveys assess various domains, including their levels of pain, depression, anxiety, fatigue,

and sleep quality on various scales (e.g., 0-10). We will also ask participants whether they have taken the study medication that day, and whether they have taken any other medications to manage their pain.

Drug Administration Protocol:
Following the behavioral visit, subjects will receive CBD or placebo. Please see "Dose and Exposure" section above for dosing and titration schedule. Participants will be treated for 4 weeks in total. Chronic CBD dosing up to 1500 mg/day has been reported to be tolerated well without AEs[84–87]; minor AEs were reported after CBD use in children with epilepsy being treated with multiple other medications in doses up to 25mg/kg twice daily[88,89]. Accordingly, we believe an upper limit of 10mg/kg twice daily orally is reasonable and safe.

Continuation of medication (e.g., NSAIDS) will be permitted on the condition that patients will be on a stable dose for at least 1 month before the baseline PET/MRI scan.

The Research Pharmacy at Massachusetts General Hospital will prepare the CBD and placebo. The bottle will contain 100mg/mL of CBD or placebo. It will also have a small ID label with a batch number printed on it. The label will explain the storage conditions, the shelf life, and the in-use shelf life. Each container will be labeled with a unique number that will be recorded by study staff at the time of administration. As soon as possible after the 4-week CBD or placebo period, patients will be re-scanned and/or re-evaluated clinically to evaluate the hypothesis that CBD compared to placebo reduces glial activation and pain/depressive symptoms. Participants will be instructed to take CBD or placebo with food, rather than in a fasted state, and not to take CBD or placebo concurrently with alcohol. Participants will be instructed to return any unused CBD or placebo at the second PET/MRI visit.

GWAS Genotyping:
The Broad Institute will perform genotyping (array-based) of subject DNA samples and subsequent in-depth analysis of the data, which will allow us to detect alterations in the genome including point mutations, small insertions and deletions, chromosomal copy number alterations, and translocations. These experiments are intended to help identify candidate genes involved in the physiopathology of neurological and psychological diseases. The molecular information generated from these samples will not be returned to subjects at any time.

Data from this study may result in communications in journals or at scientific meetings. Subjects will not be identified in those communications. To facilitate research, the genetic information generated may upon publication be deposited in protected databases (such as dbGAP) available only to bona fide researchers with specific scientific questions who promise to not try to identify individuals. The data will be sent to these banks in a coded manner and again will not contain any traditionally used identifier such as name, address, phone number, or social security number. Although we cannot predict how genetic information will be used in the future, there are many safeguards in place and we do not think that there will be further risks to patients' privacy and confidentiality by sharing such information with these banks.

The Broad Institute will not be involved in subject ascertainment. Prior to transfer of bio-specimen aliquots to the Broad Institute, samples will be re-encoded at the collaborators

institutions. No identifying patient information will be shared with Broad scientists at any time. Some limited clinical data will be obtained from collaborators. Again, all subject identifying information will remain with the collaborators and only de-identified clinical data will be shared with the Broad Institute.

Platforms for Data Collection:
Questionnaires Collected via Research Electronic Data Capture (REDCap): Surveys will be administered via REDCap, a HIPAA compliant, web-based application hosted by Partners HealthCare designed to support data capture for research studies[90], at in-person visits (or virtual if required per COVID-19 restrictions).

Data will be stored automatically and securely on a SQL Server, accessed over industry standard TLS 256 bit RSA encryption during data transfers. Data is routinely backed up locally onto a redundancy server and stored in a separate database. Long term storage on Partners servers occurs nightly and allows for incremental backup over multiple systems. Therefore, should one drive be physically damaged, there will be multiples within the chain to replace it. Both data servers are stored within PHS IS corporate firewall, in a secure, key access facility with password-protected computers. Only vetted PHS security officials will have access to physical machines storing study data. Since data are stored on a protected server, a compromise of any individual computer at a research facility will not lead to a breach of the secure database. Individual computers designated for data capture do not store participants' identifying information or study data.

Return of Research Results:
Participants should not expect to get information about the results of the study or the results of their individual participation in the study.

Incidental Findings:
In the unlikely event that evidence of physical or psychological disorder is found, with the individual's permission, the information will be shared with his or her primary care physician who can direct care as needed.

Outcomes:
Primary Outcomes:

1. Changes in neuroinflammation in the thalamus: We will test for the presence of a significant treatment effect in the brain [^{11}C]PBR28 signal in the thalamus, in order to test whether patients in the CBD arm will demonstrate significantly larger treatment-related reductions in neuroinflammation, compared to patients in the placebo arm.
    a. Time Frame: Change from Baseline to Week 4.

Secondary Outcomes:

1. Changes in neuroinflammation in limbic regions: We will test for the presence of a significant treatment effect in the brain [^{11}C]PBR28 in limbic regions (pgACC, aMCC), in order to test whether patients in the CBD arm will demonstrate significantly larger treatment-related reductions in neuroinflammation, compared to patients in the placebo arm.

a. [Time Frame: Change from Baseline to Week 4]

2. Correlation Between Reductions in Thalamic [11C]PBR28 PET Signal and Reductions in Clinical Pain Ratings: We will test whether reductions in thalamic [^{11}C]PBR28 PET signal correlate with reductions in clinical pain ratings, as assessed by the "worst pain" item of the Brief Pain Inventory - Short Form. The "worst pain" item's scale ranges from 0 - 10, with a higher score indicating worse pain intensity.
    a. [Time Frame: Change from Baseline to Week 4]

3. Correlation Between Reductions in Limbic [11C]PBR28 PET Signal and Reductions in Depressive Symptoms: We will test whether reductions in pgACC/aMCC [^{11}C]PBR28 PET signal (as measured by Standardized Uptake Value Ratio) correlate with reductions in depressive symptoms, as measured by the Beck Depression Inventory-II. The Beck Depression Inventory-II scale ranges from 0 - 63, with a higher score indicating greater depression.
    a. [Time Frame: Change from Baseline to Week 4]

4. Change in Clinical Pain Ratings: The "worst pain" item of the Brief Pain Inventory - Short Form will be used daily to assess pain intensity. The scale ranges from 0 - 10, with a higher score indicating worse pain intensity.
    a. Time Frame: We will examine change from average score during the 7 days prior to treatment (Baseline) to average score during the final week of treatment.

5. Change in Pain Bothersomeness: Pain bothersomeness will be assessed daily on a scale from 0 - 10, with a higher score indicating greater bothersomeness.
    a. Time Frame: We will examine change from average score during the 7 days prior to treatment (Baseline) to average score during the final week of treatment.

6. Change in Depressive Symptoms: The Beck Depression Inventory-II will be used to assess symptoms of depression. The scale ranges from 0 - 63, with a higher score indicating greater depression.
    a. Time Frame: Change from Baseline to Week 4.

7. Patient Global Impression of Change: The Patient Global Impression of Change scale will be used to assess participants' perceptions about their global improvement related to their low back pain. The scale ranges from 0 - 7, with a higher score indicating greater overall improvement.
    a. Time Frame: Week 4

Study Termination Criteria:
Participants will be terminated from this study if there are any significant safety concerns (e.g., actively suicidal), failure to comply with study procedures, or if the opinion of the principal investigator, can no longer safely participate. In addition, subjects will be informed that if they feel uncomfortable with the study, they can choose to terminate the study at any time. They will be informed that their refusal to participate in the study or choosing to terminate it at some point will have no effect on care and treatment received by them at MGH now or in

future. We will also discontinue EPIDIOLEX or placebo in any patients with elevations of transaminase levels greater than 3 times the ULN and bilirubin levels greater than 2 times the ULN.

If a participant decides to stop participating in the study before the planned end of the study, we will ask that they continue to follow the schedule of visits. If they are unable or unwilling to return to the MGH Charlestown Navy Yard campus for visits, we will ask if we can call them for phone interviews instead of study visits.

Study Compensation:
Subjects will be paid by check at the completion of the study for their participation.

We will pay up to $770. Payments will be as follows:

- $75 for the initial screening visit
- Up to $76 for completing daily surveys ($1 per survey completed)
    - Bonus $25 if they complete 90% of surveys
- $200 for each PET/MRI scanning visit
- $25 for each blood test to exclude pregnancy (for females of childbearing age)
- $50 for each arterial line placement
- Participants will be able to earn up to an additional $17-$22 during each Monetary Incentive Delay task

If during the imaging visit(s) we cannot inject the subject with the radioligand (e.g., due to a failure in radiosynthesis, or to issues with the scanner) and we HAVE NOT yet placed the arterial line, he/she will receive $50. If we cannot inject the subject with the radioligand, and we HAVE already placed the arterial line, he/she will receive $100.

If the subject will need to stop the scan early for any reason, he/she will still receive $50 for his/her time. Additionally, parking fees will be covered as needed.

Subjects excluded per the genotyping results of a mailed-out saliva test will receive $15.

7. Risks and Discomforts

All subjects will undergo a telephone or email pre-screening to attempt to distinguish potential subjects from those not meeting eligibility criteria. Likely candidates will undergo a characterization and training session, which will include a clinical screening procedure. This procedure will involve answering questions about subjects' medical history recording of medical history review and answering questions about their medical situation including liver disease, kidney disease, blood disorders, heart disease, alcohol and opioid use, high blood pressure, asthma, and other respiratory disorders.

Subjects will be instructed to complete the questionnaires to the best of their ability but will have the option to leave any question(s) blank. In the unlikely event that evidence of physical or psychological disorder is found, with the individual's permission, the information will be shared with his or her primary care physician who can direct care as needed.

PET/MRI Procedure: The U.S. Food and Drug Administration (FDA) recently gave the first regulatory clearance of a hybrid PET/MRI scanner in the U.S. Additionally, FDA considers investigations of MRI software and hardware operating within FDA specific parameters as non-significant risk device studies. All studies will adhere to these FDA approved safety levels for the Siemens system. These safety parameters include static magnetic field, time varying magnetic fields (dB/dt), specific absorption rate (SAR), and acoustic noise levels. Subjects will be informed about minimal risks of routine high magnetic field and non-ionizing RF radiation involved in MR imaging.

Subjects will also be informed about the PET procedure and the minor risks associated with exposure to radiation. Subjects will also be informed about small space within the magnet and noises made by switching gradients. Subjects will be informed that if they feel uncomfortable with the study, they can choose to terminate the study at any time. They will be informed that their refusal to participate in the study or choosing to terminate it at some point will have no effect on care and treatment received by them at MGH now or in future. The subjects will be informed that their personal information will be protected as per the HIPAA guidelines.

Intravenous catheter: An intravenous catheter will be placed for this study. The subject will feel a slight pinprick, similar to a bee sting, and may feel some discomfort and have some bruising or bleeding at the site where the needle goes in. Depending on the length of time the catheter is in place, a bruise may last for a day or so. Rarely an infection may occur at this site. If infection does occur, it will be treated.

Optional arterial line: An intra-arterial catheter will be placed by an individual with anesthesia training (i.e., board-certified anesthesiologist, fully licensed anesthesia resident, a certified registered nurse anesthetist), on the arm opposite to the radio-ligand injection line, for blood draws during the PET study. Local infection, swelling, and redness could occur at the sites of line placement, as well as temporary loss of pulse at the wrist. This area may have a bruise or feel uncomfortable for 2-3 days after the catheter is removed. The risks associated with having blood drawn include: bruising, local discomfort, or infection at the site of the needle puncture. Rarely an infection may occur at this site, and if an infection does occur, it will be treated. Inserting an arterial line (A-line) can hurt more than having a regular IV or having blood drawn with a needle. We will place the A-line under local anesthesia (i.e., lidocaine), which may cause an allergic reaction. Even if we numb the wrist area first, the insertion may still hurt. Once the A-line is in place, it usually does not hurt. About 24 hours after the beginning of the imaging procedures, we will give the subject a phone call to determine whether he or she is experiencing study related issues.

The subject may experience pain, bleeding, swelling or redness at the wrist, short loss of pulse at the wrist if blood flow in the artery is briefly stopped, damage to the artery wall or nearby nerves, or catheter breaking or falling out. There have been reports of decreased blood flow to the hand, which resulted in the need for surgery. This is very rare and has not been reported when catheters have been in place for only a few hours for research. Additionally, the insertion or removal of the A-line might cause temporary dizziness, nausea or fainting. After the anesthesiologist, anesthesia resident, certified nurse anesthetist, or registered nurse removes the

catheter and has held pressure for several minutes, we'll ask the subject to stay for 30 minutes (up to 60 minutes based on clinical opinion of NP or registered nurse) so we can monitor him/her in order to assess the occurrence of any adverse event. Dr. Loggia will file a report on Insight within the timeframe stipulated by the IRB (5 working days/7 days) should any adverse event occur. The subject may have a bruise or feel tenderness for 2-3 days around the area where the catheter was placed. We will instruct the subject to avoid lifting anything heavier than a small bag of flour for a day. We will instruct the subject to call us if bleeding occurs after the subject leaves (rare), and/or if the wrist area is painful or red or swollen. About 24 hours after the beginning of the imaging procedures, we will give the subject a phone call to determine whether he or she is experiencing study related issues.

Radiation exposure: The radiation exposure in this study will be small and there is no evidence that it represents a major health risk. If subjects have participated in other research studies in the past 12 months that have involved radiation exposure, they will be asked to inform the investigators or study staff (by writing initials on the consent form verifying that they have not been exposed to other radiation in the past 12 months). If it is determined that their prior radiation exposure exceeds our current guidelines, they may not be allowed to participate in this study.

We will use [^{11}C]PBR28 produced by the cyclotron/radiochemistry/radiopharmacy facility at the A. A. Martinos Center for Biomedical Imaging. The Martinos Center has studied several hundreds of people with this radioligand and have had no clinically detectable effects or side effects. Given the use of [^{11}C]PBR28 in a small clinical trial, we have obtained an IND from the FDA.

The IV injection will be administered by a licensed nuclear medicine technologist. Should there be an adverse event, Dr. Gilman will be responsible for communicating with the IRB within the stipulated time frame.

Imaging will be stopped should any untoward reaction be observed during the imaging session or if the participant so requests for whatever reason. Some subjects find it unpleasant or feel anxious when confined in the enclosed space of the scanner. If this happens, the study will be aborted. Patients will be required to use earplugs to decrease the noise perceived while in the scanner.

Saliva Genotyping: Although we cannot predict how genetic information will be used in the future, there are many safeguards in place and we do not think that there will be further risks to patients' privacy and confidentiality by sharing such information with these banks.

In addition, steps will be taken to protect confidentiality of genetic data as outlined:

1. All MGH study staff are trained to make confidentiality the first priority.
2. No genetic research data will be entered into the medical record.
3. The results of the genetic analyses will not be shared with participants, their family members or unauthorized third parties.
4. Genetic data are encoded using coded identifiers. These codes, rather than personal identifiers, are used in any analytic datasets. The code key linking coded identifiers to

personal identifiers are kept in an access-restricted, password protected electronic file and are not shared with the genetics laboratories.
5. Consent forms are stored in locked cabinets apart from demographic and diagnostic data.
6. Samples and genetic data stored in the laboratory will be identified only by the code numbers and laboratory personnel will not have access to personal identifiers.
7. The most serious risk would be identification of individuals in the publicly shared database. To prevent this, computerized data files provided to other investigators will not include any of the HIPPA-defined personal identifiers. Published material will not identify subjects.

EPIDIOLEX (CBD): CBD is an FDA-approved medication used to treat epilepsy. According to the FDA briefing document on Epidiolex, dated 04/19/2018, treatment-emergent AEs in controlled trials for Lennox-Gastaut and Dravet syndromes included decreased appetite, diarrhea, irritability, somnolence, fatigue, aggression, pneumonia, rash, and hepatic symptoms, and in a very small number of patients, an increase in suicidal thoughts. Of note, AE related to hepatic function were likely due to the interaction between CBD and anti-epileptic drugs; prescription of an anti-epileptic drug is an exclusionary criterion for this proposed study. Further, CBD may produce pharmacokinetic interaction effects when taken with opioids[91,92]. Any subjects who at baseline had elevated AST/ALT levels but still met the eligibility criteria for the study will undergo a follow-up liver function tests at 2 weeks. In addition, we will perform a liver function test in subjects who at any time point during the study develop clinical signs or symptoms suggestive of hepatic dysfunction.

Given the use of EPIDIOLEX in a small clinical trial, we have obtained an IND from the FDA.

Questionnaires: Minimal risks associated with completing questionnaires are subject fatigue and the possibility of minor psychological distress associated with answering sensitive questions regarding psychological functioning. Subjects will be instructed to complete the questionnaires to the best of their ability, but will have the option to leave any question(s) blank. In the unlikely event that evidence of physical or psychological disorder is found, with the individual's permission, the information will be shared with his or her primary care physician who can direct care as needed.

Confidentiality: As detailed, the investigators are quite careful regarding the protection of confidentiality, and multiple procedures are in place to reduce the likelihood of a breach of confidentiality. However, there is a small risk that information about subjects could become known to people outside of this study, and this risk is identified in the informed consent form.

The key investigators will meet quarterly to discuss any potential adverse event and side effects. We will involve the MGH Human Research Committee and Radiation Safety Committee if any additional potential risks arise. Adverse events and unanticipated problems involving risks to subjects or others will be reported to the PHRC in accordance with PHRC adverse event and unanticipated problems reporting guidelines, as well as FDA when appropriate.

8. Benefits

Potential benefits to participating individuals:
It is unlikely that individual subjects will benefit from taking part in this study. While this study is powered to possibly observe a statistically significant reduction in pain due to CBD, it is unclear whether the effect will be clinically meaningful.

Potential benefits to society:
Findings from these studies will help advance our understanding of the pathophysiology of pain disorders. In particular, this project will assess the role of microglia in the establishment and/or maintenance of chronic pain, and how this may be affected by CBD. As such, we envision that in the future the information obtained from the proposed research will enhance the diagnosis and management of a variety of chronic pain conditions.

9. Statistical Analysis

Data Acquisition

PET. [^{11}C]PBR28 binding will be measured on Siemens Biograph mMR, a whole-body 3T PET/MRI scanner, or a Siemens Tim Trio with a head-only PET insert. An intravenous catheter will be then placed in participants' antecubital vein to inject the radioligand. [^{11}C]PBR28 will be synthesized in-house using a procedure modified from the literature.[93] Up to 15 mCi of [^{11}C]PBR28 will be injected intravenously as a slow bolus over a 30s period.[94] In the first 90 minutes, PET/MRI data will be collected from the brain. Between 90- and 110-minutes post-injection the field of view may be repositioned to the thoracic and upper lumbar spine, so that spinal cord data can be acquired. Of note, while our primary PET imaging outcomes are brain-related (thalamic signal for "pain-related neuroinflammation" and pgACC/aMCC signal for "depression-related neuroinflammation"), we will also evaluate, in exploratory analyses in a subset of our participants (depending on the participant's availability or other factors), the signal from the most caudal segments of the spinal cord, as this regions also demonstrated neuroinflammation in our prior study of patients with lumbar radiculopathy.[95]

For the brain data, motion correction will be applied using MRI-derived motion estimates for each individual frame.[96] For the spinal cord data, frame-by-frame motion correction will be implemented using the Spinal Cord Toolbox.[97] The head attenuation map (μ-map) will be obtained using a recently implemented MR-based attenuation correction method.[98] The μ-map for the spinal data will be collected using the Dixon-VIBE sequence and using in-house developed software to additionally segment the bone.[95]

[^{11}C]PBR28 brain uptake will be measured voxelwise as Standardized Uptake Values ratio (SUVR), Volume of distribution (V_T), V_T ratio (DVR) and/or other commonly adopted metrics. In humans, SUV ratio (SUVR) estimation of [^{11}C]PBR28 binding has been used to reliably distinguish healthy volunteers from patients with Alzheimer's disease[99] as well in our own studies with chronic low back pain[36], and amyotrophic lateral sclerosis[100]. Additionally, human and animal studies indicate SUV/SUVR estimates are less variable compared to blood-derived methods[99,101]. These results suggest that SUVR estimation of [^{11}C]PBR28 binding could be a viable surrogate for arterial blood methods, and perhaps more sensitive to between group

differences. However, V_T and other more quantitative metrics will be computed as well. As an intensity normalization factor we will use the whole-brain signal or a localized pseudoreference region (e.g., the occipital cortex) for the brain data, and the lowest 1-2 spinal cord segments present in the field of view for most/all of our participants (e.g., T11-L1) for the spinal signal. Brain SUVR will be spatially normalized to the Montreal Neurological Institute (MNI) space using nonlinear registration (FNIRT, from the FSL suite; www.fmrib.ox.ac.uk/fsl [102]). Spatially-normalized SUVR images will be then spatially smoothed (full width at half maximum=8mm) to improve signal-to-noise ratio. Spinal cord SUVR will be normalized to MNI-Poly-AMU template[103] using the Spinal Cord Toolbox.

MRI. During the acquisition of brain PET data, several runs of BOLD fMRI data will be collected using whole brain T2*-weighted gradient echo BOLD EPI pulse sequence (TR/TE =2sec/30ms, flip angle=90°, voxel size=3.1x3.1x3mm, number of slices=37), for the purposes of evaluating striatal function using the MID task, performing MR-based motion-correction of PET data[136], as well as for additional exploratory analyses. In addition, a high resolution structural volume (e.g., multi-echo MPRAGE pulse; TR/TE1/TE2/TE3/T4=2530/1.64/3.5/5.36/7.22 ms, flip angle=7°, voxel size=1mm isotropic) will be collected for anatomical localization as well as attenuation correction.[155] For the spinal cord data, axial and coronal T1 (e.g., TR/TE=0.565s/13 ms; flip angle=120°; slice thickness=2mm; number of slices – 14) and T2 (e.g., TR/TE=3.38s/109 ms; flip angle=150°; slice thickness=2mm; # slices – 30) weighted images will be collected for anatomical localization and ROI definition purposes.[7]

Data Analyses

A generalized linear mixed-effects model (GLMM) will be used to quantify the association between thalamic [^{11}C]PBR28 PET signal, treatment assignment at randomization (CBD, placebo; intent-to-treat) and time (baseline, week 4). The unadjusted model will only regress PET signal onto treatment and time indicators as well as their interaction. An adjusted model will also be constructed that independently accounts for potentially confounding variables (e.g., age, depression severity, sex). Data dependencies will be accounted for using either random intercept or line (intercept and slope) parametrizations. To fully specify our GLMMs, we will initially consider the Gaussian family (identity link). Since PET signal is a strictly positive quantity, we will also consider the binomial family with the cumulative logit link. A residual analysis will be performed to assess modeling assumptions and guide our choice in determining the final model.

Our primary object of inference will be the treatment by time interaction which reflects the absolute difference in the rates of change in PET signal between treatment groups (Gaussian family) or the relative change in odds of having a higher PET signal between treatment groups (binomial family) when holding all other covariates fixed. Linear combinations of parameter estimates will also be computed to summarize secondary objects of interest including cross-sectional treatment comparisons (baseline: CBD vs. control; week 4: CBD vs. control), and treatment-specific temporal comparisons (CBD: week 4 vs. baseline; control: week 4 vs. baseline).

This analysis plan will be repeated using a per-protocol definition of treatment in which we omit subjects who did not reliably take the study medication. Additional secondary and exploratory

analyses will follow a similar analysis plan as described above. For these non-primary analyses, we will account for multiple comparisons by computing both unadjusted p-values and false discovery rate adjusted p-values.

Genotyping:
GWAS Genotyping: The Broad Institute will perform genotyping (array-based) of subject DNA samples and subsequent in-depth analysis of the data, which will allow us to detect alterations in the genome including point mutations, small insertions and deletions, chromosomal copy number alterations, and translocations. These experiments are intended to help identify candidate genes involved in the physiopathology of neurological and psychological diseases. The molecular information generated from these samples will not be returned to subjects at any time.

Data from this study may result in communications in journals or at scientific meetings. Subjects will not be identified in those communications. To facilitate research, the genetic information generated may upon publication be deposited in protected databases (such as dbGAP) available only to bona fide researchers with specific scientific questions who promise to not try to identify individuals. The data will be sent to these banks in a coded manner and again will not contain any traditionally used identifier such as name, address, phone number, or social security number. Although we cannot predict how genetic information will be used in the future, there are many safeguards in place and we do not think that there will be further risks to patients' privacy and confidentiality by sharing such information with these banks.

The Broad Institute will not be involved in subject ascertainment. Prior to transfer of bio-specimen aliquots to the Broad Institute, samples will be re-encoded at the collaborators institutions. No identifying patient information will be shared with Broad scientists at any time. Some limited clinical data will be obtained from collaborators. Again, all subject identifying information will remain with the collaborators and only de-identified clinical data will be shared with the Broad Institute.

Consideration of sex as a biological variable:
In addition to the aforementioned analyses, the effect of sex will be evaluated using ANOVAs, because animal research suggests the presence of a possible sexual dimorphism in the role of glia in pain (as pain hypersensitivity may be microglial-dependent only in males[104]). The effect of menstrual cycle status will also be evaluated by comparing women in early follicular (day 2-7 after onset of menses) and midluteal (day 20-25 after onset of menses), based on self-report[105].

Effect of coronavirus on neuroinflammation:
Finally, in addition to the aforementioned analyses, the effect of prior exposure to coronavirus (as detected by the presence of antibodies to SARS-CoV-2) will be evaluated in both ROI and voxelwise analyses, in exploratory analyses. Identifying participants who are positive to the antibodies might allow us to test the exploratory hypothesis that prior exposure to the coronavirus can lead to neuroinflammation even without having experienced overt acute COVID-19 symptoms.

Power Analysis:

Primary Aim: Using a linear mixed-effects model, we estimate the power to detect a temporal (week 4 – baseline) rate of change in thalamic [^{11}C]PBR28 PET signal between CBD and control subjects when recruiting 40 subjects per treatment group. We assume: (1) the standard deviations of the [^{11}C]PBR28 PET signal measures are 0.05,[106] (2) the correlation between repeated measurements ranges between 0.3 to 0.8, and (3) the attrition rate ranges between 5 and 15%, and the type-I error is 0.05. If the within subject correlation is 0.3, and the attrition rate for both treatment groups is 10%, then we will have 80%, and 90%, power to detect mean differences in [^{11}C]PBR28 PET signal measures of at least 0.039 and 0.045, respectively.

10. Monitoring and Quality Assurance

There will be a DSMB for this study (see attached DSMB Charter). The proposed study will be monitored for safety, with monthly staff meetings reviewing adverse events and treatment outcomes and directly reporting any adverse events. The PI will also routinely monitor and assure the validity and integrity of collected data, adherence to the IRB-approved protocol, and recordkeeping. The trained staff members who carry out the procedures will also carefully monitor the study throughout its duration. The team will evaluate the progress of the study, verify that the rights and well-being of the subjects are protected, verify that the reported study data are accurate, complete and verifiable from source documents, and the conduct of the study is in compliance with the approved protocol and amendments. Outcome monitoring and adverse events will all be reported through appropriate channels of the Human Studies Committee as well to the FDA when appropriate.

If a patient develops clinical signs or symptoms suggestive of hepatic dysfunction (e.g., unexplained nausea, vomiting, right upper quadrant abdominal pain, fatigue, anorexia, or jaundice and/or dark urine), we will promptly measure serum transaminases and total bilirubin and interrupt or discontinue treatment with EPIDIOLEX or placebo, as appropriate. We will discontinue EPIDIOLEX or placebo in any patients with elevations of transaminase levels greater than 3 times the ULN and bilirubin levels greater than 2 times the ULN. Our physician monitoring group (Drs. Mao, Zhang, Schnitzer, and Evins) will consider stopping the study if back pain becomes significantly worse in 3 or more patients. If serum liver enzyme concentrations are significantly elevated (with elevations of transaminase levels greater than 3 times the ULN and bilirubin levels greater than 2 times the ULN) in 2 or more patients, which will be considered serious adverse events, the study will be stopped. In addition, if two or more patients experience any serious adverse event, the study will be stopped.

The Siemens PET/MRI scanners have a built-in self-monitoring system that automatically shuts off if parameters exceed safe levels. For backup protection NMR technicians constantly monitor the subjects' physiological signs and the quality of the raw data.

Quality assurance of the scanner's performance is obtained by a daily quality assurance protocol. More extensive quality assurance protocols are performed monthly under the commercial service contract with Siemens Medical Systems. The daily quality assurance protocol consists of an image Signal-to-Noise measurement in a phantom and a stability run which checks the image-to-image variation in image intensity over 600 images using a standard echoplanar imaging sequence with a head-sized phantom. The images are analyzed by the technologist to provide

data on SNR (as an absolute, unitless number) and stability expressed as the peak-to-peak variation in the mean of a 15x15 pixel region of interest (ROI) in the center of the phantom expressed as a percentage of the mean of the ROI. Runs are performed at each of 3 TR values (300ms, 800ms, 1300ms). The time course of the means is also reviewed to check for periodicities (the TR values are chosen so as not to be multiples of one another). If the peak-to-peak variation is greater than 0.5% of the mean value, the Siemens Medical System service engineer is called. In addition to these daily quality assurance tests, the Siemens Medical System service engineer performs quality assurance tests once a month. These tests include a SNR test, a small sample stability test, a gradient stability test, a gradient eddy current test, a shim test, an image uniformity test, and an RF stability test.

11. Privacy and Confidentiality

- ☒ Study procedures will be conducted in a private setting
- ☒ Only data and/or specimens necessary for the conduct of the study will be collected
- ☒ Data collected (paper and/or electronic) will be maintained in a secure location with appropriate protections such as password protection, encryption, physical security measures (locked files/areas)
- ☒ Specimens collected will be maintained in a secure location with appropriate protections (e.g. locked storage spaces, laboratory areas)
- ☒ Data and specimens will only be shared with individuals who are members of the IRB-approved research team or approved for sharing as described in this IRB protocol
- ☒ Data and/or specimens requiring transportation from one location or electronic space to another will be transported only in a secure manner (e.g. encrypted files, password protection, using chain-of-custody procedures, etc.)
- ☒ All electronic communication with participants will comply with Mass General Brigham secure communication policies
- ☒ Identifiers will be coded or removed as soon as feasible and access to files linking identifiers with coded data or specimens will be limited to the minimal necessary members of the research team required to conduct the research
- ☒ All staff are trained on and will follow the Mass General Brigham policies and procedures for maintaining appropriate confidentiality of research data and specimens
- ☒ The PI will ensure that all staff implement and follow any Research Information Service Office (RISO) requirements for this research
- ☐ Additional privacy and/or confidentiality protections

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