Drug-gene-nutraceutical interactions of cannabidiol and tacrolimus

Michael T. Eadon, MD

Department of Medicine

Phone # 317-274-2502

950 W. Walnut St.

R2 Suite 202

Indianapolis, IN 46202

Indiana University School of Medicine

Sub-Investigator: Zeruesenay Desta, PhD:
Professor of Medicine
Department of Medicine
Indiana University School of Medicine

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Abbreviations

This page is optional. The list below includes some common abbreviations. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
CFR	Code of Federal Regulations
CKD	Chronic Kidney Disease
COC	Certificate of Confidentiality
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PI	Principal Investigator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOA	Schedule of Activities
UP	Unanticipated Problem
US	United States

1.0 Background & Rationale

One in seven Americans use cannabidiol (CBD)-based or cannabis-derived products[1-3]. Since the Agriculture Improvement Act of 2018[4], the availability of commercial CBD nutraceuticals has exploded with ingestible products available as pills, gummy bears, or oil which can be mixed with seltzer[5]. Cannabidiol is also an FDA approved therapy (Epidiolex) for rare forms of epilepsy and is now under investigation for chronic pain therapy[6]. However, the broad and unsupervised use of bioactive over-the-counter CBD products creates enormous potential for pharmacokinetic and pharmacodynamic interactions. This is important for all patients taking CBD, but especially in vulnerable populations, like immunosuppressed organ transplant recipients.

Cannabidiol inhibits the metabolism of drugs by CYP3A4[1, 7], the most abundant and arguably most clinically important hepatic drug metabolizing enzyme. CBD also inhibits CYP3A5[7], a similar enzyme that is more frequently expressed in African Americans (84%) than those with Asian (45%) or European (16%) ancestry[8, 9]. This causes clinically relevant drug-drug interactions (DDIs) with CYP3A substrates[10, 11]. Case reports have now implicated a DDI between CBD and the CYP3A substrate tacrolimus[12, 13]. Because CYP3A5 activity is critical to the metabolism of tacrolimus, tacrolimus dosing is adjusted for patients with altered CYP3A5 activity due to race or CYP3A5 genotype[8]. *In vitro* evidence suggests that CBD is a >5-fold more potent inhibitor of CYP3A5 than CYP3A4[7]. Thus, *we hypothesize that CBD will cause a drug-drug interaction requiring a much larger dose reduction of tacrolimus in CYP3A5 expressors than non-expressors*. We will test this hypothesis in a series of pharmacokinetic studies in CYP3A5 expressors and non-expressors using a primary outcome of tacrolimus $AUC_{0\text{-Infinity}}$ ratio before and after CBD exposure. The results will be particularly important for African Americans because they more frequently have CYP3A5 expressor genotypes. If the hypothesis is correct, it would demonstrate a clinically important nutraceutical-gene-drug interaction.

The overall goal of this study is to assess the safety of co-administration of cannabidiol with tacrolimus. CBD has the potential to immunosuppress those taking it, independent of tacrolimus concentration. For example, CBD has been shown to induce T-regulatory lymphocytes in animal models[14], and treatment with CBD increases the risk of infections[15, 16]. Thus, we will complement the pharmacokinetic investigations by examining CBD's relevant pharmacodynamic effects in the immune system with and without tacrolimus. We will assess sensitive phenotypes of immune cell stimulation, distribution, and expression signatures derived from single cell sequencing. The information gleaned from these experiments is important even if the null hypothesis is supported. If no pharmacokinetic or pharmacodynamic interactions are identified, these data provide reassurance for clinicians and patients that CBD may be safe to use. However, if our hypotheses are supported, clinicians and patients can make informed decisions to avoid CBD entirely or to appropriately adjust doses of tacrolimus and CBD. We will use the following specific aims to test our hypothesis:

Aim 1: Determine whether a CYP3A5 genotype-dependent interaction exists for cannabidiol and tacrolimus. To address aim 1, we will conduct two nearly identical pharmacokinetic studies run in parallel, one with healthy volunteers and one with subjects with chronic kidney disease (CKD). The two studies are analyzed separately and are non-contingent. Each study is a pharmacokinetic cross-over study in human CYP3A5 expressors and non-expressors with and without chronic kidney disease (CKD). Transplant recipients must be excluded ethically, but inclusion of volunteers with and without CKD will facilitate extrapolation of

results to transplant recipients regardless of renal function. Pharmacokinetic parameters will be modeled for studies of single dose tacrolimus, single dose CBD, and steady state CBD combined with single dose tacrolimus.

Aim 2: Assess the changes in lymphocyte distribution and cell expression signature caused by cannabidiol with and without concomitant tacrolimus. We will isolate lymphocytes from subjects before CBD exposure and after reaching CBD steady state. Lymphocytes isolated at baseline and lymphocytes exposed to stimulation with and without tacrolimus will undergo single cell sequencing. Alterations in the distribution and cell expression signatures of lymphocyte sub-types will be determined. The relevance of genes of interest will be interrogated using siRNA knockdown, flow cytometry, and Luminex soluble mediator assays.

The proposed studies will comprehensively define the nutraceutical-gene-drug pharmacokinetic interactions of CBD and CYP3A5 as well as immunologic pharmacodynamic interactions of CBD and tacrolimus. It is expected that this work will help practitioners advise their patients, including those taking tacrolimus, whether it is safe to take cannabidiol.

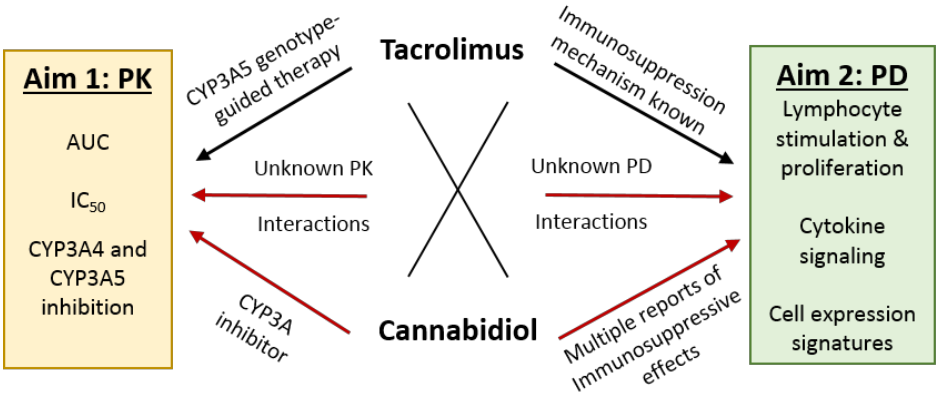


Figure 1: Overview of the experimental design. Aim 1 seeks to understand the pharmacokinetic interactions between cannabidiol and tacrolimus in a series of clinical PK studies. The primary outcome is tacrolimus $AUC_{0\text{-Infinity}}$ ratio before and after reaching steady state CBD exposure in CYP3A5 expressors and non-expressors. Secondary outcomes include other PK parameters of tacrolimus and CBD $AUC_{0\text{-Infinity}}$ between CYP3A5 genotypes in a single dose study. Aim 2 determines the pharmacodynamic interactions between cannabidiol and tacrolimus related to the immune system.

Background:

Tacrolimus is a cornerstone of immunosuppression in transplant recipients: Approximately 90% of all recipients of kidney, heart, pancreas, and lung allografts are prescribed tacrolimus as an immunosuppressant to prevent rejection and protect the function of their allograft[17-20]. Tacrolimus inhibits calcineurin which reduces cytokine expression, including interleukin-2 expression, in T lymphocytes[21]. As a result, calcineurin inhibitors (CNIs) like tacrolimus suppress activation of both B and T lymphocytes. Tacrolimus has a narrow therapeutic window and therapeutic dose monitoring is important to maintain target concentrations and avoid adverse effects such as acute kidney injury, hypertension, or hyperkalemia. Tacrolimus is metabolized by both the CYP3A4 and CYP3A5 enzymes. As a result, tacrolimus is subject to multiple important drug-drug interactions (DDIs) with CYP3A inhibitors like diltiazem or ketoconazole[22]. These DDIs place patients at risk for toxic drug levels of tacrolimus.

CYP3A5 enzyme activity is a crucial predictor of the required tacrolimus dose: Not everyone has CYP3A5 activity. CYP3A5 is a polymorphic enzyme with expression found in 84% of those with African ancestry, 45% with east Asian ancestry, and 16% with European ancestry[9]. Expression is determined by genotype as individuals with one or more *1 (wild type) allele express CYP3A5, but individuals who have two *3, *6, or *7 alleles lack expression[9]. On balance, CYP3A5 expressors require a 50% higher dose of tacrolimus to reach the same target drug concentration[8]. Lack of CYP3A4 metabolism also contribute to tacrolimus pharmacokinetics (PK), but is rare as the *22/*22 genotype frequency is only 0.2%.

Cannabidiol (CBD) is subject to multiple drug-drug interactions: 15.3% of Americans have consumed CBD-based or cannabis-derived products in the last year[2, 3]. Our understanding of CBD pharmacokinetics has evolved since the US Food and Drug Administration (FDA) approved Epidiolex in 2018 for the treatment of rare seizure disorders. However, this understanding of CBD is far from complete. We know CBD can inhibit or induce multiple CYP450 enzymes (CYP1A2, CYP2B6, CYP2C9, and CYP2C19). CBD itself is metabolized by both CYP2C19 and CYP3A4 and CBD can be inhibited or induced by drugs that affect these enzymes[23].

In contrast, we do not know whether CBD ***causes*** clinically significant inhibition of other CYP3A substrates. *In vitro* data suggests CBD does inhibit both CYP3A4 and CYP3A5; however, supportive *in vivo* data is absent. The FDA drug label suggests no *in vivo* interaction exists with CYP3A4, finding that co-administration of CBD with midazolam (a sensitive CYP3A4 substrate) did not result in changes in plasma concentrations of midazolam compared to midazolam alone. The FDA new drug application (NDA) for Epidiolex did not specifically test the relevance of CYP3A5 activity *in vitro* or *in vivo*, either by drug-inhibition studies or pharmacogenomic-stratified analyses. *In vitro* evidence suggests that CBD is a 5-fold more potent inhibitor of CYP3A5 (IC50 = 1.65 μM) than CYP3A4 (IC50 = 11.7 μM)[7]. Steady state circulating concentrations of CBD often range from 1 μM to up to 10 μM. **Thus, it is possible that circulating plasma concentrations of cannabidiol did not reach effective levels to elicit a CYP3A4 DDI with midazolam in the FDA NDA studies, but the typical circulating concentrations of CBD would be effective to potently inhibit CYP3A5, causing a DDI with tacrolimus in individuals who express CYP3A5. We hypothesize that this DDI would be greater in CYP3A5 expressors than CYP3A5 non-expressors because inhibition will have minimal effect in an individual who lacks the enzyme.** CYP3A5 expressors may be at higher risk for tacrolimus toxicity when taking CBD, while CYP3A5 non-expressors may not have pharmacokinetic alterations with CBD. The clinical pharmacology review of Epidiolex on drugs@FDA suggested that a current PBPK model incorporating induction, time-dependent inhibition, and competitive inhibition of CYP3A4 predicted a strong interaction with its substrate (for both midazolam and simvastatin).

Cannabidiol has immunomodulatory effects: A known side effect of treatment with CBD is an increase in the risk of infections[15, 16]. Multiple mechanisms have been postulated as to the immunosuppressive effects of CBD. In animal models, CBD has been shown to enhance interleukin-2 production and induce T regulatory lymphocytes[14]. Alternatively, CBD was shown *in vitro* to reduce inflammation by binding to the equilibrative nucleoside transporter 1 (ENT1), both preventing adenosine uptake and enhancing signaling[24]. Thus, CBD has the potential to further immunosuppress transplant recipients, independent of tacrolimus concentration. How the immunomodulatory effects of CBD interact with those of tacrolimus is unknown and of great interest since the mechanism of action of tacrolimus is to suppress IL-2 and other cytokine production.

Guidance is lacking on the safety of cannabidiol and tacrolimus co-administration: A knowledge gap exists because we do not know 1) whether cannabidiol will interact with tacrolimus, 2) whether CYP3A5 expressors may have a more potent DDI between CBD and tacrolimus than those that lack CYP3A5, or 3) whether the metabolism of CBD itself is CYP3A5 genotype-dependent. Case reports of CBD[12] and cannabis-induced[25] tacrolimus toxicity now exist. Further, a single case series of 5 kidney transplant patients taking artisanal CBD for pain along with their tacrolimus revealed significant inter-individual variability with 2 out of 5 individuals developing elevated tacrolimus concentrations, two developing subtherapeutic concentrations (perhaps consistent with induction), and 1 subject with an unchanged concentration[13]. The unclear immunomodulatory effects of CBD further complicate its use in immunosuppressed patients. Without additional evidence, the natural inclination of physicians will be to first do no harm and instruct transplant recipients to avoid cannabidiol. However, this recommendation would be based on a small set of case reports, not a controlled clinical trial.

Nonetheless, the use of over-the-counter CBD is now commonplace. Although the nutraceutical form of CBD has no FDA-approved medical indication, the indications for which Americans use CBD products include chronic pain, anxiety, arthritis, and headaches[1, 3]. The use of cannabidiol is under investigation as a prescriptive therapy for chronic pain as well[6]. Aside from acetaminophen, there are limited options available for pharmacologic control of chronic pain in transplant recipients. The adverse effects of non-steroidal anti-inflammatories (acute kidney injury, coronary event risk) preclude their use in most solid organ recipients[26]. Clinicians and patients may be more reticent to prescribe or take opioids as our understanding of the opioid crisis has evolved[27-29]. As a result, understanding the safety profile of CBD is important if this drug is ever to serve as an adjunctive pain therapy.

A relevant patient population: It is not ethical to recruit patients with current allografts to this study as it would require temporarily holding their tacrolimus. However, multiple precision medicine initiatives have been implemented at Indiana University from which we can recruit a relevant population. Our research team has been instrumental in implementing pharmacogenomics at the university and are uniquely qualified to pull from these patient pools to identify individuals with deep clinical phenotyping and genotypes of interest. As part of the INGENIOUS trial[30], 1315 individuals in the general population of our health system underwent broad pharmaco-genotyping panels to prevent adverse events[31]. These panels included CYP3A5 and CYP3A4 genotype, as well as CYP2C19, CYP2C9, CYP2B6, and CYP2D6 (with copy number variants). A second study examined the importance of the same metabolism genes in 580 individuals with chronic kidney disease (CKD)[32, 33]. Most of the 1895 individuals in both studies have agreed to be re-contacted for additional studies. Thus, we are able to readily identify 57 subjects with appropriate genotypes for this study without the expense of broad genotype screening. Furthermore, the breadth of known pharmacogenes enables advanced PK modeling that can incorporate additional genotype covariates for individuals we recruit to this study. Given the number of enzymes CBD is predicted to interact with, this genetic information will be of great utility. Our enriched population of individuals with CKD allows us to recruit subjects with and without CKD and model the effects of reduced renal function on any DDIs. The inclusion of subjects with CKD increases the relevance of our study to transplant recipients, since many solid organ recipients are at risk for CKD from chronic renal calcineurin inhibitor (CNI) toxicity.

Both healthy volunteers and subjects with CKD will be recruited. The reasons to include CKD include:

A. Kidney disease impacts drug exposure (PK) and response (PD). Non-renal clearance via metabolism and transport are both altered in kidney disease. These changes are related to accumulated organic anions, hormone mediated regulation, and alterations in renal mass.

B. The expected results of the PK study in CKD subjects are that the trial will inform whether there is a cannabidiol-tacrolimus-CYP3A5 interaction in subjects with CKD. We expect to identify a similar direction of effect (expected increased tacrolimus AUC) after cannabidiol exposure in both healthy volunteers and subjects with CKD. A difference in the magnitude of effect between healthy volunteers and subjects with CKD is difficult to predict because CYP3A quantity decreases in CKD (PMID: 33270249). Both tacrolimus and cannabidiol are metabolized by CYP3A, so CKD status would be predicted to cause higher exposure of both tacrolimus and cannabidiol. However, the difference in the relative change in AUC between those who express CYP3A5 and those who do not is less predictable in subjects with CKD and a potential rationale for inclusion of these subjects.

C. CKD subjects are an underrepresented population in clinical trials and drug dosing data is often not available. Transplant recipients frequently have CKD. CKD is present in over 90% of kidney transplant recipients (PMID: 14974949) and 60% of heart transplant recipients (PMID: 20305315). It is not ethical to perform this study in transplant recipients because of the risks related to altering the immunosuppression regimen; however, running the parallel study in subjects with CKD (and other related comorbidities) allows us to better extrapolate the study's results to the population of interest. Ultimately, we are performing this study to determine the safety of taking cannabidiol in transplant recipients. If the study is done in healthy volunteers alone, questions may remain in the minds of transplant physicians about the applicability of our results to their population.

2.0 Objective(s)

2.1 Primary Objective

Determine whether a CYP3A5 genotype-dependent interaction exists for cannabidiol and tacrolimus. To address aim 1, we will conduct two nearly identical pharmacokinetic studies run in parallel, one with healthy volunteers and one with subjects with chronic kidney disease (CKD). The two studies are analyzed separately and are non-contingent. Each study is a pharmacokinetic cross-over study in human CYP3A5 expressors and non-expressors with and without chronic kidney disease (CKD). Pharmacokinetic parameters will be modeled for studies of single dose tacrolimus, single dose CBD, and steady state CBD combined with single dose tacrolimus.

2.2 Secondary Objective

Assess the changes in lymphocyte distribution and cell expression signature caused by cannabidiol with and without concomitant tacrolimus. We will isolate lymphocytes from subjects before CBD exposure and after reaching CBD steady state. Lymphocytes isolated at baseline and lymphocytes exposed to stimulation with and without tacrolimus will undergo single cell sequencing. Alterations in the distribution and cell expression signatures of lymphocyte sub-types will be determined. The relevance of genes of interest will be interrogated using siRNA knockdown, flow cytometry, and Luminex soluble mediator assays.

2.3 Tertiary/Exploratory/Correlative Objectives

none

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures

The primary outcome is the $AUC_{0\text{-Infinity}}$ ratio of tacrolimus with cannabidiol divided by tacrolimus alone between CYP3A5 expressors and non-expressors. In other words, we will assess the tacrolimus interaction magnitude: tacrolimus (+CBD):tacrolimus(-CBD) $AUC_{0\text{-}\infty}$ ratio. We hypothesize that individuals with CYP3A5 expression (at least one *1 allele) will have a greater interaction magnitude (treatment:control AUC ratio) than non-expressors (*3/*3, *3/*6, *3/*7, *6/*7, etc.). Tacrolimus AUC will be measured through HPLC-MS of the serum at various time points following administration. Blood samples (~10 mL) will be drawn at 20, 40, and 60 minutes and 2, 4, 6, 12, 24, 48, and 192 hours after tacrolimus administration.

Quantification of drugs and metabolites in plasma and urine samples: We will use a highly sensitive UPLC coupled with QTRAP 6500+ MS/MS to quantify tacrolimus, cannabidiol, and their plasma and urinary metabolites that have been reported in the literature to gain insight into the specific pathways affected by CBD. Dr. Desta is the director of the Metabolism and Analytical Core at IUSM and will oversee these assays. Its unique hybrid system of a linear ion trap and triple quad allow for simultaneous quantitation and structural verification of analytes in a single experiment.

3.2 Secondary Outcome Measures

We hypothesize that cannabidiol will induce T regulatory lymphocytes (Tregs) and reduce overall lymphocyte stimulation. In addition, we expect this effect to be additive with the addition of tacrolimus. This hypothesis will be tested using six single-cell sequencing conditions (Table 2) and various cell culture techniques. Measured outcomes will include the degree of stimulation, lymphocyte population distribution, and gene expression.

Table: Six total conditions to undergo scRNA-seq

Condition	Blood Draw Date	Description
1	Day 8	Baseline lymphocyte prep: No stimulation, no CBD, no tacrolimus
2	Day 8	Baseline lymphocyte prep: + stimulation, no CBD, no tacrolimus
3	Day 8	Baseline lymphocyte prep: + stimulation, no CBD, + tacrolimus
4	Day 24	Steady State CBD lymphocyte prep: No stimulation, + CBD, no tacrolimus
5	Day 24	Steady State CBD lymphocyte prep: + stimulation, + CBD, no tacrolimus
6	Day 24	Steady State CBD lymphocyte prep: + stimulation, + CBD, + tacrolimus

Randomly selected subjects (N=6 per year) recruited and enrolled in Aim 1 will have lymphocytes isolated prior to the first dose of CBD (Day 8) and upon returning for the CBD steady state Period 3 (Day 24). Lymphocytes are isolated using a Ficoll-gradient extraction, followed by 24 hours of lectin treatment to remove monocytes, and 48 hours of stimulation with antibodies to CD3/CD28. The degree of proliferation is measured using the Cell-Titer Glo (Promega) cell viability assay in stimulated and unstimulated lymphocytes. Four of six lymphocyte conditions (Conditions 1, 2, 4, and 5) will compare the effects of cannabidiol on unstimulated and stimulated lymphocytes after scRNA-seq. In the steady-state CBD condition 5, CBD (10 μM) will be added to media during stimulation to maintain the CBD concentration that may be lost or diluted during the lymphocyte isolation process. All media will contain albumin. We will conduct methods as previously described in our prior scRNA-seq study involving human lymphocytes[43]. Briefly, scRNA-seq will be performed by the Center for Molecular Genetics sequencing core at Indiana University School of Medicine (IUSM) using the Chromium single cell system v3 (10X Genomics) and the NovaSeq 6000 Illumina sequencer. CellRanger 2.1.0 will be utilized to process the generated sequencing data and align it to the human reference genome GRCh38 using the RNAseq aligner STAR. Quality control metrics are conducted to determine cell inclusion for final feature selection, dimension reduction, and integration using the Seurat v3 anchoring method. Cells will be clustered on a UMAP and clusters identified with lymphocyte marker genes.

We expect to identify CBD-induced changes in distribution and transcriptomic signatures in unstimulated and stimulated lymphocyte sub-populations. We expect that these differences will be retained in the CKD population; however, anergy associated with renal disease may impact expression patterns. We expect to observe a higher degree of proliferation in the baseline condition that undergoes stimulation than the sample obtained after CBD treatment. Coinciding with this, we hypothesize CBD will suppress immunity and suppress lymphocyte proliferation in response to stimulation. For example, Tregs may be upregulated after CBD treatment as a mechanism to suppress the immune system. From these data, additional ex vivo targeted studies with Luminex, flow cytometry, and siRNA knockdown will be performed to understand and validate these changes in specific lymphocyte populations.

3.3 Tertiary/Exploratory/Correlative Outcome Measures

none

4.0 Eligibility Criteria

4.1 Inclusion Criteria

Inclusion criteria:

- Age 18-75
- Are judged healthy enough to participate as determined by and decided from a pre-enrollment screening session that includes medical history and laboratory tests such as blood and urine tests, and electrocardiography (EKG).
- Agree to refrain from taking any prescription medications, over-the-counter medications, and herbal, dietary, and alternative supplements that may interact with the metabolism of the study drugs at least 2 weeks prior to the start of the study and until study completion.
- Are willing to commit the time requested for this study

- Are willing to refrain from smoking or use of tobacco or marijuana for at least two weeks prior to and until the completion of the study (the entire study lasts for approximately 26 days).

Additional Criteria for the Healthy volunteer study:

- Have a GFR above 60 ml/min/1.73m^2 with one of the following: 1) proteinuria less than 100 mg/dl on urinalysis, or 2) less than 0.3 grams by urine protein to creatinine ratio or 24 hour urine collection

Additional Criteria for the CKD study:

- Have either:
- A GFR less than or equal to 60 ml/min/1.73m^2 or
- The presence of greater than 0.3 grams of proteinuria by urine protein to creatinine ratio or 24 hour urine collection, but less than 3.5 gm of nephrotic range proteinuria as hypoalbuminemia may impact protein binding.

4.2 Exclusion Criteria

Exclusion criteria:

- Unable to provide informed consent
- Have history of intolerance, allergic reactions (e.g. rash) or other forms of hypersensitivities to any of the study medications (tacrolimus or cannabidiol);
- Are currently taking sedative agents, including agents for insomnia
- Are underweight (body mass index (BMI) less than 18.5) or overweight [body mass index (BMI) greater than 35]
- Have a positive pregnancy serum or urine test obtained just prior to each study, or are breast feeding
- Are night shift workers
- Are on dialysis.
- Have compromised liver function as defined by pre-screening bilirubin, AST and ALT testing, including any elevation of bilirubin or elevation of AST or ALT to 2 times the upper limit of normal.
- Are not willing to refrain from smoking or use of marijuana for at least two weeks prior to and until the completion of the study
- Have a Hgb < 10.0 g/dL
- Have gastrointestinal (digestive) disorders such as persistent diarrhea or malabsorption that would interfere with the absorption of orally administered drugs
- Have a history of or current seizure disorder
- Are currently on immunosuppression or are immunosuppressed.
- Are recipients of a current allograft (heart, kidney, pancreas, liver, intestine, lung, stem cell transplant).
- Have baseline EKG readings that are abnormal that could place the patient at the high risk.
- Have alcohol (more than 4 alcoholic drinks per day on a regular basis) or drug abuse, including opioids, or have used tobacco products or marijuana within the past three months, and are unwilling or unable to stop taking these medications two weeks prior to and during the entire study period
- Have participated in a research study involving intensive blood sampling or have donated blood within the past two months

- Had an unplanned hospitalization in the last 6 months or two or more unplanned hospitalizations in the last 2 years.
- Are taking prescription medications, that may interfere with the metabolism of the study drugs (e.g., inhibitors or inducers of CYP3A4/5 or CYP2C19 or that significantly impact protein binding of tacrolimus or cannabidiol). Interactions will be screened according to the Flockhart table.
- Are taking over-the-counter medications, herbal or dietary supplements, and alternative medicines that may interfere with the metabolism of the study drugs (e.g., inhibitors or inducers of CYP3A4/5 or CYP2C19 or that significantly impact protein binding of tacrolimus or cannabidiol) that the subject is unwilling or unable to stop over the course of the study. Interactions will be screened according to the Flockhart table.
- Are students under supervision of any of the study investigators.
- Cannot commit the time requested for this study.
- Have a known CYP3A4 *22/*22 genotype

5.0 Study Design

This is an open-label, three-period, fixed sequence study in subjects with and without CKD who have known CYP3A4 and CYP3A5 metabolizer status (Figure 2). We will investigate the PK of a single oral dose of tacrolimus before and after oral pre-treatment with cannabidiol. Although a randomized crossover design would be ideal, we will use controls first followed by treatment for practical and logistical considerations. Each subject will serve as his or her own control.

Dose justification:

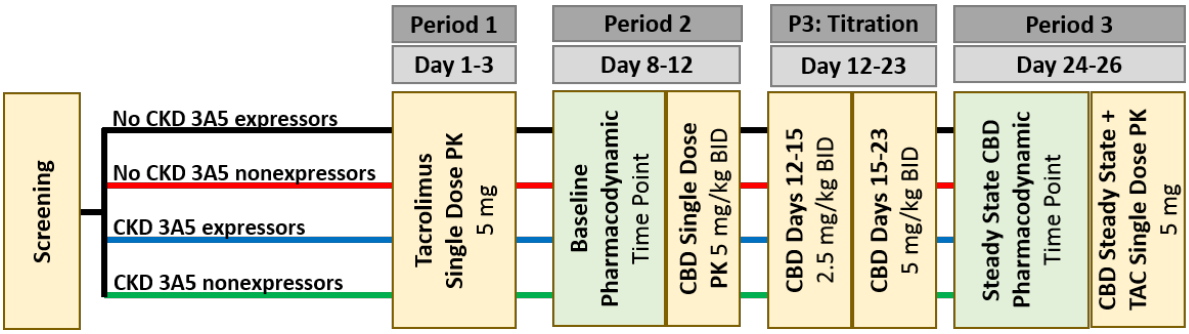


Figure 2: Study Design layout for the clinical trial. Period 1 is a tacrolimus single dose PK study, Period 2 is a CBD single dose PK study, P3 titration is a 12 day titration window of Period 3 to reach steady state. Period 3 is a tacrolimus single dose PK study while on CBD at steady state. The total number of subjects is 60 total, 30 each for the healthy volunteer and CKD studies, with an approximately even split between expressors and non-expressors (i.e. no less than 10 per group and no more than 20 per group).

The main dose of cannabidiol we will study will be 10 mg/kg/day (or 5 mg/kg twice daily). For an 80 kg person, the total daily dose would be 800 mg. CBD is available over the counter as a supplement and typical “doses” range from 100 mg to 300 mg daily. Thus, if we do not observe a drug-drug interaction at 10 mg/kg/day, it is unlikely individuals who take over the counter CBD will have a DDI unless they significantly overdose. Cannabidiol was FDA approved in 2018 as Epidiolex with indications for rare forms of seizure disorder. We will study the Epidiolex version of the drug as it is standardized and FDA approved rather than over-the-counter supplements which may contain additives or have non-standard formulations.

The Epidiolex drug comes as an oral solution at a concentration of 100 mg/ml. The recommended starting dose is 2.5 mg/kg twice daily with typical titration to 5 mg/kg twice daily

occurring after 1 week. The minimum titration interval is 48 hours. The maximum dose is 20 mg/kg/day or 10 mg/kg twice daily. The most concerning side effect of CBD is transaminase elevation, which is dose-related. Overall, ALT elevations greater than 3 times the ULN were reported in 17% of patients taking CBD 20 mg/kg/day compared with 1% in patients taking CBD 10 mg/kg/day. Most liver toxicity was observed in individuals with pre-existing elevation of hepatic liver enzymes or concomitant use of valproate or clobazam (other antiepileptic drugs). To ensure participant safety, we will not titrate to the 20 mg/kg/day dose (see full risk discussion below after the procedure). In subjects who experience significant somnolence or fatigue at 10 mg/kg/daily, we allow them to continue the study at 5 mg/kg/day total daily dose. We reserve the right not to titrate the dosage based on the clinical judgement and discretion of the PI with consideration given to participant safety, tolerability and individual response to treatment. Finally, if after 6 patients (3 CKD and 3 without CKD) or anytime thereafter, it becomes apparent that CKD patients are disproportionately having adverse effects, we will stop recruiting CKD patients and only focus on healthy volunteers. We will obtain a serum creatinine and potassium in baseline screening and again on Day 26 to ensure CKD subjects are not developing acute kidney injury or hyperkalemia due to higher than expected tacrolimus AUC levels.

The Cmax of CBD is not linear across doses and occurs 3-5 hours post dosing. There is variability of Cmax and AUC across individuals as both increase 4-5 fold with a high fat meal; however, the variability decreases when consistently ingested with food. The fasting single dose Cmax of CBD after a 750 mg dose is 336 (46.7) ng/ml and after a 1500 mg dose (20 mg/kg/day) is 525 (64.9) ng/ml. The Tmax occurred at 4.1 hours. The 336 ng/ml concentration is equivalent to a 1.08 μM concentration. Of note the *in vitro* IC50 of CYP3A5 is 1.65 μM (Yamaori S. Life Sci . 2011 Apr 11;88(15-16):730-6). Thus, these are relevant concentrations with a potential for CYP3A5 DDIs. In contrast, the in vitro IC50 for CYP3A4 was 11.7 μM; this may be why midazolam, a CYP3A4 substrate failed to elicit an inhibitory effect in the NDA trials. The Cmax increases 5 fold with food and was up to 3352 ng/ml (10.65 μM) on the 20 mg/kg/day dose while fed (the NDA did not report the Cmax under these circumstances for the 10 mg/kg/day dose). The half-life of single dose CBD is 30 hours in the NDA and extends to 56-61 hours after 7 days of twice daily dosing. Steady state concentrations were reached earlier than 4-5 half-lives at 3 days. We will plan to administer 14 total days of cannabidiol in the steady state PERIOD 3 phase below. This includes 5 mg/kg/day for 3 days, then titrated to 10 mg/kg/day for 9 days, followed by 10 mg/kg/day for 2 additional days during the tacrolimus single dose study. The rationale for the longer exposure of 9 days at 10 mg/kg/day is 1) to ensure we reach steady state since we plan to include subjects with CKD who may have different PK properties, 2) to allow for minor rescheduling (± 2 days) of the subject's fixed final inpatient study visit, and 3) to allow more time for adaptations of the immune system to take place in response to steady state concentrations (aim 2). The rationale to continue 10 mg/kg/day for 2 additional days during the tacrolimus single dose study is to ensure that if there is competitive inhibition that this inhibition continues throughout the PK monitoring of single dose tacrolimus.

The tacrolimus dose used in the single dose studies will be 5 mg oral once. Typical starting doses range from 3 to 8 mg twice daily in clinical practice, with African Americans or CYP3A5 expressors receiving a 1.5 fold higher starting dose. The 5 mg dose is within the range of typical starting tacrolimus doses and is unlikely to lead to toxicity in a single dose study even if there is CYP3A5 inhibition. Nonetheless, we will check potassium and creatinine levels on day 26 to allow us to monitor for adverse effects such as hyperkalemia and acute kidney injury during the DDI Period 3 study.

6.0 Enrollment/Randomization

This is not a randomized study. This is an open-label, three-period, fixed sequence study. Subjects will be identified through advertisement and recontacting prior study participants from the Indiana Biobank or prior clinical studies.

Following informed consent, the following activities will be performed during the screening phase.

To make sure that subjects are eligible to participate in the study, potential subjects will be initially asked some questions over the phone to see if they qualify for detailed screening. If they qualify, subjects will be asked to come to the Indiana Clinical Research Center (ICRC), a research unit at Indiana University Hospital. At the ICRC, they will be asked to read an IRB approved informed consent and allowed to ask questions they may have about the study. If they agree to participate in the study after they read the consent and have the study carefully explained to them, they will be requested to sign the IRB approved informed consent document.

Strategies for Recruitment:

Subjects will be simultaneously recruited for Aim 1 and Aim 2.

During the completion of two of the PI's prior studies, approximately 2000 individuals underwent genotyping for CYP3A4 and CYP3A5, including 580 individuals with chronic kidney disease (CKD) within the Indiana University Health system. Individuals will primarily be recruited from these cohorts as most individuals gave permission to be re-contacted for additional studies. We will recruit a mix of individuals with and without CKD because this is representative of the transplant population. We will also recruit a mix of CYP3A5 expressors and non-expressors, which likely means a close to even split between African Americans and Caucasians recruited. In the event we do not identify 57 suitable individuals who wish to participate from the 2000 with CYP3A5 genotyping in these studies, our Indiana University biobank has an additional 20,000 individuals who have either already or will soon have whole exome or whole genome sequencing completed. We would then extract CYP3A5 genotype from the sequencing of these individuals to have a larger pool of individuals to contact. Patients who have given consent to be recontacted in prior studies or as part of the Indiana Biobank will be called or sent a letter for recruitment. Additional advertising and recruiting outside of these already genotyped pools of subjects will also be done.

Our main recruitment site is the Indiana University campus. Based on previous experience, a 20% drop out rate (n=11) is expected, which means approximately 52 subjects have to be enrolled for 41 subjects to complete the entire study. As of the 8/22/25 amendment, we will close recruitment at 57 subjects (41 completions). This reduction in sample size is scientifically justified and discussed in the sample size section below.

Strategies for Retention:

To ensure retention, prospective subjects will receive a paper copy of the consent form and be counseled by our clinical trial coordinator. During screening, the coordinator or study staff will ensure that subjects understand their responsibilities by asking questions related to the study duration and procedures. Additional counseling will be performed as needed by the study pharmacist if potential dietary or drug interactions are identified during the screening. Once enrolled, subjects will be provided with a calendar of blood draws, study agent administration and clinic visits for the entire study so that planning of travels and other personal commitments

can be done in accordance with the study schedule. During the study, the research coordinator will ask subjects about any adverse events or any problems following the study protocol.

The PI (Dr. Eadon) and research coordinator working on this study will be available to speak with the subjects as necessary to address any issues, including those related to adherence, side effects or general concerns that may impact retention or safety. Our study pharmacist is available to counsel individuals on their medication regimen, potential dietary restrictions, and to be cognizant of side effects. Frequent communication will occur in-person during study visits.

Subjects will be compensated for their participation in the research study. Additionally, they will receive reimbursement for expenditures for research activities including parking and taxi costs when appropriate.

7.0 Study Procedures

This is an open-label, three-period, fixed sequence study, each period is described below.

Screening:

After a written informed consent is obtained, potential subjects will undergo a detailed pre-enrollment screening session (to be completed within a maximum of six weeks prior to enrollment into the study) at the ICRC for any medical abnormalities by medical histories, vital signs, electrocardiography (EKG) and standard clinical laboratory tests. To participate in the study, subjects must be judged healthy as determined by these screening tests. A blood sample (~15 ml) will be obtained from each subject for laboratory tests and urine samples will be obtained for urine analyses. Another blood sample (~10 mL) will be required for genotyping subjects with unknown CYP3A4 and CYP3A5 status. Laboratory tests will include urine drug screen, complete blood count, serum electrolytes, liver chemistries, prothrombin time, urine analysis, and, if female, pregnancy tests. For subjects with 100 mg/dl or greater protein on urinalysis, they will receive a urine protein to creatinine ratio to determine if they should be included in the healthy volunteer or CKD cohort. Concomitant medications and demographic characteristics will also be collected during the screening (self-identified race, bodyweight, height, gender/sex, age, and BMI calculated). Medications will be reviewed by the principal investigator or study pharmacist. Patients may be asked to stop over the counter medications or supplements which interact with cannabidiol or tacrolimus. Potential subjects who are taking interacting medications prescribed by their primary medical team will be excluded. Drug interactions will be defined according to the Flockhart Table (https://drug-interactions.medicine.iu.edu/SearchBy.aspx).

Subjects will be screened and enrolled until the target enrollment of n = 57 have completed the entire study. A portion of the blood may be collected to test CYP3A4 and CYP3A5 genotype if the genotype data is not available in the electronic health record. The total number of subjects is 60 total, 30 each for the healthy volunteer and CKD studies. We will have an approximately even split between expressors and non-expressors (i.e. 15 per group) in each study. No less than 10 per group and no more than 20 per group will be accepted during the study. If too many subjects of a certain genotype volunteer, then we will stop enrolling for that group.

PERIOD 1 (Control phase of Tacrolimus single dose pharmacokinetics)
Day 1. a) Tacrolimus pharmacokinetics. Eligible volunteers will be requested to arrive in the morning at ICRC on the first study day (Day 1) after an overnight fasting. After 11 pm the night

before admission to the ICRC, subjects will be requested not to eat anything except water. A sterile indwelling intravenous catheter will be inserted in one arm for blood collection. The principal investigator will be available for consultation. Pre-dose (baseline) blood and urine samples, and vital signs (e.g., blood pressure, respiration rate, heart/pulse rate, oral temperature, and breathing rate) will be obtained. Prior to dosing with tacrolimus, baseline blood and urine samples will be collected. In addition, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will receive a single dose of tacrolimus (5 mg) by mouth with a standardized breakfast and 250 ml water. Subjects will receive a standard lunch 4 hours after tacrolimus and free access to food and water thereafter including dinner. Blood samples (~6 mL) will be drawn into EDTA–containing tubes at 20, 40, and 60 minutes and 2, 4, 6, 12, 24, 48, and 192 hours after tacrolimus administration. The 12 hour time point may be drawn as early as 8 hours. Urine voided from drug administration to the last time point will be collected cumulatively in 1 receptacle on ice, the volume will be recorded, urine creatinine measured, and one 10-ml aliquot will be retained. Whole blood and urine aliquots will be stored at -80°C until analysis. Subjects will be discharged from the ICRC after their 12-hour blood draw and have their IV removed. We will aspire to have the study begin on a Tuesday and Wednesday so all study visits will be on weekdays.

Day 2: Subjects will return to the ICRC the next morning for the 24-hour blood draw by venipuncture to obtain blood samples (~6 mL) in EDTA-containing tubes. Vital signs will be recorded. Urine will be collected from subjects at the time of blood draw. The urine volume will be recorded. Then, a 10 ml urine aliquot will be extracted and retained. Whole blood and urine aliquots will be stored at -80°C until analysis. During the visit, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will be discharged from the ICRC after their single blood draw and urine collection. If either the 24- or 48-hour time points are missed, an additional collection may be obtained at 72 hours. The 24-, 48-, or 72-hour time points will be obtained within 12 hours of the optimal scheduled time-point.

Day 3: Subjects will return to the ICRC on Day 3 in the morning for the 48-hour blood draw by venipuncture to obtain blood samples (~6 mL) in EDTA-containing tubes. Vital signs will be recorded. Whole blood aliquots will be stored at -80°C until analysis. During the visit, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will be discharged from the ICRC after their single blood draw. If either the 24 or 48 hour time points are missed, an additional collection may be obtained at 72 hours. The 24, 48, or 72 hour time points will be obtained within 12 hours of the optimal scheduled time-point.

Notes for PERIOD 2 to 3

Cannabidiol will be dosed by the body weight (in kg) measured on Day 1. Every dose of cannabidiol will be pre-drawn into a capped syringe by the Investigational Drug Services. The dose will be rounded to nearest 10 mg.

PERIOD 2 (Cannabidiol single dose pharmacokinetics)

Day 8: b) Cannabidiol pharmacokinetics. Eligible volunteers will be requested to arrive in the morning at ICRC on study day 8 after an overnight fasting. Minor rescheduling of this visit is allowed +/- 7 days and subsequent visits would be adjusted accordingly to maintain equivalent

intervals between study visits unless otherwise noted. After 11 pm the night before admission to the ICRC, subjects will be requested not to eat anything except water. A sterile indwelling intravenous catheter will be inserted in one arm for blood collection. The principal investigator will be available for consultation. Pre-dose (baseline) blood and urine samples, and vital signs will be obtained. Prior to dosing with cannabidiol, baseline blood and urine samples will be collected. **This time point serves as one of the two blood draws for Aim 2 single cell sequencing, obtained without cannabidiol. One extra EDTA tube (~10 mL) will be drawn for this in about six individuals per funding year.** The acquisition of single cell RNA sequencing will coincide with the PERIOD 2 inpatient visit and minor rescheduling is allowed to align with the Genomics core scheduling requirements. In addition, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will receive a single dose of cannabidiol (5 mg/kg) with a standardized breakfast and 250 ml water. The bioavailability of cannabidiol increases with food. Subjects will receive a standard lunch 4 hours after cannabidiol and free access to food and water thereafter including dinner. Blood samples (~6 mL) will be drawn into EDTA–containing tubes at 20, 40, and 60 minutes and 2, 4, 6, 12, 48, and 96 hours after cannabidiol administration. The 12-hour time point may be drawn as early as 8 hours. Plasma will be separated by centrifugation as soon as possible. Urine voided from drug administration to the last time point will be collected cumulatively in one receptacle on ice, the volume will be recorded, urine creatinine measured, and one 10-ml aliquot will be retained. Plasma and urine aliquots will be stored at -80°C until analysis. We will query for side effects including symptoms of lethargy, somnolence, sleep disorder, diarrhea, or any other side effects experienced. Patients will not be allowed to drive if adverse events impair them. Subjects will be discharged from the ICRC after their 12-hour blood draw and have their IV removed. Minor rescheduling of the day 8 visit is allowed with subsequent visits adjusted accordingly.

Day 10: Subjects will return to the ICRC in 2 days for the 48-hour blood draw by venipuncture to obtain blood samples (~6 mL) in EDTA-containing tubes. Urine will be collected from subjects at the time of blood draw. The urine volume will be recorded. Then, a 10 ml urine aliquot will be extracted and retained. Vital signs will be recorded. We will query for side effects including symptoms of lethargy, somnolence, sleep disorder, diarrhea, or any other side effects experienced. Plasma will be separated by centrifugation as soon as possible. Plasma and urine aliquots will be stored at -80°C until analysis. During the visit, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will be discharged from the ICRC after their single blood draw and urine collection. Minor rescheduling of the day 10 is allowed with subsequent visits adjusted accordingly. The 48- and 96-hour time points will be obtained within 12 hours of the optimal scheduled time-point.

Day 12: Subjects will return to the ICRC on Day 12 for the 96-hour blood draw by venipuncture to obtain blood samples (~6 mL) in EDTA-containing tubes.. Vital signs will be recorded. We will query for side effects as discussed above. Plasma will be separated by centrifugation as soon as possible. Plasma aliquots will be stored at -80°C until analysis. Subjects will NOT be discharged immediately, but instead will remain for education and the start of PERIOD 3, the steady state interaction study described below. The 48- and 96-hour time points will be obtained within 12 hours of the optimal scheduled time-point.

PERIOD 3 (Cannabidiol steady state interaction study)

Day 12 continued: Subjects will be educated on the next phase of the study. Any subjects who reported significant symptoms after the cannabidiol single dose study will not proceed with the PERIOD 3. Prior to dosing with cannabidiol, subjects will be asked to complete a questionnaire

to assess the effects of cannabidiol on sleep, anxiety, and emotional state. The subject will be given 2.5 mg/kg of cannabidiol in the ICRC to start the steady state interaction study. The subject will be instructed to take cannabidiol 2.5 mg/kg twice daily with food continued for 3 total days (5 more doses) and then take 5 mg/kg twice daily with food, continued for 9 additional days (12 total days of cannabidiol). Subjects will receive a diary to record the time the medication was taken, symptoms such as lethargy, somnolence, sleep disorder, diarrhea, or any other feelings or side effects experienced during the course of the study. The subjects will be requested to determine adherence to dietary recommendations such as taking the drug with meals and avoiding grapefruit. Pill count and trough concentration on Day 24 will be used to assess compliance with cannabidiol intake. Minor rescheduling of Day 12 is allowed with subsequent visits adjusted accordingly.

Days 13- 23: Subjects will be called by study coordinators twice over this study period to assess symptomatology and adherence. These contacts will generally coincide with: 1) a reminder of the change in study drug dose and 2) a reminder to return to the ICRC for period 3.

PERIOD 3 (Cannabidiol steady state phase)

Day 24: On the morning of the 13th day of cannabidiol dosing (completed 12 total days), subjects will return to the ICRC after an overnight fast. After 11 pm the night before admission to the ICRC, subjects will be requested not to eat anything except water. Analogous to the inpatient visits on Day 1 and Day 8, a sterile indwelling intravenous catheter will be inserted in one arm for blood collection. The principal investigator will be available for consultation. Pre-dose (baseline) blood (including liver function tests), urine samples, and vital signs will be obtained. **This time point serves as the second of two blood draws for Aim 2 single cell sequencing, obtained while on cannabidiol. One extra EDTA tube (~10 mL) will be drawn for this in about six individuals enrolled in each funding year.** The acquisition of single cell RNA sequencing will coincide with the PERIOD 3 inpatient visit and minor rescheduling is allowed to align with the Genomics core scheduling requirements. Pill counts and diaries will be reviewed. Prior to dosing with cannabidiol 5 mg/kg in the ICRC, baseline blood and urine samples will be collected. Subjects will also be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will receive one dose of cannabidiol (5 mg/kg) and one dose of tacrolimus 5 mg with a standardized breakfast and 250 ml water. Subjects will receive a standard lunch 4 hours after cannabidiol/tacrolimus and free access to food and water thereafter including dinner. Blood samples will be drawn into two EDTA–containing tubes (~6 mL each) at 20, 40, and 60 minutes and 2, 4, 6, 12, 24 and 48 hours after drug administration. The 12 hour time point may be drawn as early as 8 hours. Plasma will be separated by centrifugation from one of the two tubes as soon as possible. The other tube will be stored as whole blood. Urine voided from drug administration to the last time point will be collected cumulatively in one receptacle on ice, the volume will be recorded, urine creatinine measured, and one 10-ml aliquot will be retained. Whole blood, plasma, and urine aliquots will be stored at -80°C until analysis. The subject will receive a second dose of cannabidiol (5 mg/kg) with a standardized snack prior to discharge from the ICRC. Subjects will be discharged from the ICRC after their 12-hour blood draw and second cannabidiol dose. Their IV will be removed. Minor rescheduling of the day 24 is allowed with subsequent visits adjusted accordingly.

Day 25: Subjects will return to the ICRC the next morning for the 24-hour cannabidiol/tacrolimus blood draw by venipuncture to obtain two blood samples (~6 mL) in EDTA-containing tubes. Urine will be collected from subjects at the time of blood draw. The urine volume will be recorded. Then, a 10 ml urine aliquot will be extracted and retained. Vital signs will be recorded.

We will query for side effects including symptoms of lethargy, somnolence, sleep disorder, diarrhea, or any other side effects experienced. Plasma will be separated by centrifugation from one of the two tubes as soon as possible. The other tube will be stored as whole blood. Whole blood, plasma, and urine aliquots will be stored at -80°C until analysis. Prior to dosing with cannabidiol, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. The subjects will receive a dose of cannabidiol (5 mg/kg) in the ICRC. Subjects will be discharged from the ICRC after their single blood draw, urine collection, and cannabidiol dose. Subjects will be instructed to take one additional dose of cannabidiol (5 mg/kg) in the evening to complete the full 14-day regimen. If either the Day 25 or 26 visit is missed, an additional collection may be obtained on Day 27. The Day 25 and 26 visits time points will be obtained within 12 hours of the optimal scheduled time-point.

Day 26: Subjects will return to the ICRC on Day 26 for the 48-hour cannabidiol/tacrolimus blood draw to obtain two blood samples (~6 mL) in EDTA-containing tubes. Vital signs will be recorded. Plasma will be separated by centrifugation from one of the two tubes as soon as possible. The other tube will be stored as whole blood. Whole blood, plasma, and urine aliquots will be stored at -80°C until analysis. During the visit, subjects will be asked to complete a questionnaire to assess the effects of cannabidiol on sleep, anxiety, and emotional state. Subjects will be discharged from the ICRC after their single blood draw and urine collection. If either the Day 25 or 26 visit is missed, an additional collection may be obtained on Day 27. The Day 25 and 26 visits time points will be obtained within 12 hours of the optimal scheduled time-point.

Subjects will be compensated for completion of the study. Screening visit compensation will occur at the conclusion of screening. Compensation for the other portions of the study will be done upon study completion.

Pharmacodynamic Study (Aim 2):

The study procedures for the pharmacodynamic study amount to 2 blood draws synchronized with the draws in the pharmacokinetic study above. All data acquisition is obtained via *ex vivo* cell culture.

8.0 Study Calendar

This is covered in detail in sections 5 and 7.

STUDY CALENDAR

Phase 1			
Day 1 Inpatient ICRC Tacrolimus Single Dose 5 mg	Day 1 (cont.): -IV placed and blood drawn through the IV 7 times -Collect urine -Eat ICRC diet -Estimated length: 12 hours.	Day 2: **Outpatient ICRC** -blood draw and vitals -collect urine -Estimated length: 30 min.	Day 3: **Outpatient ICRC** **(last blood draw for phase 1)** -blood draw and vitals -Estimated length: 30 min. Days 4-7: **Wash-out**
Phase 2			

Day 8 Inpatient ICRC* Cannabidiol Single Dose 5 mg/kg	Day 8 (cont.): -IV placed and blood drawn through the IV 7 times -Collect urine -Eat ICRC diet -Estimated length: 12 hours	Day 10: **Outpatient ICRC** -blood draw and vitals -collect urine -Estimated length: 30 min.	Day 12: **Outpatient ICRC** -last blood draw for phase 2 with vitals -Estimated length: 1 hour. **Phase 3 begins** *Begin taking first dose of cannabidiol 2.5 mg/kg twice daily	
*Minor rescheduling of visits is allowed and subsequent visits would be adjusted accordingly				
Phase 3 (cont.)				
Day 12-14: take cannabidiol 2.5 mg/kg twice daily	Day 15-23: take cannabidiol 5 mg/kg twice daily	Day 24: **Inpatient ICRC* Continue cannabidiol 5 mg/kg twice/day on day 24-26** -Take tacrolimus 5 mg once -IV placed and blood drawn through the IV 14 times -Collect urine -Eat ICRC diet -Estimated length: 12 hours	Day 25: **Outpatient ICRC** -blood draw and vitals -collect urine -Estimated length: 30 min.	Day 26: **Outpatient ICRC** -last blood draw for phase 3 with vitals -collect urine -Estimated length: 30 min.
*Minor rescheduling of visits is allowed and subsequent visits would be adjusted accordingly				

9.0 Reportable Events

Adverse and Serious Adverse Events: Adverse and serious adverse events (AE and SAE, respectively) will be defined according to FDA and OHRP guidelines as per IU IRB and NIH requirements.

Adverse event assessment
All adverse events will be reported to the Indiana University IRB, and FDA as required per their adverse event reporting policies. At the time of the annual review of the study, adverse events will be reported as per the Indiana University HRPP Reportable Events Policy and Guidance. An Adverse Reaction Reporting Form will be completed for any serious and unexpected adverse reaction associated with the study, and the Indiana University IRB will be notified per IU HRPP Reportable Events Policy and Guidance.

An IND safety report will be submitted to the FDA for an event that is a (1) suspected adverse reaction, (2) serious, and (3) unexpected, per FDA guidance.

> Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event.
>
> An adverse event or suspected adverse reaction is considered "serious" if, in the view of

either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the protocol.

Any unexpected fatal or life-threatening suspected adverse reaction will be submitted as an IND safety report to the FDA within seven (7) calendar days after initial receipt of information. Any potential serious risks (serious and unexpected suspected adverse reactions, findings from other studies, findings from animal or in vitro testing, or increased rate of occurrence of serious suspected adverse reactions) will be submitted as an IND safety report to the FDA within fifteen (15) calendar days after initial receipt of the information.

All adverse events will be classified and recorded according the NCI Toxicity Grading Scale. All study related toxicities will be graded (Grades 1, 2, 3 or 4) according to the Toxicity Grading Scale and will be employed along with potential study drug specific toxicities in the individual subject stopping rules. Subject and study stopping rules will be determined by the DSMB. If the DSMB recommends that the study change or be closed early for patient safety reasons or for slow accrual, the PI will act to implement the change as expeditiously as possible. If a recommendation is made to change the study for other than patient safety reasons or slow accrual, the DSMB will provide an adequate rationale for its decision.

Adverse events will be graded as follows:
Grade 0 – No adverse effects
Grade 1 - Mild adverse events
Grade 2 – Moderate adverse events
Grade 3 – Severe adverse events
Grade 4 – Life threatening or disabling adverse events
Grade 5 – Death related to adverse events

Adverse events will be assessed using the NCI Common Toxicity Criteria guidelines listed in (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf) along with potential study drug specific toxicities in the individual subject stopping rules.

Deaths will be reported to the Indiana University IRB per their institutional guidelines if they occur within 30 days of study intervention. The principal investigator will be responsible for reporting adverse effects to the IRB and other relevant bodies.

Potential Risks: The potential risks are related to venipuncture, study procedures, and study medications. These are detailed here:

Potential Risk summary/Risk to Human Subjects. The Division of Clinical Pharmacology and Dr. Desta have considerable experience in the conduct of clinical studies. These have included

several drug interaction studies without or with minimal adverse effects. The PI, Dr. Eadon, is a physician who will closely monitor for adverse effects. The risks of participating in the studies are related to blood draws and to the pharmacological effects of test drugs: tacrolimus and cannabidiol.

a) Venipuncture: Blood samples will be collected through puncture of a vein by a needle. Venipuncture may cause slight pain, bruising, and bleeding from the site of the needle puncture into the vein, and in rare cases, fainting and infection. To minimize these discomforts and risks, only trained and experienced nurses will place the intravenous catheter in the vein or perform venipuncture to collect blood samples. Aseptic technique and hospital/nursing standards will be followed.

b) Blood Loss: This study requires three inpatient admissions (12 hours each) and six brief outpatient blood draws in total for each subject. The maximum blood volume that will be collected from each subject during the course of the entire study (the study duration is 26 days) will be below 650 ml. There will be at least 7 days between the inpatient visits when intensive blood sampling will occur. To minimize the impact of blood loss, subjects accepted into the study must have a hemoglobin count of 10.0 g/dl or above. Additional measures to minimize the effects of blood loss include exclusion of subjects who have recently donated blood and who have participated in a study involving intensive blood sampling (see Exclusion criteria). Given these measures and that blood samples are drawn over a long period, we would expect the body will adequately replace this blood.

c) EKG. There are generally no risks associated with an EKG. However, the adhesive or glue used to attach the pads to the subject's body may cause a rash or skin irritation at the site where the pads were placed.

d) Study medications. Most of the risks are related to the use of the study drugs. Potential risks associated with each study medicine are outlined below.

Cannabidiol: The adverse effects of cannabidiol include somnolence, decreased appetite, diarrhea, transaminase elevations, fatigue, rash, insomnia, and poor quality sleep. The NDA and FDA label report that the amount of THC ingested and produced is negligible and that CBD produced insufficient rewarding properties to sustain reinforcement. In the NDA study, 5.3% of individuals reported a euphoric mood at the 10 mg/kg/day CBD dose which was 6 times less than that of a comparator drug dronabinol (30%) and equivalent to the benzodiazepine alprazolam in this clinical study. We will monitor for side effects in patients using a symptom diary. The principal investigator will be available to the research staff at all times during the study and will be available to outpatient study patients by paging system if necessary. The other important thing is that the single dose cannabidiol study becomes a test for tolerance of CBD for the next steady state PERIOD 3 study. The most concerning side effect is elevation of transaminase levels, which occurs in 1% of individuals at the 10 mg/kg/day total daily dose (divided in 2 doses). However, the occurrence is more frequent when also receiving clobazam or valproate and we will screen and exclude subjects with a concomitant seizure disorder. We will also screen for AST, ALT, and bilirubin levels at baseline and during the study. We have decided to limit the total daily dose to 10 mg/kg/day total dose rather than the maximum daily dose of 20 mg/kg/day because elevation of transaminases occurs in 1% of individuals at this dose, compared to 17% in the 20 mg/kg/day total dose. Finally, we will initially conduct studies in 6 subjects, and based on the assessments and recommendations made by the Data and Safety Monitoring Board we will reconsider using a lower dose (5 mg/kg/day total dose) if recommended.

In subjects who experience fatigue or other mild adverse effects at the 10 mg/kg/day dose who did not have these side effects at the 5 mg/kg/day dose, we allow them to continue the study at 5 mg/kg/day total daily dose. Finally, if after 6 patients (3 CKD and 3 without CKD) or anytime thereafter, it becomes apparent that CKD patients are disproportionately having adverse effects, we will stop recruiting CKD patients and only focus on healthy volunteers.

Tacrolimus: Tacrolimus is generally well tolerated and taken twice daily by many transplant recipients for years. Common side effects include immunosuppression, renal dysfunction, and electrolyte abnormalities. Since subjects only receive a single dose of tacrolimus on two occasions 20 days apart, we do not anticipate side effects to be a major issue. Nonetheless, we will take precautions by checking baseline labs and vitals and subjects will be closely monitored in the ICRC. We will also check serum potassium and creatinine on day 26. If substantial elevation of either is noted in the initial CKD subjects due to a higher than expected tacrolimus AUC, we will seek advice from the DSMB and convert the study to recruit only healthy volunteers.

It is also expected that AEs and SAEs may occur at a higher frequency in CKD patients compared with a healthy cohort, due to their disease and co-morbidities, rather than their participation in this study protocol. All AEs and SAEs will be adjudicated for the likelihood of relationship to the study.

Tracking of events: the following AE's are discussed in the protocol, are discussed with patients as part of the informed consent process, and will be tracked as part of the protocol:

Complications of venipuncture, blood draw, IV placement, or IV infusion
Complications related to study drug

Adverse event reporting

- Every event that is reported to either the PI or the designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented.
- An AE report will be generated for each event and each report will include: (1) description of the event; (2) when it was reported; (3) severity; and (4) a determination of attribution.
- All SAE's will be reported once the event has been verified and determination of attribution has been completed in accordance with IU IRB reporting requirements. All other AEs will be reported according to the IU IRB reporting requirements.

10.0 Data Safety Monitoring

A. Overall Framework

The intervention and measurement protocols described in the **"Protection of Humans Subjects"** section pose more than minimal risk to participants. Consequently, the data safety monitoring plan focuses on close monitoring by the PI and Co-Is in conjunction with an external Data and Safety Monitoring Board (DSMB). Members of the DSMB will include experts experienced in clinical research and patients with CKD and will consist of a Chairperson and one additional member.

The Principal Investigator or a designated co-investigator will oversee the data and safety reporting process. Members of the DSMB will include the PI (as a non-voting member) and three other members who will be appointed by the PI for the duration of the study. The voting members will select a Chair of the DSMB, who will be responsible for ensuring that the obligations of the DSMB are met. Members of the DSMB will include experts experienced in clinical research and patients with CKD. Program and statistical staff from the NIH will be permitted to serve as non-voting ex officio members of the board at the request of the NIH. Staff affiliated with Indiana University will view themselves as representing the interest of patients and not that of the institution.

Each meeting of the DSMB will be divided into three parts. A) An open session at which members of the clinical study team may be present to review the conduct of the study and to answer questions from members of the DSMB. The focus will be on accrual, protocol compliance, and adverse events. Outcome results may not be discussed in this session. B) A closed session involving DSMB members, at which interim outcome results or safety may be presented and discussed. C) A final executive session, at which the general conduct of the study and outcome results will be discussed and recommendations for the study will be developed. Confidentiality will be maintained during all phases of the study, including monitoring, preparation of interim results, review, and response to monitoring recommendations. No communication, either written or oral, of the deliberations or recommendations of the DSMB will be made outside of the DSMB, except as provided for in this policy. Outcome results are strictly confidential and may not be divulged to any non-member of the DSMB. Each member of the DSMB, including non-voting members, must sign a statement of confidentiality

Data Safety and Monitoring Board (DSMB). The DSMB will approve initiation of the protocol and will evaluate progress of Specific Aims. Primary responsibilities include:

- Review of research protocol and plan for data and safety monitoring
- Evaluate progress of study such as assessments of data quality and timeliness, subject
- recruitment, accrual, and retention, and subject risks vs. benefit
- Protect the safety of the participants by monitoring screening health measures, adverse events reported during the study, and termination of subjects according to the stop criteria.
- Make recommendations to the PI and if relevant to the IRB or NIH that include the current status of the study, summary reports of adverse events resulting from participation in the study, and determination of whether the study should continue as originally designed, should be changed, or should be terminated.
- Meetings may be convened by e-mail or conference call prior to study initiation, after 6 subjects, and every 6 months or 6 patients for duration of the study (as decided by the DSMB), and at the completion of the study.

Prior to trial initiation, Drs. Eadon and Desta will provide an initial **Data and Safety Monitoring Plan** draft to the DSMB, whose members will be able to request any modifications. The plan will contain a summary of the study purpose and protocol including potential risks and benefits to participants, informed consent documents, plans for monitoring of protocol adherence, subject compliance, data quality assessment, adverse event reporting, and trial stopping rules. The DSMB will be provided with the full IRB study protocol. During the study, all members will meet by a conference call at any time requested by the DSMB. The DSMB will be given a final report when all subjects have completed the study. The report will describe subject recruitment, accrual, retention, protocol amendments and deviations, data quality and any AEs and SAEs.

This will be prepared by the PI and study team and shared with the board at least 1 week prior to the meeting. At the end of the meeting, the Chairperson will draft a DSMB report including a recommendation to continue plans for a larger clinical trial based on this pilot study, modify the protocols, or abandon the larger trial. The DSMB report will be shared with the PI and the IRB. All DSMB discussion and proceeding will remain confidential.

Study Monitor: The Indiana CTSI will be contracted to provide an independent study monitor system to oversee the quality of the data collected and trial protocol adherence.

11.0 Study Withdrawal/Discontinuation

Taking part in this study is voluntary. Subjects have the option of participating or not participating in the study. There are no other alternative procedures or courses of treatment that may be advantageous to them. They can choose not to take part or to withdraw from this study at any time without penalty. Leaving the study will not result in any penalty or loss of benefits to which the subject is entitled. The decision of any subject to participate or not to participate will not affect current or future relationship with the research team. Subjects will be presented with new information that may affect their health, welfare, or willingness to stay in the study. The principal investigator reserves the right to withdraw a subject from the study without regard to subjects consent in the following circumstances: if risks in participating in this study outweigh their best interest in terms of safety; or if subject is unable to adhere to the study requirements. In addition, this study may be terminated by the National Institute of Health for unforeseen reasons (e.g., lack of funding), by the DSMB or IRB at their discretion.

Individuals that withdraw from the study (voluntarily or at the discretion of the PI) will be compensated corresponding to their participation for the portions of the study which were completed. Compensation will be issued at the completion/withdrawal of the study.

12.0 Statistical Considerations

Pharmacokinetic analyses: Pharmacokinetic (PK) outcomes for tacrolimus, cannabidiol, and their respective metabolites will be obtained via standard noncompartmental methods using Phoenix WinNonlin. PK outcomes will include area under the plasma concentration-time curve (AUC), maximal plasma concentration (C_{max}), time to C_{max} (T_{max}), apparent oral clearance (Cl_{PO}), and terminal elimination half-life ($t_{1/2}$). Similarly, urinary excretion kinetics will be determined via standard approaches although we understand renal excretion of both drugs is thought to be minimal. The PK interaction magnitude for tacrolimus will be quantified according to the treatment to control ratios of AUC and C_{max}. The steady state interaction magnitude will also be predicted via nonparametric superposition of the observed interaction magnitude following single tacrolimus doses. Assuming linear PK of tacrolimus, an individual accumulation ratio will be determined for each subject in the absence and presence of CBD and utilized to predict the individual, genotype-dependent, interaction magnitude at steady state. The primary outcome is the tacrolimus interaction magnitude: tacrolimus (+CBD): tacrolimus(-CBD) $AUC_{0-\infty}$ ratio between CYP3A5 expressors and non-expressors. This primary outcome will be assessed separately for the subjects with CKD and subjects without CKD as there is adequate power to do so.

Sample size consideration: The original sample size calculation suggested that 72 subjects would be recruited to have 60 patients complete the study after a predicted drop-out rate of 20%. Sixty subjects provided more than 95% power based on a coefficient of variation (CV) for

tacrolimus of 37% and a 1.5-fold difference in interaction magnitude between CYP3A5 expressor and non-expressor genotype groups. Half of the subjects would have CKD and half would not have CKD. Our interaction magnitude was more than 3-fold in the interim analysis (PMID: 39601108), suggesting that we may have more power than anticipated. A pre-specified alternate approach was to assess the primary outcome in the combined group of CKD and non-CKD patients using stratification. We will adopt this approach and close recruitment at 57 subjects (41 completions). This reduction in sample size is scientifically justified.

Statistical Analyses for pharmacodynamic study. The degree of stimulation will be calculated as the ratio of the Cell Titer Glo luminescence detected between stimulated and unstimulated lymphocytes. Each individual (n=24) will have technical triplicates averaged for each of the two stimulated conditions (i.e. conditions 2 and 5). A Wilcoxon Rank Sum test will be performed to compare the degree of stimulation between these two groups and a Dunn's Kruskal-Wallis test when stratifying by disease state. The number of high quality cells acquired in each sample varies from 5,000-10,000 cells. Performing scRNA-seq on six individuals (3 no CKD, 3 CKD) per year will result in 24 individuals per treatment group in four years. This should deliver between 120,000-240,000 cells per condition to provide ample power to detect DEGs and enriched pathways. In addition, co-clustering all 480,000-960,000 cells will provide greater accuracy to identify most lymphocyte subpopulations prior to comparisons.

In our previous study involving ~120,000 cells, we were able to identify 20 subpopulations of lymphocytes including Th0, Pre-Th1, Th1, Tregs, NK1, NK2, Pre-B, and FO B cells[43]. A chi-square test will be used to compare lymphocyte subpopulation proportions and a Wilcoxon Rank Sum test with Bonferroni adjusted *p*-values to detect differentially expressed genes between the conditions. Comparisons can be made between CKD and non-CKD individuals combined and separately to determine the interaction between CBD and CKD status. A corrected *p*-value <0.05 will be considered statistically significant. Additionally, DEGs will be tested for enrichment of pathways in the Gene Ontology, Kegg and Reactome databases. Predicted receptor-ligand interactions will be identified using CellPhoneDB. These analyses will help uncover the pharmacodynamic effects that CBD elicits on stimulated lymphocytes.

13.0 Statistical Data Management

This study utilizes the REDCap (Research Electronic Data Capture) for its database, which is provided by the IU CTSI. REDCap is a free, secure, web-based application designed to support data capture for research studies. The system was developed by a multi-institutional consortium initiated at Vanderbilt University. Data collection is customized for each study or clinical trial by the research team with guidance from IU CTSI REDCap Support Staff.

The investigator intends to register and submit results to ClinicalTrials.gov, consistent with policy and regulatory requirements. The Indiana University Office of Research Compliance, in collaboration with the Indiana CTSI, offers a robust compliance program and provides guidance to investigators in meeting their policy and regulatory obligations. The Indiana University Office of Research Compliance website outlines the institutional compliance plan and policy: http://researchcompliance.iu.edu/qio/qio_ctgov.html**.** Informed consent documents will include a specific statement relating to the posting of clinical trial information at ClinicalTrials.gov.

Primary data will be collected via phone and in person surveys and stored electronically in REDCap and OnCore. Pharmacokinetic and transcriptomic data will be de-identified and stored

in Microsoft OneDrive folders with access limited to the study team. The data will be backed up automatically daily for RedCap and weekly for OneDrive. Other data sources include data from INPC that will be stored in separate electronic files and merged with the primary data as needed. Quality assurance steps will include: built in range checks and testing of database by study team prior to moving to production mode]. Privacy/Confidentiality Issues

The study data and results are for research purposes only. To the extent allowed by law, information about a study subject's personal and medical data is considered confidential. Although we cannot guarantee absolute confidentiality, every effort will be made to keep all subject information strictly confidential. The samples collected from this study will be stored in locked freezers and only authorized personnel will have access to the samples, coded databases, and the results. The samples will be labeled with a code and not with subject names. All subject identified information will be stored in locked cabinets and in a compliant RedCap Database. For analysis, the PIs computer that is password protected and all online data will be encrypted. It will not be possible for anyone other than the investigators to determine subject specific test results without the explicit written consent of the subject or unless allowed by law. It is possible that data collected during the screening and exit lab tests, and inpatient care may become part of subject's medical record. Only research staff will have access to samples and coded databases, minimizing the risk of loss confidentiality. Personal information may be disclosed if required by law. People or organizations that may review or inspect personal information to verify the quality of the research and data analysis include the study principal investigator and his research team, the Indiana University Institutional Review Board or its designees, the study sponsor or designee (NCCIH) and the Indiana Clinical Research Center (ICRC). In addition, as allowed by law, state or federal agencies, specifically the Office for Human Research Protections (OHRP) and the Food and Drug Administration (FDA), may need to access medical and/or research records.

Samples or information will not be released to anyone other than those listed above without obtaining a written consent from the subjects who participated in the study. When the results of study are published, no names or specific identifiers will be used. In addition, we will not use this sample to characterize a subject's genetic history or determine the likelihood that they will experience a disease or not. We may know the pharmacogenotype of the subjects involved, either through direct testing as part of this study or from prior studies like INGENIOUS, the Precision Health Initiative, or the Indiana Biobank. The single cell RNA sequencing is an expression test and unlike whole genome DNA sequencing, it is extremely unlikely an individual could be reidentified through single cell RNA sequencing. This data will ultimately be stored in the NCBI Gene Expression Omnibus. Therefore, while the possibility that participation in this study may cause a loss of privacy, the steps outlined above will be implemented to significantly minimize the risk of loss confidentiality.

Finally, a description of this clinical trial will be made available on ClinicalTrials.gov, as required by U.S. Law. This Web site does not include information that can identify individual subjects. At most, the site will include a summary of the protocol and results. Subjects have the opportunity to search this freely available online resource at any time.

14.0 Follow-up and Record Retention

Recruitment of subjects to this study is expected to be completed within four years. The records (documents) will be kept to the extent allowed by law per NIH recommendations after the study is closed. All documents will be destroyed by shredding any document holding study information or through deletion of computer files using HIPPA compliant digital shredding utilities. Subjects

retain (keep) the right to have any remaining biological sample material destroyed at any time by contacting the principal investigator. The principal investigator is responsible for the destruction of biological samples at the subject's request. Any data from this study will be published without subject names.

Note: Bio-specimens (plasma and urine samples) may be stored for future research uses by the research team as specified in the consent. Involvement of other repository teams other than the research team are not planned at this point. In the event that other investigators will be involved in the future, an appropriate IRB approval will be obtained. In addition, the research team will enter into a submittal agreement through a material transfer agreement with the other investigators.

15.0 References

1. Brown, JD, Winterstein, AG: Potential Adverse Drug Events and Drug-Drug Interactions with Medical and Consumer Cannabidiol (CBD) Use. *J Clin Med,* 8, 2019.
2. Crime, UNOoDa: World Drug Report: Cannabis and Hallucinogens. 2019.
3. Brenan, M: 14% of Americans Say They Use CBD Products. *Gallup.* 2019.
4. America, TSotUSo: Agriculture Improvement Act of 2018. 115th Congress 2nd Session, 2018.
5. Corroon, J, Phillips, JA: A Cross-Sectional Study of Cannabidiol Users. *Cannabis Cannabinoid Res,* 3**:** 152-161, 2018.
6. Argueta, DA, Ventura, CM, Kiven, S, Sagi, V, Gupta, K: A Balanced Approach for Cannabidiol Use in Chronic Pain. *Frontiers in pharmacology,* 11**:** 561, 2020.
7. Yamaori, S, Ebisawa, J, Okushima, Y, Yamamoto, I, Watanabe, K: Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxyl groups in the resorcinol moiety. *Life Sci,* 88**:** 730-736, 2011.
8. Birdwell, KA, Decker, B, Barbarino, JM, Peterson, JF, Stein, CM, Sadee, W, Wang, D, Vinks, AA, He, Y, Swen, JJ, Leeder, JS, van Schaik, R, Thummel, KE, Klein, TE, Caudle, KE, MacPhee, IA: Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. *Clin Pharmacol Ther,* 98**:** 19-24, 2015.
9. Lamba, J, Hebert, JM, Schuetz, EG, Klein, TE, Altman, RB: PharmGKB summary: very important pharmacogene information for CYP3A5. *Pharmacogenet Genomics,* 22**:** 555-558, 2012.
10. Gaston, TE, Bebin, EM, Cutter, GR, Liu, Y, Szaflarski, JP, Program, UC: Interactions between cannabidiol and commonly used antiepileptic drugs. *Epilepsia,* 58**:** 1586-1592, 2017.
11. Geffrey, AL, Pollack, SF, Bruno, PL, Thiele, EA: Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia,* 56**:** 1246-1251, 2015.
12. Leino, AD, Emoto, C, Fukuda, T, Privitera, M, Vinks, AA, Alloway, RR: Evidence of a clinically significant drug-drug interaction between cannabidiol and tacrolimus. *Am J Transplant,* 19**:** 2944-2948, 2019.
13. Cunetti, L, Manzo, L, Peyraube, R, Arnaiz, J, Curi, L, Orihuela, S: Chronic Pain Treatment With Cannabidiol in Kidney Transplant Patients in Uruguay. *Transplant Proc,* 50**:** 461-464, 2018.
14. Dhital, S, Stokes, JV, Park, N, Seo, KS, Kaplan, BL: Cannabidiol (CBD) induces functional Tregs in response to low-level T cell activation. *Cell Immunol,* 312**:** 25-34, 2017.

15. Administration, USFaD: Epidiolex (Cannabidiol) FDA drug label prescribing information. 2018.
16. Hernandez-Cervantes, R, Mendez-Diaz, M, Prospero-Garcia, O, Morales-Montor, J: Immunoregulatory Role of Cannabinoids during Infectious Disease. *Neuroimmunomodulation,* 24**:** 183-199, 2017.
17. Colvin, M, Smith, JM, Skeans, MA, Edwards, LB, Uccellini, K, Snyder, JJ, Israni, AK, Kasiske, BL: OPTN/SRTR 2015 Annual Data Report: Heart. *Am J Transplant,* 17 Suppl 1**:** 286-356, 2017.
18. Kandaswamy, R, Stock, PG, Gustafson, SK, Skeans, MA, Curry, MA, Prentice, MA, Israni, AK, Snyder, JJ, Kasiske, BL: OPTN/SRTR 2015 Annual Data Report: Pancreas. *Am J Transplant,* 17 Suppl 1**:** 117-173, 2017.
19. Valapour, M, Skeans, MA, Smith, JM, Edwards, LB, Cherikh, WS, Uccellini, K, Israni, AK, Snyder, JJ, Kasiske, BL: OPTN/SRTR 2015 Annual Data Report: Lung. *Am J Transplant,* 17 Suppl 1**:** 357-424, 2017.
20. Hart, A, Smith, JM, Skeans, MA, Gustafson, SK, Stewart, DE, Cherikh, WS, Wainright, JL, Boyle, G, Snyder, JJ, Kasiske, BL, Israni, AK: Kidney. *Am J Transplant,* 16 Suppl 2**:** 11-46, 2016.
21. Thomson, AW, Bonham, CA, Zeevi, A: Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther Drug Monit,* 17**:** 584-591, 1995.
22. Christians, U, Jacobsen, W, Benet, LZ, Lampen, A: Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clin Pharmacokinet,* 41**:** 813-851, 2002.
23. Administration, USFaD: Epidiolex (Cannabidiol) Office of Clinical Pharmacology Integrated Review. 2017.
24. Carrier, EJ, Auchampach, JA, Hillard, CJ: Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci U S A,* 103**:** 7895-7900, 2006.
25. Hauser, N, Sahai, T, Richards, R, Roberts, T: High on Cannabis and Calcineurin Inhibitors: A Word of Warning in an Era of Legalized Marijuana. *Case Rep Transplant,* 2016**:** 4028492, 2016.
26. Nderitu, P, Doos, L, Jones, PW, Davies, SJ, Kadam, UT: Non-steroidal anti-inflammatory drugs and chronic kidney disease progression: a systematic review. *Fam Pract,* 30**:** 247-255, 2013.
27. Alexander, JC, Silge, J, Jones, S, Joshi, GP: Evaluation of Opioid Prescribing Habits Based on Analysis of a State Prescription Drug Monitoring Program. *Pain Physician,* 22**:** E425-E433, 2019.
28. Hussein, AI, Bekampis, CF, Jermyn, RT: Review of Opioid Prescribing in the Osteopathic and Ambulatory Setting. *J Am Osteopath Assoc,* 119**:** 820-832, 2019.
29. Allan, LD, Coyne, C, Byrnes, CM, Galet, C, Skeete, DA: Tackling the opioid epidemic: Reducing opioid prescribing while maintaining patient satisfaction with pain management after outpatient surgery. *Am J Surg*, 2020.
30. Eadon, MT, Desta, Z, Levy, KD, Decker, BS, Pierson, RC, Pratt, VM, Callaghan, JT, Rosenman, MB, Carpenter, JS, Holmes, AM, McDonald, CA, Benson, EA, Patil, AS, Vuppalanchi, R, Gufford, BT, Dave, N, Robarge, JD, Hyder, MA, Haas, DM, Kreutz, RP, Dexter, PR, Skaar, TC, Flockhart, DA: Implementation of a pharmacogenomics consult service to support the INGENIOUS trial. *Clin Pharmacol Ther,* 100**:** 63-66, 2016.
31. Rosenman, MB, Decker, B, Levy, KD, Holmes, AM, Pratt, VM, Eadon, MT: Lessons Learned When Introducing Pharmacogenomic Panel Testing into Clinical Practice. *Value Health,* 20**:** 54-59, 2017.
32. Collins, KS, Pratt, VM, Stansberry, WM, Medeiros, EB, Kannegolla, K, Swart, M, Skaar, TC, Chapman, AB, Decker, BS, Moorthi, RN, Eadon, MT: Analytical validity of a genotyping

assay for use with personalized antihypertensive and chronic kidney disease therapy. *Pharmacogenet Genomics,* 29**:** 18-22, 2019.
33. Spiech, KM, Tripathy, PR, Woodcock, AM, Sheth, NA, Collins, KS, Kannegolla, K, Sinha, AD, Sharfuddin, AA, Pratt, VM, Khalid, M, Hains, DS, Moe, SM, Skaar, TC, Moorthi, RN, Eadon, MT: Implementation of a Renal Precision Medicine Program: Clinician Attitudes and Acceptance. *Life (Basel),* 10, 2020.
34. Levy, KD, Decker, BS, Carpenter, JS, Flockhart, DA, Dexter, PR, Desta, Z, Skaar, TC: Prerequisites to implementing a pharmacogenomics program in a large health-care system. *Clin Pharmacol Ther,* 96**:** 307-309, 2014.
35. Eadon, MT: Genetic Variants Contributing to Colistin Cytotoxicity: Identification of TGIF1 and HOXD10 Using a Population Genomics Approach. 18, 2017.
36. Tillman, EM, Skaar, TC, Eadon, MT: Nephrotoxicity in a Patient With Inadequate Pain Control: Potential Role of Pharmacogenetic Testing for Cytochrome P450 2D6 and Apolipoprotein L1. *Frontiers in pharmacology,* 10**:** 1511, 2019.
37. Tillman, EM, Smetana, KS, Bantu, L, Buckley, MG: Pharmacologic Treatment for Pediatric Gastroparesis: A Review of the Literature. *J Pediatr Pharmacol Ther,* 21**:** 120-132, 2016.
38. Tillman, EM, Ireton-Jones, C: To Eat or Not to Eat: A Commentary on Eating Issues That Affect Home Parenteral Nutrition and Home Enteral Nutrition Consumers. *Nutr Clin Pract,* 31**:** 155-157, 2016.
39. Tillman, EM, Norman, JL, Huang, EY, Lazar, LF, Crill, CM: Evaluation of parenteral nutrition-associated liver disease in infants with necrotizing enterocolitis before and after the implementation of feeding guidelines. *Nutr Clin Pract,* 29**:** 234-237, 2014.
40. Collins, KS, Metzger, IF, Gufford, BT, Lu, JB, Medeiros, EB, Pratt, VM, Skaar, TC, Desta, Z: Influence of Uridine Diphosphate Glucuronosyltransferase Family 1 Member A1 and Solute Carrier Organic Anion Transporter Family 1 Member B1 Polymorphisms and Efavirenz on Bilirubin Disposition in Healthy Volunteers. *Drug Metab Dispos,* 48**:** 169-175, 2020.
41. Masters, AR, Gufford, BT, Lu, JB, Metzger, IF, Jones, DR, Desta, Z: Chiral Plasma Pharmacokinetics and Urinary Excretion of Bupropion and Metabolites in Healthy Volunteers. *J Pharmacol Exp Ther,* 358**:** 230-238, 2016.
42. Masters, AR, McCoy, M, Jones, DR, Desta, Z: Stereoselective method to quantify bupropion and its three major metabolites, hydroxybupropion, erythro-dihydrobupropion, and threo-dihydrobupropion using HPLC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci,* 1015-1016**:** 201-208, 2016.
43. Collins, KS, Cheng, YH, Ferreira, RM, Gao, H, Dollins, MD, Janosevic, D, Khan, NA, White, C, Dagher, PC, Eadon, MT: Interindividual Variability in Lymphocyte Stimulation and Transcriptomic Response Predicts Mycophenolic Acid Sensitivity in Healthy Volunteers. *Clin Transl Sci*, 2020.
44. Hato, T, Maier, B, Syed, F, Myslinski, J, Zollman, A, Plotkin, Z, Eadon, MT, Dagher, PC: Bacterial sepsis triggers an antiviral response that causes translation shutdown. *J Clin Invest,* 129**:** 296-309, 2019.
45. Fu, Y, Koh, B, Kuwahara, M, Ulrich, BJ, Kharwadkar, R, Yamashita, M, Kaplan, MH: BATF-Interacting Proteins Dictate Specificity in Th Subset Activity. *J Immunol,* 203**:** 1989-1998, 2019.
46. Kaplan, MH, Kaminski, M, Estes, JM, Gitlin, SD, Zahn, J, Elder, JT, Tejasvi, T, Gensterblum, E, Sawalha, AH, McGowan, JP, Dosik, MH, Direskeneli, H, Direskeneli, GS, Adebamowo, SN, Adebamowo, CA, Sajadi, M, Contreras-Galindo, R: Structural variation of centromeric endogenous retroviruses in human populations and their impact on cutaneous T-cell lymphoma, Sezary syndrome, and HIV infection. *BMC Med Genomics,* 12**:** 58, 2019.

47. Xie, MM, Chen, Q, Liu, H, Yang, K, Koh, B, Wu, H, Maleki, SJ, Hurlburt, BK, Cook-Mills, J, Kaplan, MH, Dent, AL: T follicular regulatory cells and IL-10 promote food antigen-specific IgE. *J Clin Invest*, 2020.
48. Eadon, MT: Implementation of a pharmacogenomics consult service to support the INGENIOUS trial. *Value Health,* 100**:** 63-66, 2016.
49. Yeung, CK, Shen, DD, Thummel, KE, Himmelfarb, J: Effects of chronic kidney disease and uremia on hepatic drug metabolism and transport. *Kidney Int,* 85**:** 522-528, 2014.
50. Cho, DY, Shen, JH, Lemler, SM, Skaar, TC, Li, L, Blievernicht, J, Zanger, UM, Kim, KB, Shin, JG, Flockhart, DA, Desta, Z: Rifampin enhances cytochrome P450 (CYP) 2B6-mediated efavirenz 8-hydroxylation in healthy volunteers. *Drug Metab Pharmacokinet,* 31**:** 107-116, 2016.
51. Robarge, JD, Metzger, IF, Lu, J, Thong, N, Skaar, TC, Desta, Z, Bies, RR: Population Pharmacokinetic Modeling To Estimate the Contributions of Genetic and Nongenetic Factors to Efavirenz Disposition. *Antimicrob Agents Chemother,* 61, 2017.
52. Desta, Z, Metzger, IF, Thong, N, Lu, JB, Callaghan, JT, Skaar, TC, Flockhart, DA, Galinsky, RE: Inhibition of Cytochrome P450 2B6 Activity by Voriconazole Profiled Using Efavirenz Disposition in Healthy Volunteers. *Antimicrob Agents Chemother,* 60**:** 6813-6822, 2016.
53. Tacrolimus CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW. In: RESEARCH, C. F. D. E. A. (Ed.), 2017.
54. Ujvary, I, Hanus, L: Human Metabolites of Cannabidiol: A Review on Their Formation, Biological Activity, and Relevance in Therapy. *Cannabis Cannabinoid Res,* 1**:** 90-101, 2016.
55. Xu, C, Quinney, SK, Guo, Y, Hall, SD, Li, L, Desta, Z: CYP2B6 pharmacogenetics-based in vitro-in vivo extrapolation of efavirenz clearance by physiologically based pharmacokinetic modeling. *Drug Metab Dispos,* 41**:** 2004-2011, 2013.
56. Jeong, S, Nguyen, PD, Desta, Z: Comprehensive in vitro analysis of voriconazole inhibition of eight cytochrome P450 (CYP) enzymes: major effect on CYPs 2B6, 2C9, 2C19, and 3A. *Antimicrob Agents Chemother,* 53**:** 541-551, 2009.
57. Couch, DG, Tasker, C, Theophilidou, E, Lund, JN, O'Sullivan, SE: Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. *Clin Sci (Lond),* 131**:** 2611-2626, 2017.
58. Petrosino, S, Verde, R, Vaia, M, Allara, M, Iuvone, T, Di Marzo, V: Anti-inflammatory Properties of Cannabidiol, a Nonpsychotropic Cannabinoid, in Experimental Allergic Contact Dermatitis. *J Pharmacol Exp Ther,* 365**:** 652-663, 2018.
59. Zgair, A, Lee, JB, Wong, JCM, Taha, DA, Aram, J, Di Virgilio, D, McArthur, JW, Cheng, YK, Hennig, IM, Barrett, DA, Fischer, PM, Constantinescu, CS, Gershkovich, P: Oral administration of cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation. *Sci Rep,* 7**:** 14542, 2017.

Appendix

Flockhart Table