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Statistical Analysis Plan

SCR-016 Clinical Study to Evaluate Cannabidiol Liver Enzyme Elevations and Drug Interactions

Sponsor: U.S. Food and Drug Administration
White Oak Building #64, Room 2072
10903 New Hampshire Avenue
Silver Spring, MD 20993

Sponsor Study Lead: David Strauss, MD, PhD
Director, Division of Applied Regulatory Science
U.S. Food and Drug Administration
Telephone: 301-796-6323
Email: david.strauss@fda.hhs.gov

Sponsor Medical Monitor: Keith Burkhart, MD
U.S. Food and Drug Administration
Telephone: 301-796-2226
Email: keith.burkhart@fda.hhs.gov

Project Manager: Jeffry Florian, PhD
U.S. Food and Drug Administration
Telephone: 301-796-4847
Email: jeffry.florian@fda.hhs.gov

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Sponsor Signatures Page

Prepared by

Pablo Salcedo -S
Digitally signed by Pablo Salcedo -S
Date: 2024.04.08 16:35:18 -04'00'

Pablo Salcedo, MD
Pharmacokineticist
Division of Applied Regulatory Science
U.S. Food and Drug Administration

Date

Jeffry Florian -S
Digitally signed by Jeffry Florian -S
Date: 2024.04.08 16:40:08 -04'00'

Approved by

Jeffry Florian, PhD
Associate Director
Division of Applied Regulatory Science
U.S. Food and Drug Administration

Date

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1 Introduction

This document outlines the proposed statistical methods for data analysis of data collection from Protocol 'SCR-016 Clinical Study to Evaluate Cannabidiol Liver Enzyme Elevations and Drug Interactions.'

2 Study Objectives

2.1 Part 1

- To characterize the effects of daily cannabidiol (CBD) use at a dose within the range of what consumers are taking as unapproved CBD products on liver enzyme elevations and endocrine measures.

2.2 Part 2 (Drug Interactions)

- To characterize the effects of daily CBD use at a dose within the range of what consumers are taking as unapproved CBD products on drug interactions.

3 Study Endpoints

3.1 Primary Endpoints

3.1.1 Part 1

- Percentage of participants with an ALT (based on consensus criteria*) or AST liver enzyme elevation greater than three times the upper limit of normal (>3 × ULN) [*Consensus criteria for ALT elevation: ULN for ALT will be 33 U/L for males and 25 U/L for females].

3.1.2 Part 2

- Comparison of the area under the plasma concentration-time curve (AUC) and maximum concentration (C_{max}) of citalopram when administered alone versus when co-administered with CBD after 7 days of CBD dosing.
- Comparison of the morphine AUC and C_{max} when administered alone versus when co-administered with the first dose of CBD and after 7 days of CBD dosing.

3.2 Secondary Endpoints

3.2.1 Part 1

- Percentage of participants meeting withdrawal criteria for potential drug-induced liver injury (DILI).
- Change from baseline in total testosterone and inhibin B in male participants after CBD administration compared to placebo.

- Change from baseline in thyroid stimulating hormone (TSH), total T3, and free T4 after CBD administration compared to placebo.

3.2.2 Part 2

- Comparison of the AUC and C_{max} of morphine metabolites when administered alone versus when co-administered with CBD.

3.3 Exploratory Endpoints

3.3.1 Part 1

- Assessment of liver-related adverse events.
- Summary of CBD and metabolite pharmacokinetics.
- Proportion of subjects with abnormal laboratory (e.g., liver, endocrine) tests.
- Changes in additional laboratory, vital signs, and plasma proteome assessments.

3.3.2 Part 2

- Additional PK parameters for citalopram, morphine, and metabolites, such as time of maximum concentration, half-life, apparent clearance, and apparent volume of distribution.
- PK parameters for CBD and metabolites.

4 Study Overview

4.1 Study Design

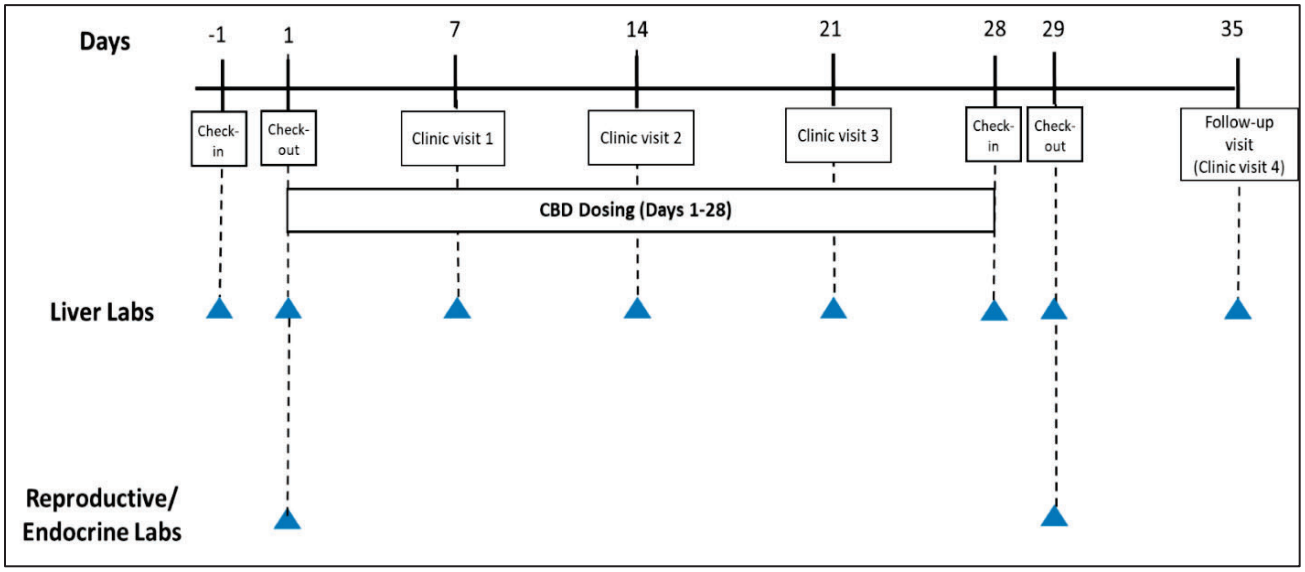
4.1.1 Part 1

Part 1 is a randomized, double-blind, placebo-controlled, parallel study in 200 subjects (150 subjects receiving 5 mg/kg/day of CBD and 50 subjects receiving placebo). Subjects will report to the study site for screening from Days -28 to -2 and then will return to the site on Day -1 for check-in, baseline assessments, and randomization. Subjects will check out from the clinical site on Day 1 (1 night in-house stay) following dosing and study assessments. Subjects will receive CBD or placebo for 28 days and will return to the study clinic on Day 28 for a single in-house day with check-out on Day 29. Subjects will have 3 clinic visits in between the in-house stays. The clinical site will contact subjects daily to confirm drug administration. Subject will have a final follow-up on Day 35.

The study design is as follows:

	CBD Dosing Days 1-28 (5mg/kg/day)						
Day -1	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 29**	**Day 35**

Check-in	Check-out	Clinic visit 1	Clinic visit 2	Clinic visit 3	Check-in	Check-out	Follow-up visit (Clinic visit 4)



4.1.2 Part 2 (Drug Interactions)

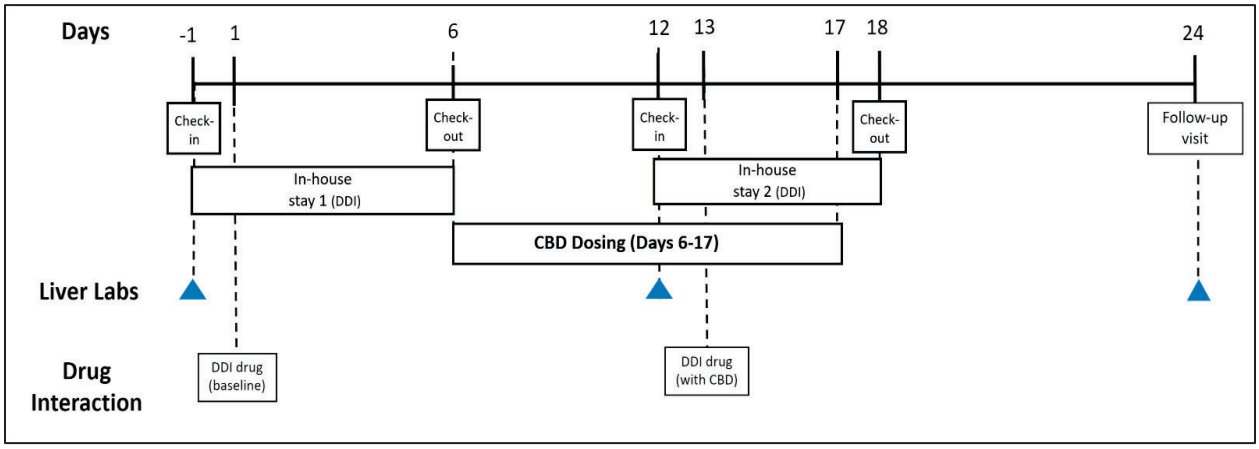
Part 2 is an open-label, sequential study in 40 subjects (two separate cohorts of 20 subjects). In the Part 2 citalopram drug-drug interaction (DDI) cohort, subjects will report to the study site for screening from Days -28 to -2 and then will return to the site on Day -1 for check-in and baseline assessments. Subjects will check-out from the clinical site on Day 6 (6 nights in-house stay) following dosing and study assessments. Subjects will return to the study clinic on Day 12 for another 6 nights in-house stay with check-out on Day 18. Subjects will have one follow-up visit after the second check-out (Day 24). Subjects will receive CBD for 12 days (Day 6 to 17) and citalopram on Day 1 and Day 13.

In the Part 2 morphine DDI cohort, subjects will report to the study site for screening from Days -28 to -2 and then will return to the site on Day -1 for check-in and baseline assessments. Subjects will check-out from the clinical site on Day 6 (6 nights in-house stay) following dosing and study assessments. Subjects will return to the study clinic on Day 10 for a 3 night in-house stay with check-out on Day 13. Subjects will receive CBD for 9 days (Day 4 to 12) and morphine on Day 1, Day 4, and Day 11. Subjects will have one follow-up visit after the second check-out (Day 19).

The study design is as follows:

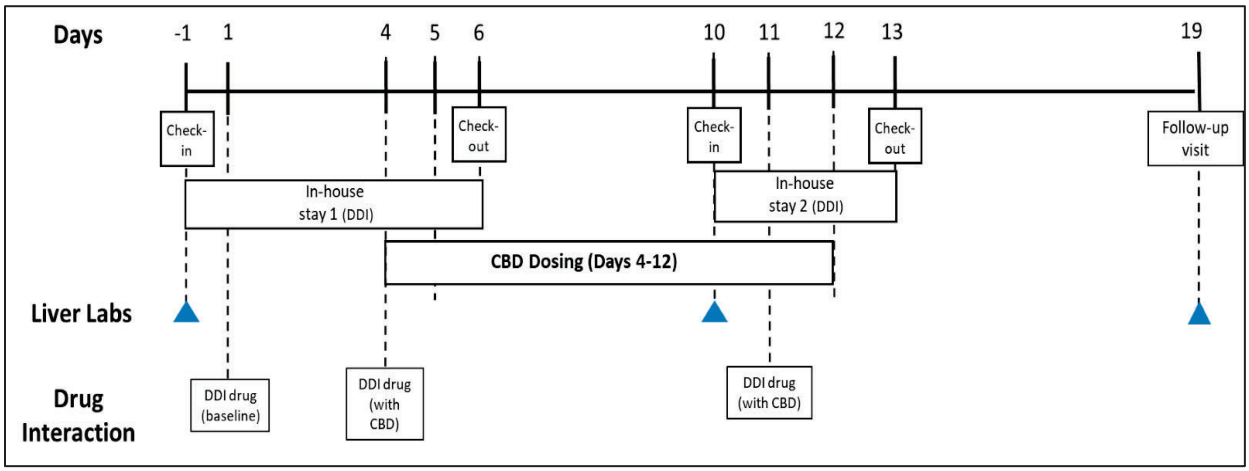
Citalopram DDI cohort

Day -1	Day 1	CBD Dosing Days 6-17 (5 mg/kg/day)			Day 18	Day 24
		Day 6	Day 12	Day 13		
Check-in	DDI drug Dosing (baseline)	Check-out	Check-in	DDI drug Dosing (w/ CBD)	Check-out	Follow-up visit



Morphine DDI cohort

Day -1	Day 1	CBD Dosing Days 4-12 (5 mg/kg/day)				Day 13	Day 19
		Day 4	Day 6	Day 10	Day 11		
Check-in	DDI drug Dosing (baseline)	DDI drug Dosing (w/ CBD)	Check-out	Check-in	DDI drug Dosing (w/ CBD)	Check-out	Follow-up visit



4.2 Sample Size

4.2.1 Part 1

A total of 240 healthy subjects are planned for enrollment and approximately 190 of these will be administered CBD. In Part 1, 200 healthy subjects will be randomized to 5 mg/kg/day of CBD (150 subjects) or placebo (50 subjects). The primary endpoint is the percentage of CBD administered participants with an ALT (based on consensus criteria[*]) or AST liver enzyme elevation greater than three times the ULN. Assuming the true event rate is 6%, the study has 95% probability of observing 5 or more events with ALT or AST liver enzyme elevation greater than three times the ULN. Assuming the true observed drug-induced liver injury event rate is 2%, the study has a 93% percent probability of observing 1 or more event.

In Part 1, change from baseline comparisons will be performed for various endocrine laboratory measures (e.g., total testosterone, inhibin B, TSH, total T3, and free T4). Sample size for the study is not powered for any of these comparisons. No adjustments are planned for multiplicity.

4.2.2 Part 2 (Drug Interactions)

In Part 2, 40 healthy subjects will receive either oral citalopram (20 subjects) or morphine (20 subjects). Comparisons will be performed on changes in exposure for two different substrate drugs following administration with CBD. Assuming no more than 20% discontinuations per arm, 30% intra-subject variability, and a two-sided test at the 0.05 significance level, the sample size would have 90% power to detect a 30% difference in exposure when the substrate drugs are co-administered with CBD.

5 Analysis Populations

5.1.1 Part 1

The liver safety analysis population will include all subjects who receive at least 1 dose of the study drug and have at least one on-treatment liver and hematology lab assessment. Subjects in the liver safety analysis population will be used for the planned primary, secondary, and exploratory analyses related to liver enzyme elevations and liver events.

Sensitivity analyses for liver safety will also be conducted for the following additional analysis populations defined based on completion of minimum durations of continuous study drug dosing:

- Subjects who complete at least 1 week of dosing and have at least one on-treatment liver and hematology lab assessment (i.e., excludes subjects who discontinue due to tolerability in the first week)

- Subjects who complete at least 2 weeks of dosing, were not discontinued due to adherence, and have at least one on-treatment liver and hematology lab assessment (i.e., excludes subjects who discontinue due to tolerability and those discontinued for repeatedly missing scheduled dosing as assessed by daily site check-ins and out-patient visit product accountability)

The laboratory analysis population will include all subjects that have endocrine, laboratory, vital signs, and proteomics measures from pre-dose Day 1 and check-out on Day 29 (time-matched to Day 1). These criteria will apply to separately to each individual endocrine, laboratory (i.e., free and total testosterone, inhibin B, TSH, total T3, free T4, lipid panel), vital sign, or proteomics measure. Subjects in the laboratory analysis population will be used for the planned secondary and exploratory analyses related to endocrine, laboratory, vital signs, and proteomics measures.

The PK population will include all subjects who receive at least one dose of study drug and have at least one on-treatment PK sample collected. Subjects in the PK analyses population will be used for the planned exploratory analyses related to CBD pharmacokinetics.

The safety population will include all subjects who receive at least one dose of any of the study drugs.

5.1.2 Part 2

The PK population will include all subjects who receive the study drug and have at least 1 estimable PK parameter ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, or Cmax) after dosing with the substrate drug alone and with the substrate drug in combination with CBD. Subjects in the PK analyses population will be used for the planned primary, secondary, and exploratory analyses related to CBD pharmacokinetics.

The safety population will include all subjects who receive at least one dose of any of the study drugs.

6 Data Screening and Quality Assurance

6.1 Missing Data

The following approaches will be used for the handling of missing and incomplete data:

- Missing data will not be imputed.
- Data that are excluded from the descriptive or inferential analyses will be included in the subject data listings. This will include data from unscheduled visits or extra measurements that may arise from 2 or more analyses of the biofluid sample at the same time point.
- PK measurements below the lower limit of quantification will be set to zero for all analyses.

6.2 Data Quality Assurance

Completed eCRFs are required for each subject randomly assigned to treatment. Electronic data entry will be accomplished through the ClinSpark® remote electronic data capture system, which allows for on-site data entry and data management. This system provides immediate, direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records.

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system. The completed dataset and their associated files are the sole property of the sponsor and should not be made available in any form to third parties, except for appropriate governmental health or regulatory authorities, without written permission of the sponsor.

6.3 Data Sharing

De-identified subject-level data may be released to other researchers (including through a data warehouse or as a part of a publication) to enable secondary research. Additional secondary research may also be performed by the sponsor.

7 General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings, but not included in the calculation of summary statistics. Demographic and baseline characteristics will be summarized by study Part and by treatment for all subjects.

7.1 Subject Disposition

The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented for each study Part. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized for each study Part.

7.2 Demographic and Baseline Characteristics

Descriptive statistics will be used to summarize demographic and baseline subject characteristics for each study Part. For continuous variables, the mean, median, standard deviation (SD), minimum, and maximum values will be reported. For categorical (nominal) variables, the number and percentage of subjects (or observations) will be reported.

7.3 Primary Analysis

7.3.1 Part 1

Percentage of participants with an ALT (based on consensus criteria) or AST liver enzyme elevation greater than three times the upper limit of normal (> 3 × ULN)

The primary endpoint for Part 1 is the percentage of subjects with an ALT (based on consensus criteria) or AST liver enzyme elevation greater than three times the upper limit of normal (ULN). The proportions of subjects exceeding 3x ULN for ALT and AST will be summarized separately for each treatment group. Results will be reported as percentages with a 95% confidence interval (CI) based on a Kaplan-Meier analysis. The analysis will be performed on the liver safety analysis population. Separate percentages will be reported for both treatment groups. The analysis will be repeated for all populations described as part of sensitivity analyses.

7.3.2 Part 2 (Drug Interactions)

Comparison of the area under the plasma concentration-time curve (AUC) and C_{max} of citalopram when administered alone versus when co-administered with CBD after 7 days of CBD dosing

The primary analysis for the Part 2, citalopram cohort will include a comparison of the area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\text{-}inf}$), AUC from time 0 to the last quantifiable concentration ($AUC_{0\text{-}t}$), and C_{max} of citalopram when administered alone versus when co-administered with CBD.

The PK parameters ($AUC_{0\text{-}inf}$ and $AUC_{0\text{-}t}$) will be analyzed using noncompartmental methods based on actual sampling times, using dosing of citalopram as time zero. $AUC_{0\text{-}inf}$ will only be included if the subject has 3 or more concentration values on the terminal portion of the pharmacokinetic curve and with an adjusted coefficient of determination (R^2) greater than 0.80. C_{max} will be based on maximum observed concentration of citalopram.

Using linear mixed-effects modeling, comparisons of log-transformed PK parameters will be performed. Treatment will be a fixed effect. Subject will be included as a random effect on the intercept. Treatment differences on the log-scale will be estimated for the PK parameters. Geometric mean ratio and 90% CI will be obtained by exponentiation of the treatment effect and 90% CI based on the log-transformed scale. Testing will be two-sided, and a significant change will be concluded if the two-sided 90% CI excludes 1. The analysis will be performed on the Part 2 PK population. No adjustment will be performed for multiplicity.

Comparison of the morphine AUC and Cmax when administered alone versus when co-administered with the first dose of CBD and after 7 days of CBD dosing

The primary analysis for Part 2 morphine cohort will also include a comparison of the $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and C_{max} of morphine when administered alone versus when co-administered with CBD. Comparisons will be performed between morphine administration alone and after co-administration with CBD for 1-day and 7-days. The PK parameters will be analyzed using the same methods described above. Testing will be two-sided, and a significant change will be concluded if the two-sided 90% CI excludes 1. The analysis will be performed on the Part 2 PK population. No adjustment will be performed for multiplicity.

7.4 Secondary Analyses

7.4.1 Part 1

Percentage of participants meeting withdrawal criteria for potential drug-induced liver injury (DILI)

The secondary liver safety endpoint is the percentage of participants meeting withdrawal criteria for potential drug-induced liver injury, as defined in section 4.7.3.2.1 of the study protocol (Assessment and Reporting of Potential Cases of Drug-Induced Liver Injury). Analysis and reporting will be performed in similar fashion to the Part 1 primary endpoint using the liver safety analysis population and populations described as part of sensitivity analyses.

Change from baseline in total testosterone and inhibin B in male participants after CBD administration compared to placebo

Change from baseline in total testosterone and inhibin B in males after CBD administration compared to placebo will be analyzed using a mixed-effect analysis approach. The model will include treatment as fixed effects and subject as a random effect. Contrasts from the model will be used to compare CBD vs placebo. Testing will be two-sided, and a significant increase will be concluded if the two-sided 95% CI excludes 0.

Change from baseline in thyroid stimulating hormone (TSH), total T3, and free T4 after CBD administration compared to placebo

Change from baseline in thyroid stimulating hormone, total T3, and free T4 in both males and females after CBD administration compared to placebo will also be assessed. These analyses will be conducted in the same fashion as described above for total testosterone and inhibin B. The analysis will be conducted on the laboratory analysis population.

7.4.2 Part 2 (Drug Interactions)

Comparison of the AUC and C_{max} of morphine metabolites when administered alone versus when co-administered with CBD

Part 2 secondary PK endpoints, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and C_{max} for morphine metabolites (alone and when co-administered with CBD), will be analyzed using the same approach as the Part 2 primary PK endpoints described above in 7.3.2. The analysis will be conducted using the Part 2 PK population (morphine cohort).

7.5 Exploratory Analyses

7.5.1 Part 1

Assessment of liver-related adverse events

As part of the exploratory analyses for Part 1, liver-related adverse events will be assessed according to FDA's Guidance for Industry on Drug-Induced Liver Injury: Premarketing Clinical Evaluation [1]. Categorical and visual summaries of liver-related adverse events will be produced based on the following criteria by treatment arm. Results will be reported as percentages with a 95% CI. The analysis will be performed on the liver safety analysis population and repeated for all populations described as part of sensitivity analyses:

- Elevations of 3x, 5x, 10x, and 20x the ULN for both ALT and AST.
- Any elevations in bilirubin.
- Any elevations in TBL (Total Bilirubin) > 2x ULN.
- Any elevations in ALP (Alkaline Phosphatase) > 1.5x ULN.
- Elevations in ALT or AST > 3x ULN accompanied by elevated bilirubin (> 1.5x ULN, >2x ULN).
- Elevations in ALT or AST in temporal association with nausea, vomiting, anorexia, abdominal pain, or fatigue.

Summary of CBD and metabolite pharmacokinetics

Steady state CBD and metabolite drug concentrations will be determined using descriptive statistics and the Part 1, PK population.

Percentage of subjects with abnormal laboratory (e.g., liver, endocrine) tests

The percentage of subjects meeting predefined criteria for abnormal liver, endocrine, and other laboratory tests will be summarized along with 95% CI for each treatment group. All analyses will be conducted using the safety population.

Changes in additional laboratory assessments

For other laboratory measures, the change from baseline will be compared between treatment groups (CBD and placebo) using a mixed-effect analysis approach. These analyses will be conducted in the same fashion as described for total testosterone and inhibin B. The analysis will be conducted on the laboratory analysis population. An analysis plan for plasma proteomic markers will be specified in a separate document.

7.5.2 Part 2 (Drug Interactions)

Additional PK parameters for citalopram, morphine, and metabolites, such as time of maximum concentration, half-life, apparent clearance, and apparent volume of distribution.

Additional exploratory PK parameters for citalopram, morphine, and metabolites, such as time of maximum concentration (T_{max}), half-life ($t_{1/2}$), terminal elimination rate constant (K_{el}), apparent clearance (CL/F), and apparent volume of distribution (V/F) will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using statistical software. Mean and individual concentration time profiles will be presented in graphs.

- T_{max} – Time of maximum concentration corresponding to observed C_{max}
- K_{el} - The terminal elimination rate constant, which will be determined from the terminal slope of the log-concentration curve using linear regression
- Half-life ($t_{1/2}$) - calculated using the elimination rate constant as $\ln(2)/K_{el}$
- CL/F – calculated using dose/$AUC_{0\text{-}inf}$
- V/F – calculated using CL/F / K_{el}

PK parameters for CBD and metabolites

PK parameters for CBD and metabolites, such as AUC_{ss} (steady state AUC from time of CBD dosing with the substrate to time of next CBD dose), C_{max}, T_{max}, $t_{1/2}$, K_{el}, CL/F, and V/F will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using statistical software. Parameters will be calculated for the first CBD dose on Day 4 and 11 (morphine cohort) and Day 13 (citalopram cohort). Mean and individual concentration time profiles will be presented in graphs.

8 Safety Analyses

8.1.1 Adverse Events

All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. The incidence of TEAEs, organized by system organ class and frequency, will be

summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided.

8.1.2 Clinical Laboratory Tests (Other Than Specified in Planned Analyses)

Clinical laboratory results (hematology, serum chemistry, and urinalysis) will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum). Clinical laboratory results will be classified as normal or abnormal, according to the reference ranges of the individual parameter. The number and percentage of subjects with abnormal laboratory results will be provided. No statistical testing will be performed on clinical laboratory data.

8.1.3 Vital Sign Measurements (Other Than Specified in Planned Analyses)

Vital sign measurements and changes from baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time points as specified in the protocol.

8.1.4 Safety 12-lead Electrocardiograms

12-lead ECGs will be obtained with the subjects in the supine position for a minimum of 10 minutes before recording. ECGs will be overread by a physician. If an abnormality is observed, the subject will be instructed to follow up with his or her personal physician.

8.1.5 Physical Examinations

Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs.

8.1.6 Other Safety Data

All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided informed consent will be summarized for each subject.

9 References

1. U.S. Food and Drug Administration (FDA) Science Board. Drug-Induced Liver Injury: Premarking Clinical Evaluation. Retrieved from https://www.fda.gov/media/116737/download.