Cyclerion Therapeutics, Inc.

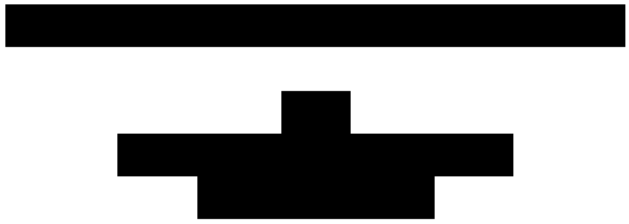
C6463-201

Phase 2a safety, tolerability, pharmacokinetic, and pharmacodynamic study in individuals with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)

21 March 2022

Statistical Analysis Plan

Version *2*



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MODIFICATION HISTORY

Version 2 of this SAP was created to align with changes made in C6463-201 Protocol Amendment 2, dated 23 April 2021 and Amendment 3, dated 23 July 2021. Major changes made to this SAP are summarized below.

Item	Section(s)	Description of Change(s)
1	1 (Introduction)	Updated to reflect the current version of the protocol, C6463-201 Protocol Amendment 3, dated 23 July 2021.
2	3.1 (Overall Study Design and Plan)	Figure 1 was replaced with the updated figure in Protocol Amendment 3
3	16.1 Schedule of event	Table 3 was replaced with the updated table in Protocol Amendment 3
4	3.2.2 (Exploratory Endpoints)	Updated to reflect Protocol Amendment 3
5	4.3.3	Updated definition of PD Evaluable Population
6	5.1 (Disposition), 5.2 (Protocol Deviations)	Inserted phrase 'if applicable' for summaries related to the PD Evaluable population
7	7.2.2 (Compliance)	Revised text from ~~Compliance is defined as the total doses taken divided by the total doses expected to be taken, times 100.~~ to Compliance is defined as the total dosage taken divided by the total dosage expected to be taken, times 100.
8	9.1.8 Adverse Events of Special Interest	Updated text to clarify that AESIs will be presented as collected in the eCRFs, and removed Table 1 (Criteria for AESIs) to be consistent with Protocol Amendments 2 & 3
9	9.2 (Clinical Laboratory)	For urinalysis, summary tables will be performed as data permit.
10	9.3 (Vital Sign Measurements)	Added descriptive statistics for change from baseline in SBP, DBP, MAP, and PCI criteria for standing and orthostatic SBP, DBP, and pulse.
11	10 (Questionnaires)	Revised analyses to be conducted on the safety population instead of the PD evaluable population.
12	11 (PK)	Revised imputation of BLQ values for calculation of summary statistics. Also included additional text from protocol.
15	12. (PD)	Clarified endpoints to be summarized and those presented only in listings

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List of Abbreviations

1. Introduction

Study C6463-201 is an open-label, single-arm study in approximately 20 participants diagnosed with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of IW-6463 administered daily for up to 29 days.

The statistical analysis plan (SAP) provides a more technical and detailed elaboration of the data presentations and statistical analyses of the efficacy and safety data as specified in the final protocol for Study C6463-201 (C6463-201 Protocol Amendment 3, dated 23 July 2021). Specifications for tables, figures, and data listings are contained in a separate document.

2. Objectives

2.1. Primary Objective

The primary objective of the study is to evaluate the safety and tolerability of IW-6463 when administered to participants with MELAS.

2.2. Exploratory Objectives

The exploratory objectives of the study are as follows:

- To evaluate the PK of IW-6463 when administered to participants with MELAS.
- To evaluate the effect of IW-6463 on exploratory plasma biomarkers in participants with MELAS.
- To evaluate the effect of IW-6463 on CBF in participants with MELAS.
- To evaluate the effect of IW-6463 on brain activity as measured by functional magnetic resonance imaging (fMRI) in participants with MELAS.
- To evaluate the effect of IW-6463 on brain metabolites as measured by magnetic resonance spectroscopy (MRS).
- To evaluate the effect of IW-6463 on fatigue as measured by the Modified Fatigue Impact Scale (MFIS).
- To evaluate the effect on IW-6463 on cognitive function as measured by the Patient-Reported Outcomes Measurement Information System (PROMIS) Item Bank v2.0–Cognitive function.

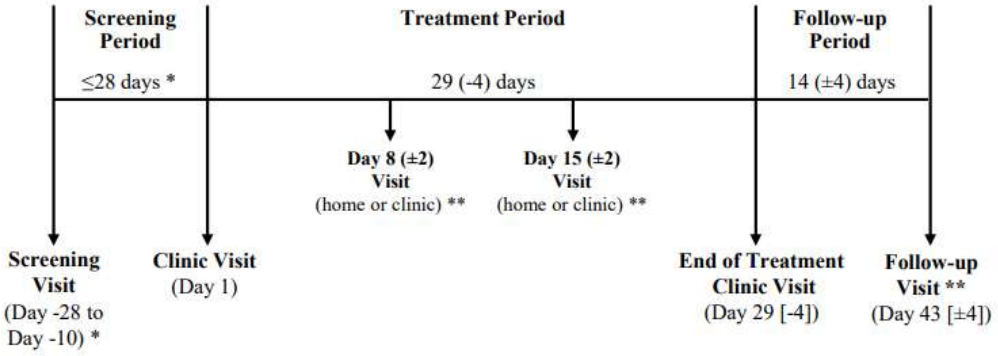
3. Investigational Plan

3.1. Overall Study Design and Plan

C6463-201 is an open-label, single-arm study evaluating the safety, tolerability, PK, and PD of IW-6463 when administered for up to 29 days to participants diagnosed with MELAS. Because this is the first study evaluating IW-6463 in a patient population, the primary objective is safety and tolerability; see Section 2 and Section 3.2 for a complete list of study objectives and their corresponding endpoints.

To complete the study, each participant will progress through 3 distinct periods as described below. For details regarding the timing of the specific procedures and assessments, see the Schedule of Events (**Table**). A study schematic is shown in **Figure 1**.

Figure 1: Study Schematic



All participants will receive open-label IW-6463 once daily for up to 29 days during the Treatment Period.

* Screening assessments may take place over more than 1 day. However, all Screening procedures must be completed at least 10 but not more than 28 days before the start of the Treatment Period.

** The Day 8, Day 15, and Follow-up visits can be conducted either at home or in the study clinic, per participant/Study Center preference.

3.2. Study Endpoints

3.2.1. Primary Endpoint

The primary endpoints of the study are as follows:

- Number of participants with study drug dose reductions or discontinuations due to ≥1 treatment-emergent adverse event (TEAE)
- Incidence and severity of AEs

3.2.2. Exploratory Endpoints

The exploratory endpoints of the study are as follows:

- Plasma and cerebral spinal fluid (CSF) concentrations of IW-6463 as data permit at assessed timepoints.
- PK parameters as data permit, including but not limited to area under the concentration-time curve from time zero (predose) to 24 hours postdose (AUC_{tau}), maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), apparent terminal half-life ($t_{1/2}$), plasma concentration observed at the end of a dosing interval (C_{trough}), apparent total plasma clearance (CL/F), apparent volume of distribution (V_z/F), and CSF: plasma concentration ratio.
- Change from baseline in plasma biomarker concentrations on Day 29. [redacted]
- Change from baseline in CBF as measured by arterial spin labeling (ASL) on Day 29
- Change from baseline in fMRI-blood-oxygen-level-dependent (BOLD) signals during resting state and a visual stimulus on Day 29, as data permit.
- Change from baseline in brain metabolite [redacted] levels by H-MRS on Day 29.
- Change from baseline in MFIS scores (total, physical, cognitive, and psychosocial subscores) on Day 29.
- Change from baseline in PROMIS Item Bank v2.0–Cognitive function total score on Day 29.

3.3. Treatments

All eligible participants will receive open-label IW-6463 at a starting dose of 15 mg once a day (QD). Participants will be instructed to take IW-6463 once per day on Days 2 through 28 at a time consistent (preferably ±1 hour) with the in-clinic study drug administration on Day 1.

Exception/Note: For the Day 8, 15, and 29/ End of Treatment (EOT) visits, participants will take their dose during the visit to allow for the appropriate timing of pre- and post-

dose assessments. Attempts should be made to schedule the Day 8 and 15 visits (at home or in clinic) such that dosing can still occur at approximately the same time as the Day 1 and subsequent doses.

Participants are to record the date and time of each at-home dose administration in their diary.

3.4. Dose Adjustment/Modifications

The decision to de-escalate IW-6463 dosing in an individual participant will be made jointly by the Investigator and PPD Medical Monitor, on a per-participant basis.

Participants who require a dose adjustment from the 15-mg QD dose will be de-escalated to a 10-mg QD dose. Initiation of the new dose level should occur only after the Treatment-emergent AE(s) prompting the dose reduction has/have resolved or has improved to be considered mild in severity.

If the 10-mg IW-6463 QD dose is not tolerated, the participant will be discontinued from study drug.

4. General Statistical Considerations

Continuous variables will be summarized using the following descriptive statistics: n, mean, median, standard deviation, minimum, maximum. For the summary of continuous clinical outcomes, vital signs, and PD data, the 95% confidence intervals (CI) will also be presented. For the summary statistics of all numerical variables, unless otherwise specified, minimum and maximum will be displayed to the same level of precision as reported. Mean and median will be displayed to one level of precision greater than the data collected. Standard deviation will be displayed to two levels of precision greater than the data collected.

Categorical and count variables will be summarized by the number of participants (n) and the percent of participants in each category. Percentages will be presented as whole numbers. When count data are presented, the percentage will be suppressed when the count is zero. A row denoted “Missing” will be included in count tabulations only if there are missing values. The denominator for all percentages will be the number of participants within the population of interest, unless otherwise specified.

Baseline will be defined as the last non-missing assessment before administration of study drug on Day 1, unless otherwise specified.

4.1. Sample Size

A maximum of 20 participants will be assigned to receive IW-6463 such that approximately 12 evaluable participants complete the study.

The sample size selected is not based on statistical considerations. A sample size of approximately 12 evaluable participants in a rare disease indication is considered sufficient to address the primary and research objective.

4.2. Randomization, Stratification, and Blinding

Not applicable.

4.3. Analysis Population

4.3.1. Screened Population

The Screened population will include all participants who sign the informed consent form (ICF).

4.3.2. Safety Population

The safety population will include all participants who sign the ICF and take at least 1 dose of study drug.

4.3.3. PD Evaluable Population

The PD evaluable population will include all participants who complete the baseline and EOT Visit assessments, have ≥80% compliance with study drug dosing, and do not have any major protocol violation(s) that could affect their PD assessment. The PD Evaluable population will be defined only if at least 12 participants are enrolled in the study.

4.3.4. PK Evaluable Population

The PK Evaluable population will include all participants who complete the EOT Visit and have ≥1 evaluable post-dose PK assessment.

5. Participant Disposition

5.1. Disposition

A summary of the analysis populations will include the number and percentage of participants for the following categories: participants in the Screened population, Screen Failures, Safety population, PD Evaluable population (if applicable), and PK Evaluable population. All percentages will be based on the number of participants in the Screened population. Screen failures will be defined as participants who consent to participate in the clinical study but are not subsequently administered study drug (i.e., are not part of the Safety Population).
A disposition of participants will include the number and percentage of participants in the Safety population for the following categories: participants who completed the study or discontinued early, as well as the reasons for discontinuation.

A summary of the participants excluded from PD Evaluable (if applicable) and PK Evaluable analysis population and the reasons for exclusion will be presented. Percentages for exclusion reasons will be based on the number of participants excluded from the specific analysis population being summarized. Participants could be excluded from an analysis set for more than one reason.

Disposition data will be presented in a listing for the Safety population.

5.2. Protocol Deviations

Major protocol deviations will be identified and documented based on review of protocol deviations before database lock, and will be used to define the PD Evaluable Population (if applicable). The categories of major protocol deviations to be reviewed include, but are not limited to, participants who:

- Did not meet key inclusion/exclusion criteria in the judgment of the evaluability committee
- Received disallowed concomitant medication that could meaningfully impact results
- Had overall treatment compliance rate <80% or >120%

The number and percentage of participants with major protocol deviations will be summarized for the Safety Population by type of deviation. All protocol deviations will be presented in a data listing.

6. Demographics and Baseline Characteristics

6.1. Demographics

A summary of demographics and baseline information will be presented. The demographic characteristics consist of age (year), sex, race, and ethnicity. The baseline characteristics consist of baseline height (cm), baseline weight (kg), and baseline body mass index (BMI) (kg/m^2). Body mass index is calculated as (body weight in kilograms) / (height in meters) ^2.

A participant's age in years is calculated using the date of the informed consent and date of birth. Age (year), baseline height (cm), baseline weight (kg), and baseline BMI (kg/m^2) will be summarized using descriptive statistics. The number and percentage of participants by sex (Male, Female), race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, and Other) and ethnicity (Hispanic or Latino, Not Hispanic or Latino), will also be reported. Percentages will be based on the total number of participants in the Safety population.

Participant demographic and baseline characteristics will be presented in a listing for the Safety population.

6.2. Drug and Alcohol Screen

Drug and alcohol screening test will be conducted as scheduled in **Table** . Test results will be presented in a listing for participants in the Safety population.

6.3. Smoking History

Smoking history will be presented in a listing for participants in Safety population. It includes the following categories: Smoking Status (Current, Previous, Never), Type of Tobacco Substance (Cigarettes, Cigars, Pipes, Chewing Tobacco, Vaping (e-cigarette)), start date /end date and number of uses per day.

6.4. Medical History

6.4.1. General Medical History

The number and percentage of participants with any medical history will be presented by system organ class (SOC) and preferred term (PT). At each level of summarization, a participant will be counted once if the participant reported one or more events. The

summary will be presented alphabetically by SOC and within each SOC, by decreasing total incidence of PT. If the incidence for any two or more PTs is equal, the PTs will be presented in alphabetical order. . Body systems will be included as recorded on the electronic case report form (eCRF). Percentages will be calculated based on the number of participants in the Safety population.

Medical history data will be presented in a listing for participants in the Safety population.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

All medications that the participant is receiving at the time of Screening until the Follow-up Visit will be collected on the case report form (CRF). All medications will be coded according to the World Health Organization drug dictionary (WHODRUG 2019 Mar).

Prior medication will be defined as any medication with the start date prior to the date of the first dose of the investigational product. Concomitant medication will be defined as any medication with a start date prior to the date of the first dose of study drug and continuing after the first dose of study drug or with a start date between the dates of the first dose of study drug and the follow up visit date, inclusive. Incomplete medication dates will be imputed as described in Section 16.3.

Prior and concomitant medication use will be summarized for the Safety Population by the number and percentage of participants receiving each medication within each therapeutic class. Multiple medications used by a participant in the same category (based on Anatomical-Therapeutic-Chemical classification) will be counted only once. All prior and concomitant medications will be presented in a listing for participants in the Safety population.

7.2. Study Treatments

All eligible participants will receive open-label IW-6463 at a starting dose of 15 mg QD. The appropriate amount of study drug will be dispensed to participants on Day 1 of the Treatment Period. Drug will be resupplied as needed. Participants will be asked to record the date and time of each at-home study drug administration in their daily diary; these diaries will be checked for dosing compliance at each scheduled visit during the Treatment Period.

Participants will be asked to bring their study drug supply and daily diary to each in-clinic visit during the Treatment Period.

7.2.1. Extent of Exposure

Duration of exposure is defined as the total number of days a participant is exposed to any study drug and will be presented as the total number of days from the first dose date (Day 1) to the last dose date (date of last dose minus the date of first dose + 1) as recorded on the exposure page on the CRF. The duration of exposure to study drug will be summarized for all participants in the Safety population and will be presented in a table by summary statistics.

The total number of doses taken for the entire study will be calculated and summarized for all participants in the Safety population and will be presented in a table by summary statistics.

Each participant's study drug administration information will be presented in a listing.

7.2.2. Treatment Compliance and Modifications

Compliance is defined as the total dosage taken divided by the total dosage expected to be taken, times 100. The treatment compliance will be summarized for all participants in the Safety population. Compliance rates will also be categorized as missing, <80%, ≥80% and ≤120%, and >120% and will be summarized. Overall compliance will be listed for all participants in the Safety population.

8. Efficacy Analysis

Not applicable.

9. Safety Analysis

If not otherwise specified, the safety analyses will be performed using the Safety population. If not otherwise specified, the baseline value is defined as the last non-missing value measured (scheduled or unscheduled measurements) before administration of study drug on Day 1.

If there is more than 1 safety measurement (e.g., for a lab endpoint) at a postbaseline timepoint, only the last measurement will be used for by visit summary. All postbaseline assessments including unscheduled assessments, if any, will be used for shift tables, and all assessments will be presented in listings.

A listing of all participants affected by the coronavirus disease (COVID-19) related study disruption will be presented for participants in Safety population.

9.1. Adverse Events

Adverse events will be coded using MedDRA Version 24.0.

Pretreatment AEs are defined as AEs with a start date between the informed consent date and the date of the first dose of study drug.

Treatment-emergent AEs are defined as AEs reported in the clinical database with a start date on or after the first dose of study drug until the end of the follow-up period.

An overall summary of the number of participants in the Safety population with AEs will be presented, including the number and percentage of participants with AEs, pretreatment AEs, TEAEs, severe TEAEs, drug-related TEAEs, serious AEs, AEs leading to death, TEAEs leading to study drug discontinuation and Treatment-emergent adverse events of special interest (AESIs).

All AEs will be presented in listings for the Safety population, and AEs will be flagged to denote pretreatment AEs and TEAEs. Incomplete AE start dates will be imputed as described in Section 16.2.

9.1.1. Dose Reductions or Discontinuations due to TEAE

The number and percentage of participants with study drug dose reductions, or with study drug discontinuations due to TEAE (that is, participants who have a TEAE with a study treatment action taken of “Dose Reduced” or “Drug Withdrawn”) will be summarized for participants in the Safety population. A participant will be counted once if they experienced both these events. Percentages will be calculated based on the number of participants in the Safety population.

All TEAEs lead to study drug dose reductions or study drug discontinuations will be presented in a listing for the Safety population.

9.1.2. Incidence of Adverse Events

Incidence of pretreatment AEs and TEAEs will be summarized by the number and percentage of participants with at least one AE. The number and percentage of participants will also be presented by system organ class (SOC) and preferred term (PT). At each level of summarization, a participant will be counted once if the participant reported one or more

events. Percentages will be calculated based on the number of participants in the Safety population. The summary will be presented alphabetically by SOC and within each SOC, by decreasing total incidence of PT. If the incidence for any two or more PTs is equal, the PTs will be presented in alphabetical order.

9.1.3. Severity of Adverse Event

A summary of pretreatment AEs and TEAEs by severity ("Mild", "Moderate" and "Severe") as assessed by the Investigator will be presented by SOC, PT, and severity in a manner similar to that described in Section 9.1.1. In the severity table, if a participant reported multiple occurrences of the same AE, only the most severe will be presented. Percentages will be calculated based on the number of participants in the Safety population.

If severity is missing for an AE, all efforts should be made to obtain the severity from the Investigator. If it is still missing after all efforts, then a severity of "Mild" will be assigned for pre-treatment AEs and a severity of "Severe" will be assigned for TEAEs. The imputed values for the missing severity assessment will be used for the incidence summary, while the actual missing values will be presented in listings.

All severe AEs will be presented in a listing for participants in Safety population, and AEs will be flagged to denote pretreatment AEs and TEAEs.

9.1.4. Relationship of Adverse Events to Study Drug

A summary of TEAEs by relationship ("Related" and "Unrelated") to study drug per investigator will be presented by SOC, PT, and relationship in a manner similar to that described in Section 9.1.1. Percentages will be calculated based on the number of participants in the Safety population.

If a participant reported more than 1 TEAE within an SOC or PT, then the TEAE with the strongest causality relationship to study drug per investigator within each SOC and each PT will be included. If the relationship to study drug is missing for a TEAE, all efforts should be made to obtain the relationship from the Investigator. If it is still missing after all efforts, a study drug causality of "Related" will be assigned for TEAEs. The imputed values for the missing relationship to study drug will be used only for incidence summary, while the actual missing values will be presented in listings.

All drug-related AEs will be presented in a listing for the Safety population, and AEs will be flagged to denote pretreatment AEs and TEAEs.

9.1.5. Serious Adverse Events

Serious TEAE will be presented by SOC and PT in a manner similar to that described in Section 9.1.12. Percentages will be calculated based on the number of participants in the Safety population.

All participants in the Safety population who have an SAE will be presented in a listing and AE will be flagged to denote pretreatment AEs and TEAEs.

9.1.6. Adverse Events Leading to Study Drug Discontinuation

A summary of study drug discontinuations due to TEAE (that is, participants who have a TEAE with a study treatment action taken of "Drug Withdrawn") will be presented by SOC and PT in a manner similar to that described in Section 9.1.1. At each level of participant summarization, a participant is counted once if the participant reported one or more events. Percentages will be calculated based on the number of participants in the Safety population.

All participants in the Safety population who have an AE leading to study drug discontinuations or an AE leading to study discontinuation (that is, participants who answer "Yes" to the question of "Caused Study Discontinuation") will be presented in a listing and AEs will be flagged to denote pretreatment AEs and TEAEs.

9.1.7. Death

All participants in the Safety population with an AE leading to death (that is, participants who have an AE with an outcome of "Death Related to Adverse Event") will be presented a listing and AEs will be flagged to denote pretreatment AEs and TEAEs.

9.1.8. Adverse Events of Special Interest (AESIs)

A summary of AESIs as collected in the eCRFs will be presented by SOC and PT in a manner similar to that described in Section 9.1.1. At each level of participant summarization, a participant is counted once if the participant reported one or more events. Percentages will be calculated based on the number of participants in the Safety population within the subgroup category.

All participants in the Safety population who have an AESIs will be presented in a listing

9.2. Clinical Laboratory Evaluations

Tests detailed in **Table 1** will be performed by the Study Center and sent to the Central laboratory as scheduled in **Table 3**. Participants will fast for 3 to 4 hours before safety laboratory sample collections.

Descriptive statistics for observed values (SI unit) at each scheduled visit and changes from baseline at each scheduled post-baseline visit will be summarized for clinical laboratory tests with numeric values for participants in the Safety population. For urinalysis, summary tables will be performed as data permit.

All laboratory data will be presented in listings for participants in Safety population and abnormal values will be flagged.

Pregnancy test lab data will be presented in a listing for female participants in Safety population.

Table 1 Protocol-Required Safety Laboratory Assessments

Assessment	Parameter			
Hematology	Hematocrit	RBC indices:	WBC count w/differential:	
	Hemoglobin	-- MCH	-- Basophils	-- Monocytes
	Platelet count	-- MCV	-- Eosinophils	-- Neutrophils
	RBC count	-- %Reticulocytes	-- Lymphocytes	
Clinical chemistry (serum)	Alkaline phosphatase	BUN	Creatine kinase	Potassium
	ALT/SGPT	Calcium	GGT	Protein (total)
	AST/SGOT	Creatinine	Glucose (fasting)	Sodium
	Bilirubin (total, direct)			
Coagulation	aPTT	INR	Prothrombin time	
Routine urinalysis	Specific gravity pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick Microscopic examination (if blood or protein is abnormal)			
Additional tests	Follicle-stimulating hormone and estradiol (women of nonreproductive potential, only) [1] Human chorionic gonadotropin (hCG) pregnancy test (for women of reproductive potential, only)[2] Urine drug screen: amphetamines, cocaine, opiates, and cannabinoids [1]			

ALT=alanine aminotransferase; aPTT=activated partial thromboplastin time AST=aspartate aminotransferase; BUN=blood urea nitrogen; GGT=gamma glutamyl transferase; INR=international

normalized ratio; MCH=mean corpuscular hemoglobin; MCV=mean corpuscular volume; RBC=red blood cell; SGOT=serum glutamic-oxaloacetic transaminase; SGPT=serum glutamic-pyruvic transaminase; WBC=white blood cell
Note: All events of ALT ≥ 3×upper limit of normal (ULN) and bilirubin ≥2×ULN (>35% direct bilirubin) or ALT ≥3×ULN and international normalized ratio (INR) >1.5, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE.

[1] Test is necessary at the Screening Visit, only

2 Pregnancy tests will be serum based at Screening and at the Follow-up Visit. Urine-based tests are acceptable for all other visits, unless serum testing is required by local regulation or IRB/IEC.

9.3. Vital Sign Measurements

Supine Blood Pressure (BP), orthostatic BP and pulse, respiratory rate, oxygen saturation, and temperature will be performed as scheduled in **Table 3.**

Descriptive statistics (include 95% CI of means) for observed values at each scheduled visit and changes from baseline at each scheduled post-baseline visit will be summarized for respiratory rate, oxygen saturation, and temperature tests. Observed values and changes from baseline by position (supine, standing) at each scheduled visit will also be summarized for Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP), and pulse for participants in the Safety population.

Descriptive statistics (including 95% CI of means) for observed values by position (supine, standing and orthostatic) at each scheduled visit will be summarized for SBP, DBP, MAP, and pulse. Mean and 95% CI plots in observed value of SBP, DBP, MAP, and pulse will be presented over time.

The number and percentage of participants who meet the potentially clinically important (PCI) criteria presented in **Table 2** at any postdose timepoint will be summarized. Percentages will be calculated relative to the number of participants with non-PCI baseline values and at least 1 assessment in the corresponding post-baseline period. The numerator will be the total number of participants with available non-PCI baselines and at least 1 PCI value in the postbaseline period.

Table 2 Criteria for Vital Signs Results

Vital Sign Parameter	Flag	PCI Criteria
		Observed Value
Supine/Standing Systolic Blood Pressure (mmHg)	High	≥ 180
	Low	≤ 90
Supine/Standing Diastolic Blood Pressure (mmHg)	High	≥ 105
	Low	≤ 60
Supine/Standing Pulse Rate (bpm)	High	≥ 110
	Low	≤ 50
Orthostatic Systolic Blood Pressure (mmHg)	Decrease	≥ 20
Orthostatic Diastolic Blood Pressure (mmHg)	Decrease	≥ 10
Orthostatic Pulse (bpm)	Increase	≥ 30

All vital signs data will be listed for the Safety population.

9.4. Physical Examination

At Screening and Follow-up visit, a complete physical examination will be performed by the Investigator and documented on the appropriate eCRF. All other examinations may be symptom directed at the Investigator's discretion. A complete physical examination will include examination and assessment of the following:

General appearance	Lymph nodes	Nervous system
Cardiovascular system	Head, eyes, ears, nose, and throat	Skin
Respiratory system	Neck	Mental status
Abdomen/liver/spleen	Musculoskeletal system	

Breast, genitourinary, and rectal examinations are optional and may be performed at the discretion of the Investigator.

Physical examination results for all participants in the Safety population will be presented in a listing.

9.5. Electrocardiogram

All ECGs must be obtained after the participant has rested supine for ≥5 minutes and should be obtained before blood draws (or ≤10 m after a blood draw, if necessary). When timing coincides, ECGs and vital signs can be assessed together.

If a QTc result (corrected using Fridericia's formula) is outside of the normal range (>450 ms), the ECG should be repeated twice and the average of the 3 results calculated.

Descriptive statistics for observed values and changes from baseline will be summarized for ventricular rate (bpm), PR Interval (msec), QRS Duration (msec), QT Interval (msec), QTc Interval (msec), QTcF Interval (msec) for participants in the Safety population at each scheduled visit in 3. All ECG results for all participants in the Safety population will be presented in a listing.

The number and percentage of participants with absolute QTcF intervals in the following categories will be examined overall and by sex: QTcF≤450 msec, 450 msec <QTcF≤480 msec, 480 msec <QTcF≤500 msec, and QTcF>500 msec. Shift tables will be presented comparing values between baseline and EOT visit as well as baseline and end of study visit.

The number and percentage of participants with increase from baseline QTcF intervals in the following categories will be examined overall and by sex: QTcF interval increases by >30 msec, but ≤60 msec, and QTcF interval increases by >60 msec.

The number and percentage of participants with ECG interpretations at each scheduled visit for the Safety population will be presented. Interpretation results include Normal, Abnormal Not Clinically Significant, Abnormal Clinically Significant and Not Done.

9.6. Other Safety Data

9.6.1. Echocardiogram

All data for participants in the Safety population who have Screening Echocardiogram will be presented in a listing.

9.6.2. Suicidality

The Columbia Suicidality Severity Rating Scale (C-SSRS) will be administered according to 2, starting pre-dose on Day 1 of the Treatment Period and at all subsequent visits where clinical assessments are conducted, including at any unscheduled visit.

The C-SSRS (1) contains two required items pertaining to suicidal ideation, four required items pertaining to suicidal behavior, and one required item pertaining to non-suicidal self-injurious behavior. There are eight additional suicidal ideation items and two additional suicidal behavior items which are completed in cases of positive responses for other items, as well as two items for suicide and suicide behavior present during the interview. Most items are rated on a dichotomous scale (yes or no) or 5-point Likert scale. In addition, the total number of attempts is recorded. In the event of a positive categorical response the interviewer can provide text or narrative that further describes the thought or behavior.

Two versions of the C-SSRS are used in this study:

- The “Baseline/Screening” version will be administered at the Day 1 visit and will be completed for all participants. It includes questions about suicidal ideation in the past 12 months or lifetime history.
- The “Since Last Assessment” version will be completed for all participants at all study visits after the Day 1 Visit.

The number and percentage of participants with a positive response for suicidal ideation and suicidal behavior will be summarized by participants in Safety population at each scheduled visit and overall. Total number of attempts for each suicidal behavior will be summarized for participants in Safety population at each scheduled visit and overall.

Listings of the C-SSRS data will be provided for participants in the Safety population with a positive response for suicidal ideation and/or suicidal behavior.

10. Clinical Outcomes Assessments/Participant Questionnaires

10.1. Patient-reported Outcomes Measurement Information System (PROMIS)–Cognitive Function

The PROMIS Item Bank v2.0–Cognitive Function is a self-administered questionnaire that assesses multiple aspects of mental fatigue and cognitive function in the past 7 days prior to the administration of the questionnaire. (2) It uses a Likert-type rating scale (eg, "5=Never" to "1=Very often"). A total raw score ranging from 0 to 160 scores is calculated by summing participants' responses to each item. Higher scores represent better cognitive function. All questions must be answered in order to calculate total scores.

Descriptive statistics for observed values and changes from baseline in total PROMIS score will be summarized for each domain of the questionnaires for participants in the Safety population at each scheduled visit in 3.

Listings of the PROMIS data will be provided for participants in the Safety population and participants will be flagged to denote PD Evaluable population, if applicable.

10.2. Patient Global Impression of Change (PGIC)

The Patient Global Impression of Change (PGIC) is a single-item questionnaire that assesses the participant's perception of change in his/her overall health status since the start of the study using a Likert-type rating scale (e.g., "Very much improved" to "Very much worse").

The number and percentage of participants in each level of PGIC will be summarized by participant in Safety population at end of treatment visit.

Descriptive statistics for observed values will be summarized for participants in the Safety population at end of treatment.

Listings of the PGIC data will be provided for participants in the Safety population and participants will be flagged to denote PD Evaluable population, if applicable.

10.3. Modified Fatigue Impact Scale (MFIS)

The MFIS is a self-administered questionnaire that assesses the impact of fatigue. (3) Participants respond using a likert-type rating scale (e.g., "Never" to "Almost always"). Items on the MFIS can be aggregated into three subscales (physical, cognitive, and psychosocial), as well as into a total MFIS score. All items are scaled so that higher scores indicate a greater impact of fatigue on a person’s activities.

The MFIS questions yields 3 subscales and one total score:

- Physical

This scale can range from 0 to 36. It is computed by adding raw scores on the following items: 4+6+7+10+13+14+17+20+21. If more than 4 items are missing, then the scale score will be missing. Otherwise the scale score will be imputed by multiplying 9 by the average score of the rest of non-missing items. For example, if one item is missing, the scale score will be derived as the total score of rest of non-missing 8 items divided by 8 and multiplied by 9.

- Cognitive

This scale can range from 0 to 40. It is computed by adding raw scores on the following items: 1+2+3+5+11+12+15+16+18+19. If more than 5 items are missing, then the scale score will be missing. Otherwise the scale score will be imputed by multiplying 10 by the average score of the rest of non-missing items. For example, if one item is missing, the scale score will be derived as the total score of rest of non-missing 9 items divided by 9 and multiplied by 10.

- Psychosocial

This scale can range from 0 to 8. It is computed by adding raw scores on the following items: 8+9. If one item is missing, the scale score will be imputed by multiplying 2 by the non-missing item. If more than one item is missing, then the scale score will be missing.

- Total MFIS

The total MFIS score can range from 0 to 84. It is computed by adding scores on the physical, cognitive, and psychosocial subscales. If any subscale is missing, the total score will be missing.

Descriptive statistics for observed values and changes from baseline will be summarized for MFIS total scores and each subscale for participants in the Safety population at each scheduled visit in 3.

Listings of the MFIS data will be provided for participants in the Safety population and participants will be flagged to denote PD Evaluable population, if applicable.

11. Pharmacokinetics

PK samples (Plasma and CSF) will be collected as scheduled in **Table** 3. The actual date and time (24h clock time) of each sample collection will be recorded in the source documents and eCRF.

The lower limit of quantification (LLOQ) for IW-6463 is 1.0 ng/mL. Plasma and CSF concentrations below the limit (BLQ) of quantification will be assigned a value of ½ LLOQ for the calculation of summary statistics and will be presented as BLQ in the listing.

Descriptive statistics for plasma and CSF concentrations of IW-6463 will be summarized for participants in PK Evaluable population at each scheduled assessed timepoint in **Table**

3. The calculation and summary of PK parameters may be described and reported outside of this SAP.

Listings of the plasma and CSF concentrations of IW-6463 data will be provided for participants in the PK Evaluable population.

A population PK approach based on sparse PK data will be used to determine exposure and oral clearance of IW-6463. If data allow, influence of participant demographics (eg, age, race) and effects of concomitant medications on IW-6463 PK exposure will be evaluated. In addition, exposure-effect relationships (such as hemodynamic, biomarkers, and safety parameters) may also be explored. PK parameters of interest include but are not limited to the area under the concentration-time curve from time zero (predose) to 24 hours postdose (AUCtau), time to Cmax (Tmax), t1/2, maximum observed plasma concentration (Cmax), plasma concentration observed at the end of a dosing interval (Ctrough), apparent total plasma clearance (CL/F), apparent volume of distribution (Vz/F), and CSF:plasma concentration ratio, and will be calculated and presented as data permit.

These analyses will be performed outside of the SAP and reported separately outside of the clinical study report.

12. Pharmacodynamics

Descriptive statistics for plasma biomarkers and imaging endpoints from MRS and ASL at each assessment time point and the change from baseline at each post-baseline time point will be summarized for participants in the Safety and PD Evaluable population (if applicable). Of the imaging endpoints from fMRI, only the fractional amplitude of low-frequency fluctuations in the different networks at each assessment timepoint and the respective change from baseline at each post-baseline time point will be summarized. Assessment of the change from baseline in fMRI-blood-oxygen-level-dependent (BOLD) signals with a visual stimulus, will be described and conducted outside of this SAP and reported separately.

Listings will be provided for all participants in Safety population.

13. Immunogenicity

Immunogenicity testing will not be conducted in this study.

14. Changes in the Planned Analysis

The following changes/clarifications will be made to the analyses described in the study protocol.

- Updated to present all the protocol deviations instead of Major protocol deviations in a data listing.
- Removed Shift tables for QTcF interval increases category: >30 ms, but ≤60 ms, and QTcF interval increases by >60 ms.
- Added clarification for definition of a PD Evaluable Population (Section 4.3) only if the full planned number of participants enrolled in the study.
- Added that the assessment of the change from baseline in fMRI-blood-oxygen-level-dependent (BOLD) signals with a visual stimulus (Section 12) will be described and conducted outside of this SAP and reported separately.
- Analyses of the clinical outcomes assessments/participant questionnaire data in Section 10 was revised to be conducted on the safety population instead of the PD Evaluable population
- Medical History is analyzed by SOC and PT
- Blood pressure (supine and standing) will be described by each value and change from baseline
- Potentially clinically important (PCI) criteria will also be assessed for Standing and Orthostatic Criteria

15. References

1. Posner K 2007. Suicidality issues in clinical trials: Columbia suicidal adverse event identification in FDA safety analyses. Division of Metabolism and Endocrinology Products Advisory Committee Meeting, Food and Drug Administration.
2. www.healthmeasures.net/images/PROMIS/manuals/PROMIS_Cognitive_Function_Scoring_Manual.pdf.
3. Larson RD. Psychometric properties of the modified fatigue impact scale. Int J MS Care. 2013;15(1):15‐20. doi:10.7224/1537-2073.2012-019

16. Appendices

16.1. Schedule of Study Procedures

Table 3: Schedule of Events

Study Period →	**Screening**	**Treatment Period (assessments are predose unless noted)**							**F/U**
Visit Type/Name →	**In-clinic Screening**	**In-clinic Visit**	**At-home Visit [1]**	**At-home Visit [1]**	**In-clinic EOT Visit [2]**				**At-home Visit [1]**
Visit Day (window) →	**Day -28 (-28 to -10 days)**	**Day 1**	**Day 8 (±2 days)**	**Day 15 (±2 days)**	**Day 29 (-4 days)**				**Day 43 (±4 days)**
					Pre	**2 h**	**4 h**	**6 h**	
Study Procedure ↓						**(±30 m)**			
ICF signed	X								
Eligibility evaluation	X	X							
Demographics	X								
Drug/alcohol screen [2]	X								
Medical history	X	X							
Physical examination [3]	X	X	X	X	X				X
Weight (W), height (H)	W, H	W		W	W				W
12-lead ECG [4]	X	X			X				X
ECHO [5]	X								
BP (including orthostatic) & pulse [6]	X	X	X	X	X			X	X
Oral temp, O_2, & RR	X	X	X	X	X			X	X
Clinical safety samples [7] (chemistry, coag/hematology, UA)	X	X [8]	X	X	X				X
Pregnancy test [9]	X	X	X	X	X				X
C-SSRS		At each scheduled and unscheduled visit							
Study drug supply/ accountability/return [10]		X	X	X	X				
Participant diary	Issued at Screening; reviewed at each scheduled visit; collected at Follow-up								

Study Period →	**Screening**	**Treatment Period (assessments are predose unless noted)**							**F/U**
Visit Type/Name →	**In-clinic Screening**	**In-clinic Visit**	**At-home Visit [1]**	**At-home Visit [1]**	**In-clinic EOT Visit [2]**				**At-home Visit [1]**
Visit Day (window) →	**Day -28 (-28 to -10 days)**	**Day 1**	**Day 8 (±2 days)**	**Day 15 (±2 days)**	**Day 29 (-4 days)**				**Day 43 (±4 days)**
					Pre	**2 h**	**4 h**	**6 h**	
Study Procedure ↓						**(±30 m)**			
Study drug dosing [10,11]		QD Days 2–28, including at home, at a consistent time (±1 h of Day 1 dose time)			In clinic				
AE/SAE evaluation	From Screening through Follow-up								
Prior/concom. med. rev.	From Screening through Follow-up								
Blood sample for PK			X	X	X	X	X	X	
CSF sample for PK								X	
Blood sample for PD biomarkers [7]	X	X		X	X				X
Neuroimaging [12]	X	X					X		
MFIS		X			X				
PGIC					X				
PROMIS–Cognitive function		X			X				
Visit discharge [13]		~4h post							
Study completion									X

AE=adverse event; BP=blood pressure; coag=coagulation; concom=concomitant; CSF=cerebral spinal fluid; C-SSRS=Columbia Suicide Severity Rating Scale; ECG=electrocardiogram; ECHO=echocardiogram; EOT=end of treatment; F/U=follow-up; h=hour(s); H=height; ICF=informed consent form; m=minute(s); med=medication(s); MFIS=Modified Fatigue Impact Scale; O_2=oxygen saturation; PD=pharmacodynamic; PGIC=Patient Global Impression of Change; PK=pharmacokinetic(s); post=postdose; Pre=predose; PROMIS=Patient-Reported Outcomes Measurement Information System; QD=once daily; rev=review; RR=respiratory rate; SAE=serious adverse event; UA=urinalysis; VS=vital signs; W=weight

[1]Visit will be conducted within the allowable timeframe, either at the participant's home or in the Study Center per participant/Study Center preference. If the Follow-up Visit is conducted at home: The investigator will perform a medical review of the safety assessments conducted during the at-home F/U visit and may contact the participant via telephone if further follow-up is needed.

[2] All participants will be asked to return to the Study Center for the EOT visits.
For premature study drug discontinuations: If possible, participants who prematurely discontinue study drug will be asked to complete the EOT assessments prior to departure and then complete the Follow up Visit as scheduled

[2] See Protocol Section **Error! Reference source not found.** for a list of drugs to be screened.

[3] A complete physical exam will be done at Screening and Follow-up; a symptom-directed exam can be performed at all other visits.

[4] ECGs will be performed after the participant has been resting supine for ≥5 minutes and should be obtained before blood draws (or ≥10 m after a blood draw, if necessary). See Protocol Section **Error! Reference source not found.** for details. When timing coincides, ECGs and vital signs can be assessed together.

[5] Screening ECHO is necessary only in the absence of a documented ECHO performed within 3 months of Day 1

[6] All BP and pulse measurements will be obtained with an automated BP device (left arm preferred) prior to blood draws (or ≥10 m after a blood draw, if necessary). Manual readings are allowed only if automatic is not available.
--*Supine BP at Screening (only):* Record the average of 2 measurements obtained at 2-minute intervals after the participant has rested quietly in a semi-recumbent/supine position for ≥5 minutes
--*Orthostatic vital signs (all scheduled visits):* Participant must rest quietly in a supine/semi-recumbent position for ≥5 minutes before supine measurements are recorded, then assume sitting position for ≥1 minute, and finally assume standing position for 2 (±1) minute before standing measurements are recorded. Results will be used to calculate and record orthostatic BP and pulse.

[7] Although IW-6463 can be taken with or without food, participants are requested to fast for 3 to 4 hours prior to all clinical safety and PD laboratory sample collections. Water and a light snack (if previously approved), are allowed upon awakening on the morning of the visit. **For in-clinic visits**, because participants may be vulnerable to metabolic decompensation during any catabolic state, a standardized meal with a low glycemic index will be administered just prior to the start of this fasting period. **For at-home visits**, participants will either be counseled on what to consume prior to the fasting period, or will be given a standardized meal by home health services. For details regarding standardized snacks and meals, see the Nutrition Guidelines for this study.

[8] Safety laboratory results must be assessed for clinical significance prior to initiation of study drug dosing on Day 1; local laboratory results can be used for the Day 1 eligibility assessments; a second set of samples must also be sent to the Central laboratory.

[9] For women of reproductive potential (defined in Protocol Section **Error! Reference source not found.**), a serum pregnancy test will be conducted at Screening and at the Follow-up Visit; urine-based pregnancy tests will be acceptable at all other scheduled visits (unless serum testing is required by local regulation or the institutional review board/independent ethics committee [IRB/IEC]). A reviewed and documented negative result is required at Screening and again prior to study drug initiation on Day 1. A confirmed positive result at any time during the study will result in immediate discontinuation of study drug. See Protocol Section **Error! Reference source not found.**.

[10] Study drug supply will be distributed on Day 1 and will be resupplied as applicable. Dosing compliance (via tablet count) will be recorded at each scheduled visit during the Treatment Period. Participant diaries will also be checked at each visit for completeness. Participants will be instructed to bring in any unused study drug supply and their diary to each scheduled visit through EOT.

[11] Participants will be instructed to take their study drug dose on Days 2 through 28 at a consistent time each day, corresponding to the Day 1 dose administration time (±1 h). *Exception/Note:* For the Days 8, 15, and 29/EOT visits, they will take their dose during the visit to allow for appropriate timing of pre- and postdose assessments. Attempts should be made to schedule the Day 8 and 15 visits (at home or in clinic) such that dosing can still occur at approximately the same time as the Day 1 and subsequent doses.

[12] Includes magnetic resonance spectroscopy (MRS): arterial spin labeling (ASL), and functional magnetic resonance imaging blood-oxygen-level-dependent (fMRI-BOLD) imaging. Scanners that lack fMRI capabilities may be considered for use in this study with Sponsor approval. See Protocol Section **Error! Reference source not found.** and the study's scanning guidelines document for details.

[13] Participants will be observed for ≥4 hours postdose and thereafter may be released from the study center per Investigator discretion

16.2. Missing Date Information for Adverse Events

The following imputation rules apply to cases in which the start date is incomplete (i.e., partial missing) for adverse events.

Missing day and month

- If the year is the same as the year of the date of the first dose of double-blind study drug, then the day and month of the date of the first dose of double-blind study drug will be assigned to the missing fields.

- If the year is prior to the year of the date of the first dose of double-blind study drug, then December 31 will be assigned to the missing fields.

- If the year is after the year of the date of the first dose of double-blind study drug, then January 1 will be assigned to the missing fields.

Missing month only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

Missing day only

- If the month and year are the same as the month and year of the date of the first dose of double-blind study drug, then the date of the first dose of double-blind study drug will be assigned to the missing day.

- If either the year is before the year of the date of the first dose of double-blind study drug or if both years are the same but the month is before the month of the date of the first dose of double-blind study drug, then the last day of the month will be assigned to the missing day.

- If either the year is after the year of the date of the first dose of double-blind study drug or if both years are the same but the month is after the month of the date of the first dose of double-blind study drug, then the first day of the month will be assigned to the missing day.

If the stop date is complete and the imputed start date as above is after the stop date, the start date will be imputed by the stop date.

If the start date is completely missing and the stop date is complete, then the following algorithm is used to impute the start date:

- If the stop date is after the date of the first dose of double-blind study drug, the date of the first dose of double-blind study drug will be assigned to the missing start date.

- If the stop date is before the date of the first dose of double-blind study drug, the stop date will be assigned to the missing start date.

16.3. Missing Date Information for Prior or Concomitant Medications

For prior or concomitant medications, incomplete (i.e., partially missing) start date and/or stop date will be imputed. When the start date and the stop date are both incomplete for a patient, impute the start date first.

16.3.1. Incomplete Start Date

The following rules will be applied to impute the missing numerical fields. If the stop date is complete and the imputed start date is after the stop date, then the start date will be imputed using the stop date.

Missing day and month

- If the year of the incomplete start date is the same as the year of the date of the first dose of double-blind study drug, then the day and month of the date of the first dose will be assigned to the missing fields.

- If the year of the incomplete start date is prior to the year of the date of the first dose of double-blind study drug, then December 31 will be assigned to the missing fields.

Missing month only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

Missing day only

- If the month and year of the incomplete start date are the same as the month and year of the date of the first dose of double-blind study drug, then the day of the date of the first dose will be assigned to the missing day.

- If either the year is before the year of the date of the first dose of double-blind study drug or if both years are the same but the month is before the month of the date of the first dose of double-blind study drug, then the last day of the month will be assigned to the missing day.

- If either the year is after the year of the date of the first dose of double-blind study drug or if both years are the same but the month is after the month of the date of the first

dose of double-blind study drug, then the first day of the month will be assigned to the missing day.

16.3.2. Incomplete Stop Date

The following rules will be applied to impute the missing numerical fields. If the date of the last dose of double-blind study drug is missing, replace it with the last diary call date. If the imputed stop date is before the start date (imputed or non-imputed start date), then the imputed stop date will be equal to the start date.

Missing day and month

- If the year of the incomplete stop date is prior to the year of the date of the last dose of double-blind study drug, then December 31 will be assigned to the missing fields.

Missing month only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

Missing day only

- If either the year is before the year of the date of the last dose of double-blind study drug or if both years are the same but the month is before the month of the date of the last dose of double-blind study drug, then the last day of the month will be assigned to the missing day.