STUDY DRUG:
IW-6463

PROTOCOL NUMBER:
C6463-201

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

REGULATORY AGENCY IDENTIFIERS:
US IND: 147088
NCT: 04475549

SPONSOR:
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Original Protocol Date:	10 February 2020
Amendment 1:	16 March 2020
Amendment 2:	23 April 2021
Amendment 3:	23 July 2021

1. PROTOCOL SUMMARY

1.1. Synopsis

Name of Sponsor/Company: Cyclerion Therapeutics, Inc.		
Name of Investigational Product: IW-6463		
Name of Active Ingredient: IW-6463		
Protocol number: C6463-201	**Phase:** 2a	**Country:** United States (US)
Study title: A Phase 2a safety, tolerability, pharmacokinetic, and pharmacodynamic study in individuals with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)		
Primary objective and endpoints *Objective*: To evaluate the safety and tolerability of IW-6463 when administered to participants with MELAS *Endpoints*: ● Number of participants with study drug dose reductions or discontinuations due to ≥1 TEAE ● Incidence and severity of adverse events		
Study design: C6463-201 is an open-label, single-arm study evaluating the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of IW-6463 in participants diagnosed with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). Because this is the first study evaluating IW-6463 in a patient population, the primary objective is safety and tolerability. To complete the study, each participant will progress through 3 distinct periods: Screening, Treatment, and Follow-up. Specific procedures and assessments will be conducted according to the Schedule of Events (Table 1 of the protocol).		
Number of participants (planned): It is expected that approximately 20 participants will be included in the Safety Population to result in approximately 12 evaluable participants.		
Diagnosis and main criteria for study inclusion: Participants will be 18 years of age or older on the day of consent and will have prior genetic confirmation of a known mitochondrial disease mutation; neurological feature(s) of MELAS; and a plasma lactate level of at least 1.0 at Screening.		
Study drug and mode of administration: IW-6463 tablets will be administered orally on a once-daily (QD) regimen		
Duration of study drug dosing and study participation for each participant: Participants will receive QD doses for up to 29 days. The overall study duration per participant will be a maximum of 75 days, including the Screening, Treatment, and Follow-up periods.		
Reference therapy: Not applicable		
Safety assessments: Safety will be assessed by the monitoring of adverse events, clinical safety laboratory test results, vital signs, and 12-lead electrocardiograms.		

Statistical methods:

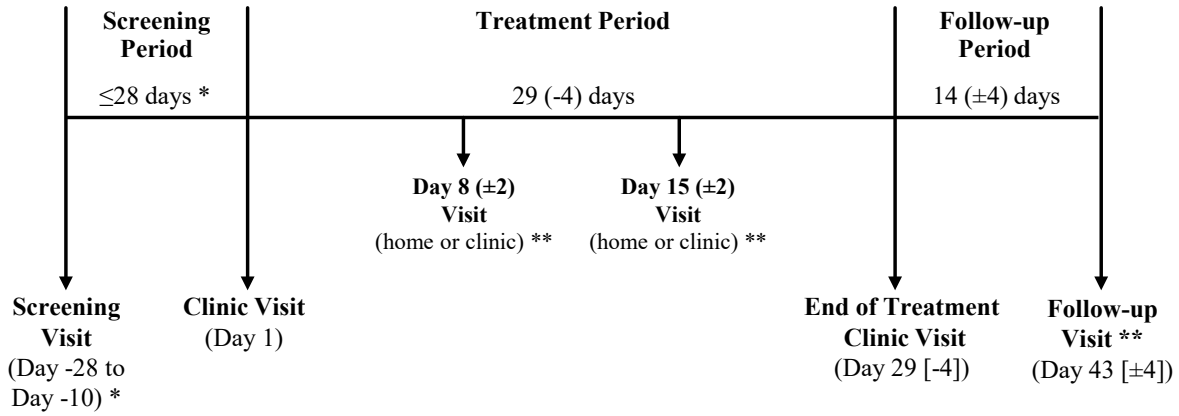
Sample size justification: The sample size selected is not based on statistical considerations due to the developmental stage of this study. A sample size of approximately 12 evaluable participants in a rare disease indication is considered sufficient to address the primary research objective.

General considerations: Descriptive statistics (n, mean, standard deviation, minimum, maximum, median, and interquartile range) will be calculated to summarize continuous variables. Frequency and percentage of participants in each category will be calculated to summarize categorical variables.

Due to the developmental stage of this study, no adjustments will be made for multiplicity. Details of the data handling methods will be specified in the statistical analysis plan. Inferential statistics will be used for descriptive purposes only. If not otherwise specified, the baseline value is defined as the last non-missing value measured before administration of study drug on Day 1.

1.2. Study Schematic

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.

1.3. Schedule of Events

Table 1 presents the Schedule of Events for this study. Please also refer to Section 7 as well as the study's scanning guidelines, and laboratory and pharmacy manuals and any other study-specific manual(s) for additional details.

Table 1: 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 to Day -10**	**Day 1**	**Day 8 (±2 days)**	**Day 15 (±2 days)**	**Day 29 (-4 days)**				**Day 43 (±4 days)**
Study Procedure ↓					**Pre**	**2 h**	**4 h**	**6 h**	
						(±30 m)			
ICF signed	X								
Eligibility evaluation	X	X							
Demographics	X								
Drug/alcohol screen [3]	X								
Medical history	X	X							
Physical examination [4]	X	X	X	X	X				X
Weight (W), height (H)	W, H	W		W	W				W
12-lead ECG [5]	X	X			X				X
ECHO [6]	X								
BP (including orthostatic) & pulse [7]	X	X	X	X	X			X	X
Oral temp, O_2, & RR	X	X	X	X	X			X	X
Clinical safety samples [8] (chemistry, coag/ hematology, UA)	X	X [9]	X	X	X				X
Pregnancy test [10]	X	X	X	X	X				X
C-SSRS		At each scheduled and unscheduled visit							
Study drug supply/ accountability/return [11]		X	X	X	X				
Participant diary (see Section 8.4)	Issued at Screening; reviewed at each scheduled visit; collected at Follow-up								
Study drug dosing [11,12]		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 (optional)								X	
Blood sample for PD biomarkers [8]	X	X		X	X				X

Table 1: 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 to Day -10**	**Day 1**	**Day 8 (±2 days)**	**Day 15 (±2 days)**	**Day 29 (-4 days)**				**Day 43 (±4 days)**
Study Procedure ↓					**Pre**	**2 h**	**4 h**	**6 h**	
						(±30 m)			
Neuroimaging [13]	X	X					X		
MFIS		X			X				
PGIC					X				
PROMIS–Cognitive function		X			X				
Visit discharge [14]		~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 visit. *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.

[3] See Section 8.3.6 for a list of drugs to be screened.

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

[5] 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 Section 8.3.4 for details. When timing coincides, ECGs and vital signs can be assessed together.

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

[7] 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.

[8] 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.

[9] 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.

[10] For women of reproductive potential (defined in Section 5.3.4.1), 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 Section 8.3.6.1.

[11] 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.

[12] 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.

[13] 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 Section 8.7 and the study's scanning guidelines document for details.

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

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation/Term	Definition
AE	adverse event
ASL	arterial spin labeling
AUC	area under the plasma concentration time curve
AUC_{tau}	area under the concentration-time curve during a dosing interval
BOLD	blood-oxygen-level-dependent
BP	blood pressure
CBF	cerebral blood flow
CFB	change from baseline
CFR	Code of Federal Regulations
cGMP	cyclic guanosine 3',5'-monophosphate
CL/F	apparent total plasma clearance
C_{max}	maximum observed plasma concentration
CNS	central nervous system
CRO	clinical research organization
CSF	cerebral spinal fluid
C-SSRS	Columbia Suicide Severity Rating Scale
C_{trough}	plasma concentration observed at the end of a dosing interval (ie, trough; collected before the next administration)
ECG	electrocardiogram
ECHO	echocardiogram
eCRF	electronic case report form
EOT	end of treatment
FDA	Food and Drug Administration
FIH	first in human
fMRI	functional magnetic resonance imaging
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
h	hour(s)
HRT	hormone replacement therapy
IB	Investigator's Brochure
ICF	informed consent form

Abbreviation/Term	Definition
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
m	minute(s)
MELAS	mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MFIS	Modified Fatigue Impact Scale
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NO	nitric oxide
PD	pharmacodynamic(s)
PDE5	phosphodiesterase 5
PGIC	Patient Global Impression of Change
PK	pharmacokinetic(s)
PROMIS	Patient-Reported Outcomes Measurement Information System
QD	once daily
QT	the time between the beginning of the QRS (ventricular polarization) complex and the end of the T-wave
QTcF	QT interval corrected for HR using Fridericia's formula
SAE	serious adverse event
SD	standard deviation
sGC	soluble guanylate cyclase
SLE	stroke-like episode
SOP	standard operating procedure
$t_{1/2}$	apparent terminal half-life
TEAE	treatment-emergent adverse event
T_{max}	time to C_{max}
V_z/F	apparent volume of distribution

2. BACKGROUND INFORMATION AND STUDY RATIONALE

2.1. Name and Description of Study Drug

IW-6463 is being investigated as a treatment for mitochondrial diseases. It is intended for administration only to eligible study participants in accordance with this protocol. Additional drug product details are provided in Section 7.

2.2. Introduction and Rationale

IW-6463 is an orally administered central nervous system (CNS)-penetrant stimulator of soluble guanylate cyclase (sGC), a signaling enzyme that catalyzes the formation of cyclic guanosine 3',5'-monophosphate (cGMP) from guanosine triphosphate in response to nitric oxide (NO) binding. Intracellular cGMP regulates vascular tone and regional blood flow, fibrosis, and inflammation, and has been implicated in neuronal survival and cognitive function. Impaired NO-sGC-cGMP signaling is believed to play an important role in the pathogenesis of mitochondrial diseases such as mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS).(1)

Mitochondrial diseases are a group of rare genetic disorders that occur when mitochondria fail to produce enough energy for the body to function properly. The NO signaling pathway is critical for the regulation of mitochondrial function and mitochondrial biogenesis.(2-6) NO pathway dysregulation is recognized as a major contributing factor in mitochondrial disease, and leads to impaired cerebral blood flow (CBF), oxidative stress, inflammation and metabolic crises.(7, 8) There are clear links observed between NO signaling, stroke-like episodes (SLEs), and dysregulated CBF in patients with mitochondrial disease.(9)

In the absence of approved therapies for MELAS, citrulline and L-arginine, precursors of NO, are hypothesized to provide benefit in this patient population. The consensus guidelines from the Mitochondrial Medicine Society recommend acute arginine administration to improve clinical symptoms associated with SLEs in patients with MELAS.(9-12) Mechanistically, L-arginine is converted directly into NO, the starting point of the NO-sGC-cGMP pathway. sGC stimulators act synergistically with NO on the native, heme-containing form of the enzyme to produce cGMP and thereby increase NO signaling. As a core node in the NO-sGC-cGMP pathway, it is hypothesized that an sGC stimulator is in a key position to potentially enhance mitochondrial function and biogenesis.(6)

As the first CNS-targeted sGC stimulator, IW-6463 may offer unique therapeutic benefit for patients with mitochondrial disease. Data from nonclinical studies evaluating IW-6463 support the hypothesis that sGC stimulation may have beneficial effects on several key mechanisms of mitochondrial disease pathologies and thus may be of value in treating mitochondrial diseases. Please see the IW-6463 Investigator's Brochure (IB) for details.

2.3. Findings from Nonclinical and Clinical Studies

2.3.1. Nonclinical Studies

Results from nonclinical studies of IW-6463 supporting the evaluation of IW-6463 in this clinical study population are summarized in the IW-6463 IB.

2.3.2. Clinical Studies

Preliminary safety and pharmacokinetic (PK) data from the first-in-human (FIH) study C6463-101 and from a second Phase 1 study (C6463-102) provide support for further clinical investigation of IW-6463. See Section 2.4 and the most recent IB for details.

2.3.2.1. Clinical Studies in Children

IW-6463 has not been administered to participants younger than 18 years of age.

[redacted]

2.5. Risk Assessment

No safety concerns were identified in the Phase 1 studies conducted to date (see Section 2.4). Thus, the risk to participants in this Phase 2 study are expected to be minimal.

Procedures and assessments included in this protocol are generally consistent with standard of care for monitoring disease in mitochondrial disease patients. Although magnetic resonance imaging (MRI), blood sampling, and (the optional) lumbar puncture represent the highest burden for participants, these assessments are associated with minimal risks and a low probability of harm. The duration and frequency of MRI procedures, blood sampling volumes and frequency, and the number of lumbar punctures (limited to not more than 1) have been minimized to reduce the risk to study participants and to collect only the information necessary to support further development of IW-6463.

2.5.1. Potential Benefits of Study Participation

Because clinical evaluation of IW-6463 has been limited to administration in healthy participants thus far, any direct benefit to a patient population has not yet been determined. Indirect benefits to participants include increased medical care/attention, a better understanding of their disease, and satisfaction knowing that their participation will contribute to mitochondrial disease research and potential therapeutics.

2.5.2. Risk-Benefit Assessment

Because this is the first study of IW-6463 in this population, a full risk-benefit assessment cannot be conducted. However, evaluation of IW-6463 in individuals with mitochondrial disease is justified based on the minimal risk profile reported in healthy participants who have received IW-6463, along with the potential benefit to study participants based on the pharmacological effects observed in nonclinical studies. Results from the nonclinical efficacy and safety assessment program support a favorable benefit:risk assessment; see the IB for more information.

2.6. Compliance Statement

See Section 11.3.2.

3. OBJECTIVES AND ENDPOINTS

Objective	Endpoint
Primary:	
• To evaluate the safety and tolerability of IW-6463 when administered to participants with MELAS	• Number of participants with study drug dose reductions or discontinuations due to ≥1 TEAE • Incidence and severity of AEs
Exploratory:	
• To evaluate the PK of IW-6463 when administered to participants with MELAS	• Plasma and CSF concentrations of IW-6463 as data permit, at assessed timepoints • PK parameters as data permit, including but not limited to AUC_{tau}, T_{max}, $t_{1/2}$, C_{max}, C_{trough}, CL/F, V_z/F, and the CSF:plasma concentration ratio
• To evaluate the effect of IW-6463 on exploratory plasma biomarkers in participants with MELAS	• Change from baseline in plasma biomarker concentrations on Day 29 *
• To evaluate the effect of IW-6463 on CBF in participants with MELAS	• Change from baseline in CBF as measured by ASL on Day 29
• To evaluate the effect of IW-6463 on brain activity as measured by fMRI in participants with MELAS	• Change from baseline in fMRI-BOLD signals during resting state and a visual stimulus on Day 29, as data permit
• To evaluate the effect of IW-6463 on brain metabolites as measured by MRS	• Change from baseline in brain metabolite [redacted] levels by ^{1}H-MRS on Day 29
• To evaluate the effect of IW-6463 on fatigue as measured by the MFIS	• Change from baseline in MFIS scores (total, physical, cognitive, and psychosocial subscores) on Day 29
• To evaluate the effect on IW-6463 on cognitive function as measured by the PROMIS Item Bank v2.0–Cognitive function	• Change from baseline in PROMIS Item Bank v2.0–Cognitive function total score on Day 29

^{1}H=proton; AE=adverse event; ASL=arterial spin labeling; AUC_{tau}=area under the concentration-time curve during a dosing interval; BOLD=blood-oxygen-level-dependent; CBF=cerebral blood flow; CL/F=apparent total plasma clearance; C_{max}=maximum observed plasma concentration; CNS=central nervous system; CSF=cerebral spinal fluid; C_{trough}=trough plasma concentration observed at the end of a dosing interval [collected before the next administration]; fMRI=functional magnetic resonance imaging; MELAS=mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MFIS=Modified Fatigue Impact Scale; MRS=magnetic resonance spectroscopy; PK=pharmacokinetic(s); PROMIS=Patient-Reported Outcomes Measurement Information System, SLE=stroke-like episode; $t_{1/2}$=apparent terminal half-life; TEAE=treatment-emergent adverse event T_{max}=time to C_{max}; V_z/F=apparent volume of distribution

[redacted]

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design

C6463-201 is an open-label, single-arm study evaluating the safety, tolerability, PK, and pharmacodynamics (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 3 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 1). A study schematic is shown in Figure 1.

Criteria for premature discontinuation of individual participants are provided in Section 6.2.

4.1.1. Screening Period

The Screening Period begins with the execution of the informed consent form (ICF) at the Screening Visit. After signing the ICF, each participant's study eligibility will be assessed according to the protocol inclusion and exclusion criteria (Section 5).

Screening assessments may be conducted 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.

After the Screening Visit, participants who are eligible for the study must begin complying with the lifestyle restrictions detailed in Section 5.3. They will also begin completing their daily diary for this study (see Section 8.4).

The end of the Screening Period will coincide with the start of the Treatment Period.

Collection of adverse events (AEs) will begin after the ICF is signed and will continue through the Follow-up Period.

4.1.2. Treatment Period

The Treatment Period begins on Day 1 (there is no "Day 0") when participants return to the Study Center to undergo baseline procedures and receive their first daily dose of study drug. Participants will return to the Study Center on Day 29 (-4 days) for the End-of-Treatment (EOT) Visit; all other scheduled visits during this period will be completed within the allowable timeframe either at home or in the study center, per participant preference. Throughout the Treatment Period, participants will continue completing their daily diary (see Section 8.4).

The end of the Treatment Period coincides with the beginning of the Follow-up Period.

4.1.3. Follow-up Period

The Follow-up Period begins immediately after the EOT Visit. During this period, participants will continue to comply with all lifestyle restrictions as detailed in Section 5.3 and will continue to complete their daily diary (Section 8.4).

See Table 1 for Follow-up Visit instructions.

4.2. Estimated Total Blood and CSF Volumes to be Collected from Each Participant

An estimate of the total blood volume that will be collected from each participant over the course of the study will be a minimum of approximately 100 mL and will not exceed 500 mL. In addition to the blood samples, 1 CSF sample (~4 mL) will be collected from each participant who does not withhold consent for the procedure. Collection timepoints are detailed in Table 1.

Biological samples collected from participants in this study may be stored for up to 5 years for future analysis.

4.3. Number of Study Centers and Participants

The study will be conducted at approximately 5 investigative sites in the United States. It is expected that approximately 20 participants will be included in the Safety Population to result in approximately 12 evaluable participants.

4.4. Treatment Assignment and Blinding

Not applicable; this is an open-label study.

4.5. Safety Stopping Criteria for the Overall Study

The decision to prematurely terminate this study for safety reasons will be made by the Sponsor after consultation with the Trial Safety Committee (Section 11.5).

All dosing will be stopped and the study will be terminated if an overall pattern of clinically significant TEAEs or changes in any safety parameter occurs that may appear minor in terms of an individual event but, in the opinion of the Sponsor, collectively represent a safety concern.

See Section 5.6 for discontinuation criteria for individual participants.

4.6. Duration of Individual Participation

The overall study duration per participant will be a maximum of 75 days, including the Screening (≤28 days), Treatment (≤29 days), and Follow-up (≤18 days) periods.

4.7. End of Study Definition

The end of the study is defined as the date of the last visit of the last participant in the study.

5. PARTICIPANT SELECTION, LIFESTYLE RESTRICTIONS, AND DISCONTINUATION

Please note:

- 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.

- Participants may be rescreened per Investigator judgment after consultation with the Sponsor's Medical Monitor. Laboratory assessments may be repeated if an error is suspected. Laboratory, electrocardiogram (ECG), or blood pressure (BP) values that are outside of the range specified in the protocol's eligibility criteria may be repeated after consultation with the contract research organization (CRO)-based medical monitor.
- *Supine BP measurement at Screening (only):* Record the average of 2 measurements obtained using an automated BP device (left arm preferred) at 2-minute intervals after the participant has rested quietly for ≥5 minutes in a semi-recumbent/supine position.
- *Orthostatic vital sign measurements (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 a standing position for 2 (±1) minutes before standing measurements are recorded. Results will be used to calculate and record orthostatic BP and pulse.
- The Investigator will determine whether a particular finding is clinically significant. In making this determination, the Investigator will consider whether the finding could prevent the participant from performing any of the protocol-specified assessments, could represent a condition that would exclude the participant from the study, could represent a safety concern if the participant participates in the study, or could confound the study-specified assessments.

5.1. Inclusion Criteria

Each participant must meet <u>each</u> of the following criteria to be eligible for entering this study.

1. Signed an ICF (either the participant or his/her legal representative/guardian, where appropriate) before any study-specific procedures are performed
2. 18 years of age or older on the day of consent
3. Prior genetic confirmation of a known mitochondrial disease mutation
4. Neurological features of MELAS (can be based on medical history)
5. Elevated plasma lactate during Screening (≥1.0 mmol/L)
6. Agrees to adhere to all study requirements, including lifestyle restrictions noted in Section 5.3
7. Agrees to refrain from making any major lifestyle changes (eg, start a new diet or change an exercise pattern) from the time of the ICF signature until after the Follow-up Visit (and beyond, vis-à-vis the contraception requirements)
8. If receiving chronic medication(s), has had no change in regimen(s) for ≥4 weeks before first day of dosing and has no plans to change regimen(s) during the study

9. If female, meets 1 of the 2 following criteria:
    a. Confirmed as being postmenopausal (no menses for ≥1 year or ≥12 consecutive months) or surgically sterile (bilateral oophorectomy, hysterectomy, or tubal sterilization [tie, clip, band, or burn])

       —*or*—

    b. If of reproductive potential (see Section 5.3.4.1):
        i. Is not pregnant or breastfeeding at the time of the Screening Visit and
        ii. Has negative pregnancy test results at the Screening Visit and predose at Day 1
        iii. Agrees to contraception (see Inclusion #10) for duration of study and for ≥90 days after the final study drug dose
10. Male and female participants of reproductive potential must agree to use ≥1 of the following effective contraception methods from the date of signing the ICF until ≥90 days after receiving his/her final study drug dose:
    a. Completely abstain from heterosexual intercourse
       —*or*—
    b. If heterosexually active, use:
        i. Intrauterine device ("IUD") and/or a progesterone implant –*or*—
        ii. Combination of 2 highly effective birth control methods (eg, condom with spermicide + diaphragm or cervical cap; hormonal contraceptive [eg, oral or transdermal patch or progesterone implant] + barrier method) –*or*—
        iii. Maintenance of a monogamous relationship with a partner who has been surgically sterilized either by vasectomy (conducted ≥60 days before the Screening Visit or confirmed via sperm analysis) or by bilateral oophorectomy, hysterectomy or tubal sterilization; or is not sterile due to postmenopausal status
11. Male participants must agree to refrain from donating sperm from the Screening Visit through 90 days after their final dose of study drug
12. Agrees to not receive an investigational therapy or device in any other study while participating in this study, through the Follow-up Visit
13. No clinically significant findings on ECG (as assessed at Screening and predose on Day 1) and echocardiogram (ECHO; within 3 months of Day 1)

5.2. Exclusion Criteria

A participant who meets any of the following criteria will be excluded from entering this study.

1. Severe visual, auditory, or cognitive impairment as determined by the Investigator that may affect the ability to adhere to protocol requirements or to complete required assessments

2. Used any nicotine-containing products (eg, cigarettes, e-cigarettes, vape pens, cigars, chewing tobacco, gum, patches) within 1 month of enrollment
3. Positive pregnancy test at Screening or on Day 1 (or at any other point during the study)
4. Inpatient hospitalization for alcoholism or drug addiction in the 12 months before the Screening Visit and/or has positive drug or alcohol test results at Screening or predose at Day 1. Drug screen will include amphetamines, cocaine, opiates, and cannabinoids.

   Note:

   - Use of cannabis and cannabidiol products for medical purposes is permitted in this study *except* for 24 hours before the Screening, Day 1, and EOT visits, and for 4 hours prior to all other visits.
   - A participant should be excluded from study entry if there is a known cannabis abuse or dependence that, in the opinion of the Investigator, will impact the ability of that individual to comply with the protocol or may lead to harm to the individual.
5. Clinically significant hypersensitivity or allergy to any of the inactive ingredients contained in the IW-6463 drug product
6. Hypotension, defined as systolic BP ≤90 mmHg or diastolic BP ≤60 mmHg at Screening or predose at Day 1
7. Hypertension, defined as systolic BP >160 mmHg or diastolic BP >100 mmHg, at Screening or predose at Day 1
8. Orthostatic hypotension at Screening or predose at Day 1, defined as a decrease in systolic BP of ≥20 mmHg or a decrease in diastolic BP of ≥10 mmHg when measured after assuming a standing position from a semi-recumbent/supine position
9. Uncontrolled diabetes mellitus with an HbA1c >11% or as determined by the Investigator
10. Lymphoma, leukemia, or any malignancy within the past 5 years
    Exception: Basal cell or squamous epithelial carcinomas of the skin that have been resected with no evidence of metastatic disease for 3 years
11. Severe gastrointestinal dysmotility (eg, history of dyspepsia, stomach aches, nausea, vomiting, recurrent pancreatitis, constipation) as determined by the Investigator that may impact compliance and/or oral drug administration, absorption, and exposure
12. Unable to fast (ie, no food or fluid; water allowed as required) for 3 to 4 hours after a meal
13. Family history of short QT syndrome or long QT syndrome
14. Clinically significant cardiac involvement or an ECG with corrected QT interval using Fridericia's formula (QTcF interval) >500 ms
15. Current or past history of clinically significant cardiomyopathy and/or cardiac conduction abnormality

16. History of platelet dysfunction, hemophilia, von Willebrand disease, coagulation disorder, other bleeding diathesis condition(s), or significant, nontraumatic bleeding episodes
17. Current use of aspirin ≥325 mg/day, any P2Y12 inhibitor, any anticoagulant medication, specific inhibitors of phosphodiesterase 5 (PDE5), nonspecific inhibitors of PDE5 (including dipyridamole and theophylline), any supplement for the treatment of erectile dysfunction, riociguat, and/or any nitrate. These medications are prohibited from the Screening Visit through the duration of the study. See Appendix 1 for details.
Note: Participants who are taking arginine or citrulline for the treatment of mitochondrial disease will be eligible and allowed to continue these therapies.
18. Participated in any study of investigational therapies for mitochondrial disease and/or for the symptoms of mitochondrial disease within 1 month before Day 1
19. Any contraindication to MRI procedures
20. Hospitalized for any illness, trauma, surgical procedure, or mitochondrial disease-related complication within 4 weeks before Screening
21. Unable or unwilling to adhere to the study schedule, lifestyle restrictions (see Section 5.3), and assessment requirements or, in the clinical judgment of the Investigator, is otherwise not suitable for study participation
22. Patient or his/her legal representative/guardian (where appropriate) is unable or unwilling to provide written, informed consent to participate in this study

5.3. Lifestyle Restrictions

Participants are to abide by the following lifestyle restrictions throughout the study, starting from the Screening Visit through the Follow-up Visit, unless otherwise indicated.

5.3.1. Meals and Dietary Restrictions

- Fasting (ie, no food or fluid; water allowed as required) is required for 3 to 4 hours prior to the collection of clinical safety and PD laboratory samples at each scheduled visit, including at any at-home visit.
  - Because participants may be vulnerable to metabolic decompensation during any catabolic state, on the day of each visit, participants will fast upon awakening (water and a light snack, if previously approved, are allowed) and then will be given a standardized meal with a low glycemic index in the clinic, 3 to 4 hours before the collection of clinical safety and PD laboratory samples. 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.
  - *On days when neuroimaging is conducted (Screening, Day 1, and Day 29 visits):* After the safety and PD laboratory samples have been collected, participants can eat a light standardized snack; heavy meals, however, should be avoided until after all imaging scans have been conducted.

– For more details regarding approved snacks and standardized meals, please refer to the Nutrition Guidelines for this study.

5.3.2. Caffeine, Alcohol, and Tobacco

- Caffeine- or xanthine-containing products (eg, coffee, tea, cola drinks, and chocolate) should not be ingested for 24 hours before the Screening, Day 1, and EOT visits, and for 4 hours prior to all other scheduled visits. These products can impact the assessments conducted in this study.
- The use of alcohol is not permitted during the 24 hours prior to any scheduled visit
- Tobacco- or nicotine-containing products (eg, e-cigarettes, patches) should not be used during this study until after the final study visit

5.3.3. Medications, Vitamins, Supplements, and other Substances

Participants are to be advised that the following are prohibited as indicated below.

- Any medical treatment for erectile dysfunction beginning with Screening through the Follow-up Visit
- "Illicit" drugs beginning 1 month before Screening through the Follow-up Visit.
- Use of cannabis and cannabidiol products for medical purposes is permitted *except* for the 24-hour period before Screening, the Day 1 and EOT visits, and for 4 hours before all other visits.
- Foods containing poppy seeds should be avoided throughout the study because they can cause a positive "drug" result

Investigators must refer to Appendix 1 for comprehensive list of medications that are prohibited or are allowed with caution. Also see Section 6.3.

5.3.4. Contraception Requirements (Both Sexes)

Each participant (of either sex) of reproductive potential must use effective contraception as defined in Inclusion Criteria 10 and 11 (Section 5.1), as applicable. Details regarding pregnancy testing and reporting are provided in Section 8.3.6.1 and Section 10.1.4, respectively.

5.3.4.1. Definition of Reproductive Potential in Female Participants

A premenopausal woman is considered fertile following menarche **unless** she has undergone a documented hysterectomy, bilateral salpingectomy, or bilateral oophorectomy. (Documentation can come from the Study Center personnel's review of the participant's medical records, medical examination, or medical history interview.)

A postmenopausal state is defined as no menses for ≥12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. A female participant on HRT whose menopausal status is in doubt is required to use 1 of the nonestrogen hormonal highly effective contraception methods if HRT

is continued during the study. Otherwise, HRT must be discontinued to allow confirmation of postmenopausal status before study enrollment.

5.3.5. Breastfeeding

Breastfeeding is not allowed from the Screening Visit through 90 days after the final dose of study drug.

5.3.6. Strenuous Activity

- Participants will abstain from strenuous exercise 48 hours before each blood collection for clinical laboratory tests
- Participants may engage in light recreational activities during the study, but should maintain the same level of physical activity from Screening through their Follow-up Visit.

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently administered study drug (ie, are not part of the Safety Population). A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants in compliance with the Consolidated Standards of Reporting Trials requirements and to respond to queries from regulatory authorities. Minimal information includes reporting of demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the eligibility criteria for participation in this study (ie, screen failure) may be rescreened. Rescreened participants will be assigned a new screening number that is different from the number assigned at the initial screening.

5.5. Lost to Follow-up

The following actions must be taken if a participant fails to complete/be present for an at-home visit or fails to return to the clinic for a required visit:

- The Study Center must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule.
- Before a participant is deemed as being lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 phone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's electronic case report form (eCRF).
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

5.6. Participant Completion/Withdrawal

5.6.1. Completion

A participant is considered to have completed the study after completing all study periods (Screening, Treatment, and Follow-up). It will be documented in the eCRF whether or not each participant completes the study.

5.6.2. Premature Study Drug Discontinuation for Individual Participants

Participants will be informed that they may withdraw from the study at any time without prejudice to their care.

A confirmed positive pregnancy test result in a participant requires immediate discontinuation of study drug. See Section 8.3.6.1.

The Investigator may discontinue study drug administration in an individual participant if, in the Investigator's opinion, it is not in the best interest of that individual to continue. See Section 6.2 for study drug dose adjustment instructions.

Immediate safety concerns should be discussed with the study's designated contract research organization (CRO)-based Medical Monitor upon occurrence or awareness. Changes in the participant's health status since the previous visit or previous study drug administration must be checked, including laboratory results, as applicable.

Participants may also be discontinued from the study by the Investigator or the Sponsor at any time for any reason, including the following:

- Adverse event(s)
- Any symptomatic hypotension-related AE (eg, syncope) that requires intervention and is considered related to study drug
- Any ECG change from baseline that is considered to be clinically meaningful by the Investigator (eg, abnormal Q waves, prolonged QT)
- Protocol violation, including lack of compliance
- Lost to follow-up (see Section 5.5)
- Withdrawal of consent (attempts should be made to determine the reason for the withdrawal, if possible)
  Note: The participant may request destruction of any samples collected but not yet tested; the Investigator must document this request in the study records. If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected prior to the withdrawal of consent.
- Symptomatic deterioration
- Study termination by the Sponsor

Participants who discontinue from study drug administration should complete all EOT Visit assessments, as appropriate, at the time of their discontinuation. If possible, they should also

return to the study center for the Follow-up Visit. Refer to Table 1 for the assessments to be conducted at EOT and Follow-up.

Participants who discontinue from the study due to a TEAE will be followed until resolution of all of their TEAEs or until the unresolved TEAEs are judged by the Investigator to have stabilized.

The Sponsor will be notified of any premature discontinuation. The date the participant is withdrawn from the study and the reason for discontinuation will be recorded on the study termination form of the eCRF.

The Sponsor and/or Investigator may contact participants after completion of the study or participant discontinuations to inquire about health status and/or experiences in the study.

5.7. Replacement of Prematurely Discontinued Participants

Participants who withdraw from the study after receiving ≥1 dose of study drug will not be replaced.

6. TREATMENT OF PARTICIPANTS

6.1. Study Drug Regimen and Administration

All eligible participants will receive open-label IW-6463 at a starting dose of 15 mg 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/EOT** visits, participants will take their dose during the visit to allow for the 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.

Participants are to record the date and time of each at-home dose administration in their diary (Section 8.4).

Permitted concomitant medications may be taken with study drug. Participants will be asked to record in their diary each dosing day, time, and dose strength of any concomitant medication(s) they take.

While IW-6463 can be taken with or without food, participants are requested to fast for 3 to 4 hours prior to in-clinic and at-home clinical safety and PD laboratory sample collections.

Study drug supply and dosing compliance assessment details are provided in Section 7.4.

6.2. Dose Adjustment Criteria for Individual Participants

6.2.1. Safety Criteria

The decision to de-escalate IW-6463 dosing in an individual participant will be made jointly by the Investigator and the designated CRO-based 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 TEAE(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. See Section 5.6.2 for study drug discontinuation procedures for individual participants.

6.2.2. PK Criteria

No PK criteria will be used to inform dose adjustment or stopping decisions. All dose adjustments and stopping decisions will be based on emerging clinical findings and TEAEs.

6.3. Concomitant Medications and Procedures

6.3.1. Recording of Medications

Any medication (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements), vaccine, or other therapeutic/procedure that the participant is receiving at the time of Screening until the Follow-up Visit will be recorded along with:

- Reason(s) for use
- Dates of administration including start and end dates
- Dosage information including dose, route of administration, and frequency

6.3.2. Prohibited Medications

Comprehensive drug-drug interaction studies with IW-6463 have not been conducted in humans. See Appendix 1 for a complete list of medications that are either prohibited or are allowed with caution.

The decision and need to switch medications and/or dose-reduce concomitant medications prior to or during IW-6463 exposure will be made by the Investigator in consultation with the Study Center Pharmacist to assess the risk:benefit.

7. STUDY DRUG MATERIALS AND MANAGEMENT

Please also refer to the study's pharmacy manual.

7.1. Description of Study Drug

Table 2 described the study drug and dosing regimen that will be administered in this study.

Table 2: Study Drug Description

Product name	IW-6463
Dosage form	round tablet
Unit dose and schedule	15 mg once daily (QD) for up to 29 days
Route of administration	oral
Physical description	white to off-white solid

Dose adjustment criteria are provided in Section 6.2.

7.2. Packaging and Labeling

IW-6463 tablets are supplied in white, induction-sealed 60-cc high-density polyethylene bottles that are fitted with a polypropylene child-resistant cap; each bottle contains a count of 35 tablets and a 3-g silica gel desiccant pack.

7.3. Storage

All study drug must be stored in a secure, monitored (manual or automated) area in accordance with the labeled storage conditions and with access limited to the Investigator and authorized Study Center staff.

Study drug should be kept refrigerated (2°–8°C) until the bottles are opened (ie, the induction seal is broken). After the bottles have been opened, they should be stored at ambient temperatures and may be used for up to 30 days. The bottles must be kept tightly closed.

Participants will be advised to maintain their study drug supply at room temperature (ie, 50°–86°F), and to report any excursion outside of this temperature range to Study Center staff.

7.4. Treatment Compliance and Adherence

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 (Section 8.4); 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.

Study drug dosing compliance in the eCRF will be based on tablet counts conducted at each scheduled in-clinic and at-home visit during the Treatment Period.

7.5. Accountability

The Investigator/designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received and any discrepancies are reported and resolved before use of the study drug.

Only participants who are eligible per this protocol for study drug administration may receive study drug. Only authorized Study Center staff may supply study drug to the participants.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). A copy of the final Drug Accountability Log will be provided to the Sponsor or designee.

7.6. Handling and Disposal

All unused and reconciled study drug will be returned to the Sponsor or designee; refer to the study's Pharmacy Manual for instruction. No study drug is to be destroyed without prior written permission of the Sponsor.

8. STUDY EVALUATIONS AND MEASUREMENTS

The following safety, PK, and PD assessments/procedures are required per protocol and will be conducted according to Table 1 (Schedule of Events) and documented in the eCRF. Additional details are included below.

During any unscheduled visit, safety assessments will be conducted per Investigator discretion; results will be documented in the source document and the appropriate eCRF.

8.1. Informed Consent

Before a potential study participant undergoes any study-specific evaluations or procedures, he or she (or his/her legal representative/guardian) must provide written, informed consent. See Section 11.3.3 for more information.

8.2. Efficacy Evaluations

Efficacy will not be evaluated in this study.

8.3. Safety Evaluations and Parameters

8.3.1. Demographic and Medical Record Review (Screening Visit only)

At the Screening Visit, demographic characteristics (eg, age, sex, race) will be recorded along with a complete medical history that will include the participant’s diagnosis of MELAS, smoking history, prior and current medications, surgeries, and any additional relevant medical history.

Note: Prior therapies, medications, surgeries, etc. (taken before initiation of study drug) should be differentiated from **concomitant** therapies (taken after initiation of study drug); see Section 6.3.1.

8.3.2. Physical Examination

At Screening and at Follow-up, 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 should 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.

Clinically significant changes since the Screening Visit that are not already associated with an ongoing AE will be recorded as AEs.

8.3.3. Height and Weight

Height will be measured only at Screening. Weight will be measured throughout the study per Table 1.

8.3.4. Electrocardiogram (ECG) and Echocardiogram (ECHO)

All ECGs and the Screening ECHO (if not documented in medical history within 3 months prior to Day 1; see Inclusion Criterion 13) 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.

All ECGs will be evaluated by an Investigator or qualified designee for the presence of abnormalities. Results will be reported as "normal," "abnormal clinically significant," or "abnormal not clinically significant." Abnormalities of clinical significance will be recorded as an AE.

If a confirmed clinically significant abnormal result is obtained, the Study Center should follow standard institutional procedure until the result resolves to baseline. If concerns remain, escalate the issue to the Sponsor's Medical Monitor.

8.3.5. Vital Signs

8.3.5.1. Blood Pressure and Heart Rate (Pulse)

All BP and pulse measurements will be obtained with an automated BP device (left arm is preferred) prior to blood draws (or ≥10 m after a blood draw, if necessary).

- S*upine 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 BP and pulse measurements are recorded, then assume sitting position for ≥1 minute, and finally assume a standing position for 2 (±1) minutes before standing measurements are recorded. Values from these measurements will be used to calculate and record orthostatic BP and pulse.

8.3.5.2. Respiratory Rate, Oxygen Saturation, and Temperature

Respiratory rate and oral temperature (°C) should be measured after the participant has been resting supine /seated for ≥5 minutes.

Oxygen saturation measurements should be taken by pulse oximeter on room air.

> **Note:** If oxygen saturation declines <85% or if decreasing oxygen saturation (decreasing values across 2 sequential visits), the participant should be assessed (eg, cardiovascular exam, chest x-ray, as indicated) for pulmonary edema or other causes of decreased oxygen saturation.

8.3.6. Clinical Laboratory Assessments

Tests detailed in Table 3 will be performed by the Study Center and sent to the Central laboratory.

Clinical safety laboratory (clinical chemistry, coagulation, hematology, and urinalysis) and pregnancy results on Day 1 must be reviewed and assessed for clinical significance prior to initiation of study drug dosing; local laboratory values may be used for this purpose. A separate set of Day 1 samples will be sent to the Central laboratory and processing for eCRF recording.

Protocol-specific laboratory parameter requirements for inclusion or exclusion of participants are detailed in Section 5.

Participants will fast for 3 to 4 hours before safety laboratory sample collections.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or as required by local regulations. Laboratory assessments may be repeated if an error is suspected. However, the maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.

Throughout the study, the Investigator must review each laboratory report, document this review, and file the laboratory reports with the source documents.

Table 3: Protocol-Required Safety Laboratory Assessments

Assessment	Parameter			
Hematology	Hematocrit Hemoglobin Platelet count RBC count	RBC indices: -- MCH -- MCV -- %Reticulocytes	WBC count w/differential: -- Basophils -- Monocytes -- Eosinophils -- Neutrophils -- Lymphocytes	
Clinical chemistry (serum)	Alkaline phosphatase ALT/SGPT AST/SGOT Bilirubin (total, direct)	BUN Calcium Creatinine	Creatine kinase GGT Glucose (fasting)	Potassium Protein (total) Sodium
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. Also see Section 8.3.6.1.

8.3.6.1. Pregnancy Testing

Contraception requirements for male and female participants are provided in Inclusion Criteria 9, 10, and 11 (Section 5.1).

For women of reproductive potential (defined in Section 5.3.4.1), a pregnancy test result from each scheduled visit must be documented in the eCRF. Negative results must be confirmed and documented at Screening and at predose on Day 1 for study entry eligibility. Additional testing will be performed when a menstrual cycle is missed or when pregnancy is otherwise suspected.

A confirmed pregnancy test result will result in immediate discontinuation from study drug (see Section 5.6.2). See Section 10.1.4 for pregnancy reporting requirements.

8.3.6.2. Genetic Evaluations

Genetic evaluation in this study is limited to confirmation of MELAS (by medical history) at Screening for study eligibility determination.

8.3.7. Suicidality

IW-6463 is a CNS-active investigational drug. Although IW-6463 and other similar drugs in this class have not been associated with an increased risk of suicidal thinking or behavior, the Sponsor considers it important to monitor for such ideation or behavior before and during this clinical study. Therefore, participants should be appropriately monitored and closely observed for suicidal ideation and behavior or any other unusual change in behavior. The Columbia Suicidality Severity Rating Scale (C-SSRS) (13, 14) will be administered according to Table 1, starting predose on Day 1 of the Treatment Period and at all subsequent visits where clinical assessments are conducted, including at any unscheduled visit. Immediate consultation with the Medical Monitor should be sought for participants who experience signs of suicidal ideation or behavior, and consideration given to discontinuing study drug.

8.3.8. Adverse Event and SAE Monitoring

Adverse events (AEs) will be monitored and recorded throughout the study starting from ICF execution through the Follow-up Visit. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

Investigators are not obligated to actively collect AE information after conclusion of the participant's involvement in the study. However, if the Investigator learns of any SAE or death at any time after a participant has been discharged and the Investigator considers the event to be reasonably related to the study drug or to study participation, he/she must promptly notify the Sponsor.

See Section 10 for details regarding AE management and reporting requirements.

8.4. Participant Diary for Recording Changes in Health Status, Daily Dosing, and Concomitant Medications

At the Screening Visit, each participant will be issued a paper (source) diary in which they (or their legal representative/guardian) will be asked to record on a daily basis the name of any other medication(s) they the take, along with the date, time, and dose strength of the medication(s); and any changes in their health status (including the dates, times, and brief descriptions).

Starting on Day 1 of the Treatment Period, they will be instructed to also record the date and time of each study drug dose taken at home, in addition to any other medications they take (including name, date, time, and dose strength), and any changes in their health status (including the dates, times, and brief descriptions).

Participants will be asked to have the diaries available at each scheduled at-home and in-clinic visit during the Treatment Period for monitoring by the study staff.

During the Follow-up Period, participants will be asked to continue to record in the same manner any concomitant medications and changes in their health status. The diaries will be collected from the participants at the Follow-up Visit.

8.5. Clinical Outcomes Assessments/Participant Questionnaires

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

The Patient-reported Outcomes Measurement Information System (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. It uses a Likert-type rating scale (eg, "Never" to "Very often").

8.5.2. 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 (eg, "Very much improved" to "Very much worse").

8.5.3. Modified Fatigue Impact Scale (MFIS)

The Modified Fatigue Impact Scale (MFIS) is a self-administered questionnaire that assesses the impact of fatigue in terms of physical, cognitive, and psychosocial functioning over the past 4 weeks. Patients respond using a Likert-type rating scale. (eg, "Never" to "Almost always").

8.6. PK Evaluations (Plasma and CSF)

PK samples will be collected as scheduled in Table 1. The actual date and time (24-h clock time) of each sample collection will be recorded in the source documents and eCRF. Each sample will be divided into 2 aliquots (1 each for PK and a back-up). Specific instructions for the collection and handling of biological samples are provided in the study's laboratory manual.

- Sparse whole-blood samples of approximately 2 mL will be collected for measurement of plasma concentrations of IW-6463 using a validated liquid chromatography-tandem mass spectrometry bioanalytical method.
- From each participant who does not withhold consent for the procedure, a CSF sample of approximately 4 mL will be collected for measurement of IW-6463 CSF concentration using a validated liquid chromatography-tandem mass spectrometry bioanalytical method.

The timing of sampling may be altered during the course of the study based on emerging data (eg, to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Samples collected for analyses of IW-6463 concentration may also be used to evaluate safety aspects related to concerns arising during or after the study.

Biological samples collected from participants in this study may be stored for future analysis for not more than 5 years.

8.7. PD Evaluations and Parameters (Neuroimaging)

Two exploratory functional neuroimaging modalities, arterial spin labeling (ASL) and functional magnetic resonance imaging (fMRI), will be used to measure the potential PD effects of IW-6463 in the brain. While ASL quantifies regional CBF during the resting state, fMRI is a relative measure based on the blood-oxygen-level-dependent (BOLD) effect and will be conducted during both a resting state and a visual stimulus to measure brain activity.

See Table 1 for specific imaging time points. Refer to the study's imaging manual for detailed aspects of each MRI procedure, including the scanning guidelines. Each individual MRI scanning session will take approximately 45 minutes (maximum of ~1 hour). *Note*: Scanners that lack fMRI capabilities *may* be considered for use in this study with Sponsor approval.

8.8. Immunogenicity

Immunogenicity testing will not be conducted in this study.

8.9. Biomarkers

8.9.1. Circulating Biomarkers in Blood

Plasma and serum samples for biomarkers will be collected from each participant as scheduled in Table 1. These biomarkers will test the target engagement of IW-6463 as well as the impact of IW-6463 [redacted]

8.9.2. Brain Metabolites

The IW-6463 effect on the neuronal profile of the brain will be measured in each participant by proton magnetic resonance spectroscopy (^{1}H-MRS) at the timepoints specified in Table 1. [redacted]. Detailed aspects of the MRS procedures are provided in the separate imaging manual provided for this study.

8.10. Medical Resource Utilization and Health Economics

Because C6463-201 is a limited-duration, first-in-patient study, health economics and health-care utilization endpoints will not be evaluated.

9. STATISTICAL CONSIDERATIONS

See Section 3 for a list of the study objectives and their corresponding endpoints. Section 4.5 provides overall study stopping criteria.

9.1. Statistical Hypotheses

Within-treatment statistical hypothesis testing will be performed on postdose change from baseline (CFB) exploratory endpoint. The null hypothesis assumes the CFB=0; rejecting the null hypothesis supports the alternative hypothesis (CFB≠0).

9.2. Sample Size and Power

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.

9.3. Statistical Methods

9.3.1. General Considerations

Descriptive statistics (n, mean, standard deviation [SD], minimum, maximum, median, and interquartile range) will be calculated to summarize continuous variables. Frequency and percentage of participants in each category will be calculated to summarize categorical variables.

Due to the developmental stage of this study, no adjustments will be made for multiplicity. Details of the data handling methods will be specified in the statistical analysis plan. Inferential statistics will be used for descriptive purposes only. If not otherwise specified, the baseline value is defined as the last non-missing value measured before administration of study drug on Day 1.

9.3.2. Populations for Analyses

Analysis populations are defined as follows.

Population	Description
Screened	All participants who sign the ICF
Safety	All participants who sign the ICF and take ≥1 dose of study drug
PD Evaluable	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
PK Evaluable	All participants who complete the EOT Visit and have ≥1 evaluable postdose PK assessment

9.3.3. Disposition

The total number of screened participants and the number of participants who are screen failures will be tabulated. The number and percentage of participants included in each population (Screened, Safety, PD Evaluable, and PK Evaluable) will be presented. The number and percentage of participants who completed the study or discontinued early, as well as the reasons for discontinuation will be presented for the Safety Population.

9.3.4. Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (eg, means and SDs for continuous variables such as age and percentages for categorical variables such as sex).

9.3.5. Drug Exposure and Compliance

Exposure to open-label study drug, calculated as the number of days from the first dose taken to the date of the last dose taken, inclusive, will be summarized for the Safety Population. The total number of doses taken between specified visits and overall for the entire study will be calculated for each participant. Compliance will be based on the number of doses expected to be taken. Percent compliance will be summarized for each scheduled visit and overall. Compliance rates will also be categorized as missing, <80%, ≥80% and ≤120%, and >120% and will be summarized.

9.3.6. Prior and Concomitant Medications and Procedures

See definitions for prior and concomitant medications and procedures in Section 6.3. 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.

9.3.7. Major 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. 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 major protocol deviations will be presented in a data listing.

9.3.8. Safety Analyses

Incidence of AEs will be summarized by Medical Dictionary for Regulatory Activities system organ class and preferred term. By-participant listings will be provided for pretreatment AEs, TEAEs, severe AEs, drug-related AEs, SAEs, and AEs leading to study discontinuation or death. All safety analyses will be conducted on the Safety Population.

ECGs, vital signs, and clinical laboratory tests (in International System [SI] units) at each assessment time point and the change from baseline at each postbaseline time point will be summarized using descriptive statistics unless noted otherwise. Listings will be provided for participants with abnormal values.

A TEAE is defined as an adverse event that emerges during treatment, having been absent pretreatment, or that worsens relative to the pretreatment state.

9.3.8.1. ECGs

The number and percentage of participants with absolute QTcF intervals in the following categories will be examined: QTcF≤450 ms, 450 ms<QTcF≤480 ms, 480 ms<QTcF≤500 ms, and QTcF>500 ms. Shift tables will be presented comparing values between baseline and end of study.

Additionally, the number and percentage of participants with a change from baseline in QTcF interval according to the following categories will be examined: QTcF interval increases by >30 ms, but ≤60 ms, and QTcF interval increases by >60 ms. Shift tables will be presented comparing values between baseline and end of study.

9.3.9. Patient-Reported Outcomes Analyses

Health outcomes analyses (Section 8.5) will be based on the PD Evaluable Population. Descriptive summaries for each domain of the questionnaires will be presented for each assessment time point and the change from baseline at each postbaseline time point, unless noted otherwise.

9.3.10. PK Analyses

Descriptive statistics will be calculated and summarized for plasma and CSF concentrations of IW-6463 at each assessed timepoint.

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. The results of these analyses may be reported separately outside of the clinical study report.

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 (AUC_{tau}), time to C_{max} (T_{max}), $t_{1/2}$, maximum observed plasma concentration (C_{max}), 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, and will be calculated and presented as data permit.

9.3.11. PD Analyses

Pharmacodynamic analyses will be conducted using the Safety and PD populations.

Plasma biomarkers and imaging endpoints at each assessment time point and the change from baseline at each postbaseline time point will be summarized using descriptive statistics unless noted otherwise. Listings will be provided for all participants.

9.4. Controlling for Multiplicity

Due to the developmental stage of this study, no adjustments will be made for multiple comparisons.

9.5. Interim Analysis

An interim analysis is not planned.

9.6. Independent Data Monitoring Committee

Because this is an open-label study, a data monitoring committee will not be utilized.

10. SAFETY MANAGEMENT AND REPORTING OF ADVERSE EVENTS

10.1. Monitoring and Reporting Details

All AEs (related and unrelated; serious or nonserious) will be monitored and recorded from the signing of the ICF until the Follow-up Visit in accordance with regulatory, Sponsor, and Good Clinical Practice (GCP) guidelines and requirements.

Changes in laboratory values that are not already part of an ongoing AE *and* that are considered clinically significant are to be reported as AEs. For example, an ALT >3×the upper limit of normal range in the context of a diagnosis of hepatitis would not be reported as an AE. However, if the same elevation occurs outside of the context of an existing diagnosis and is considered clinically significant, it would be reported as an AE.

The AE term should be reported in standard medical terminology. For each AE, the Investigator will evaluate and report the onset (date and time), outcome/resolution (date and time), seriousness, severity (intensity), causality, action(s) taken with study drug, and whether any therapies or medications were administered in response to the AE.

10.1.1. SAE Reporting

All SAEs must be reported to the Sponsor using the AE eCRF page within 24 hours of the Investigator's first awareness of the event. In all cases, the Investigator must verify the accuracy of the information with the corresponding source documents.

10.1.2. IRB/IEC Notification of SAEs and Other Unanticipated Events

The Sponsor or designee will notify FDA and all investigators of all unexpected, serious, study drug-related (per Sponsor assessment) events (ie, 7- or 15-day FDA safety reports) that occur at any Study Center during the study. The Principal Investigator at each site will be responsible for notifying the relevant IRB/IEC of these additional SAEs, according to local standard operating procedures (SOPs).

10.1.3. Follow-up Reports

The Investigator is responsible for ensuring that all AESIs and SAEs are followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (see Section 5.5).

The investigator will submit via the AE eCRF any updated SAE information within 24 hours of the investigator's first awareness of the information.

10.1.4. Reporting of Pregnancy in a Participant or Participant's Partner

A female participant who reports pregnancy **before** initiation of study drug dosing on Day 1 will be withdrawn from study participation; the discontinuation will be recorded as a screen failure.

Should a pregnancy occur in either a participant or a participant's partner after initiation of study drug, it must be reported and recorded on the study-specific pregnancy form. The Study Center must notify Syneos Health GSPV (see Section 10.1.1 for contact details) within 24 hours from the time that Center personnel first learn of the pregnancy.

The Study Center should make reasonable efforts to follow the pregnancy to term, notifying Syneos Health GSPV of the pregnancy outcome (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) within 24 hours of awareness.

All reports of congenital abnormalities/birth defects and still births will be considered SAEs. Spontaneous miscarriages, elective termination without complications, and normal births without congenital abnormality will not be reported or handled as SAEs, but will be reported as the outcome of the respective pregnancy.

If the pregnancy is associated with an SAE, a separate SAE form will be completed.

10.2. Definition of Adverse Events

10.2.1. Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. Regardless of causality, all AEs must be recorded and reported on the forms provided by the Sponsor from the time that a participant signs the ICF at the Screening Visit until completion of the Follow-up Visit.

10.2.1.1. Definition of TEAE

A TEAE is defined as an adverse event that emerges during treatment, having been absent pretreatment, or that worsens relative to the pretreatment state.

10.2.2. Serious Adverse Event (SAE)

An SAE is an AE occurring during any study phase (ie, baseline, treatment, washout, or follow-up), and at any dose of the study drug, comparator or placebo, that fulfills 1 or more of the following:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity: a substantial disruption of a person's ability to conduct normal daily functions
- Results in a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the participant or may require medical intervention to prevent 1 of the outcomes listed above.

All SAEs that occur after any participant has been enrolled, before treatment, during treatment, or within the Follow-up Period after the cessation of treatment, whether or not they are related to the study, must be recorded on forms provided by the Sponsor.

10.2.3. Adverse Events of Special Interest (AESIs)

Based on the mechanism of action of sGC and anticipated vasodilation effects and based on the fact that IW-6463 is a CNS-penetrant stimulator of sGC, the Sponsor will collect AESIs in a timely manner.

The AESIs relate to symptomatic hypotensive events and/or tachycardia, dizziness, syncope, and TEAEs related to change of neurobehaviors (ie, suicidality or euphoria). In addition, because riociguat, a marketed sGC stimulator, includes bleeding events as a warning and precaution on its prescribing information (15), bleeding events will also be considered AESIs for IW-6463 in

this study. The specific list of TEAE terms will be provided in the C6463-201 safety management plan.

An AESI classification means that although an event might be nonserious, it will nonetheless be reported to the Sponsor by completing an SAE form, filling in the AESI box, and submitting to Syneos Health (see Section 10.1.1) within 5 business days of the Investigator's awareness of the event.

10.3. Categorization of Events

10.3.1. Relationship to Study Drug

For all TEAEs, the Investigator must provide an assessment of causal relationship to study drug. The causality assessment must be recorded in the patient's source documentation and on the AE page of the subject's eCRF. Causal relationship must be assessed according to the following:

Related:	An event where there is at least a reasonable possibility of a causal relationship between the event and the study drug. "Reasonable possibility" means there is evidence to suggest a potential causal relationship between the drug and the TEAE.
Unrelated:	Any other event

10.3.2. Severity (Intensity)

The intensity (severity) of each AE will be assessed according to the following scale:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is different than seriousness. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 10.2.2. An AE of severe intensity may not necessarily be considered serious, and vice versa.

11. STUDY ADMINISTRATION AND ETHICAL CONSIDERATIONS

11.1. Data Collection and Management

- The Sponsor's procedures include quality control and assurance checks on all clinical studies that it conducts. Before enrolling any participants into this study, Sponsor personnel and/or a trained designated representative will review with the Investigator: the protocol, the IB, the eCRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs.
- All participant data relating to the study will be recorded on eCRFs unless transmitted electronically to the Sponsor or designee (eg, laboratory data).

- The Investigator is responsible for verifying that all data entries on the eCRFs are accurate and correct and ensure that all data are entered in a timely manner, as soon as possible after information is collected. An explanation should be provided for all missing data.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- Data entered into the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records. In addition, current medical records must be available.

11.1.1. Participant Confidentiality and Data Protection

- After ICF execution (see Section 11.3.3), each participant will be assigned a unique identifier by the Study Center. Any participant records or datasets that are transferred to the Sponsor will contain only the identifier; participant names or any information that would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records (eg, eCRFs) recorded during the study may be examined by Research Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

11.2. Monitoring and Inspection of Data and Study Centers

11.2.1. Monitoring of Study Center and Inspection of Records

The Sponsor will be allowed to conduct site visits to the study center(s) for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, participant charts and study source documents, and other records relative to study conduct.

Before the Study Center can enter a participant into the study, a representative of the Sponsor will visit the Center to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the Sponsor or its representatives. This will be documented in a Clinical Study Agreement between the Sponsor and the Investigator.

During the study, a monitor from the Sponsor or its representative will have regular contacts with the Study Center, for the following:

- Provide information and support to the Investigator(s)

- Confirm that facilities remain acceptable
- Confirm that the study team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that study drug accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRFs with the participant's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each participant (eg, clinic charts).
- Record and report any protocol deviations not previously sent to the Sponsor.
- Confirm AEs, AESIs, and SAEs have been properly documented on eCRFs and confirm that:
  - All SAEs and AESIs have been forwarded to the Sponsor within the requested timelines
  - SAEs that met criteria for reporting have been forwarded to the IRB

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

11.2.2. Audits and Inspections

Authorized representatives of the Sponsor, regulatory authority, IEC, or IRB may visit the Study Center to perform audits or inspections, including source data verification. The purpose of an audit or inspection by the Sponsor is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and that data were recorded, analyzed, and accurately reported according to the protocol, International Council on Harmonisation (ICH) GCP guidelines, and any applicable regulatory requirements.

The Investigator should immediately contact the Sponsor if contacted by a regulatory agency about an inspection.

11.2.3. Retention and Archiving of Records

The Principal Investigator at each Study Center must maintain all documentation relating to the study including eCRFs, source documents (including signed ICFs, laboratory test results, and medication inventory records), and audit trails, in accordance with locally applicable regulatory requirements; and, in any event, for a period of 2 years after the last marketing application approval, or if not approved, 2 years after the discontinuance of the study drug for investigation. If it becomes necessary for the Sponsor or Regulatory Authority to review any documentation relating to the study, the Investigator must permit access to such records.

No study records will be destroyed without notifying the Sponsor and giving the Sponsor the opportunity to take such study records or authorizing in writing the destruction of records after the required retention period.

11.2.4. Quality Control and Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, the Sponsor may conduct a quality assurance audit. See Section 11.2.2 for details.

11.3. Ethics

11.3.1. IRB/IEC and Investigator Requirements

The Principal Investigator at each Study Center must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRB for this study including the final version of the ICF and recruitment materials must be maintained by the Principal Investigator and made available for inspection.

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by the IRB/IEC as appropriate. The Principal Investigator at a given Study Center must submit written approval to the Sponsor before enrolling any participant into the study.

The Principal Investigator is responsible for informing the IRB/IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB/IEC must approve all advertising used to recruit participants for the study. The protocol must be re-approved by the IRB/IEC upon receipt of amendments and annually, as local regulations require.

Progress reports and notifications of SAEs will be provided to the IRB or IEC by the Investigator according to local regulations and guidelines.

11.3.2. Ethical Conduct of the Study/Compliance Statement

This study will be conducted in full accordance all applicable local, state, and federal laws, in addition to 45 Code of Federal Regulations (CFR) 46, 21 CFR Parts 50, 54, 56, 312, and 314 and ICH GCPs. All episodes of noncompliance will be documented.

All Investigators will perform the study in accordance with this protocol, will obtain written, informed consent, and will report unanticipated problems involving risks to participants or others in accordance with local SOPs and federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research participants during and after the study.

11.3.3. Written Informed Consent

Informed consent procedures will comply with the CFR 21, Parts 50 and 312, and ICH E6(R2) guidelines. The written ICF must be pre-approved by the IRB/IEC for the purposes of obtaining and documenting consent.

Before entry into the study, the Investigator(s) at each Study Center will ensure that each participant, if applicable, is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. All participants must be notified that they are free to discontinue from the study at any time. They must be given the opportunity to ask questions and allowed time to consider the information provided.

Each participant's signed and dated informed consent must be obtained before conducting any study procedures on that participant. The participant will receive a copy of the signed and dated documents. The Investigator must retain each participant's original signed ICF.

If new information becomes available that may be relevant to the participant's consent and willingness to participate in the study, the ICF will be revised and the participant will be reconsented. The revised ICF(s) must be submitted to the IRB/IEC for review and approval before being used.

11.4. Changes in the Conduct of the Study or Planned Analyses

Any amendment to this protocol will be provided to the Investigator in writing by the Sponsor or its designee. Before implementation, any protocol amendment regarding reportable deviations (as defined by the IRB/IEC) must be approved by the IRB/IEC and the signature page must be signed by the Investigator and received by the Sponsor or its designee, with the following exception: If the protocol is amended to eliminate or reduce the risk to participants, the amendment may be implemented before IRB/IEC review and approval. However, the IRB/IEC must be informed in writing of such an amendment, and approval must be obtained within reasonable time limits.

Deviating from the protocol is permitted only if absolutely necessary for the safety of the participants and must immediately be reported to the Sponsor or its designee.

11.5. Trial Safety Committee Structure and Safety Data Reviews

The Trial Safety Committee will include, at a minimum, the Sponsor's Medical Monitor, the Sponsor's Safety Monitor, and the Lead Principal Investigator or designee. The Sponsor, when appropriate, will invite other specialists to participate as necessary, such as PK scientists, statisticians, and clinical specialists.

The Committee will review available safety data after 3 participants have completed the study treatment. They will also meet on an ad hoc basis during the study if an unanticipated trend or signal emerges.

[redacted]

13. LIST OF REFERENCES

1. El-Hattab AW, Emrick LT, Craigen WJ, Scaglia F. Citrulline and arginine utility in treating nitric oxide deficiency in mitochondrial disorders. Mol Genet Metab. 2012;107(3):247-52.
2. Onyango IG, Lu J, Rodova M, Lezi E, Crafter AB, Swerdlow RH. Regulation of neuron mitochondrial biogenesis and relevance to brain health. Biochim Biophys Acta. 2010;1802(1):228-34.
3. Navarro A, Boveris A. Mitochondrial nitric oxide synthase, mitochondrial brain dysfunction in aging, and mitochondria-targeted antioxidants. Adv Drug Deliv Rev. 2008;60(13-14):1534-44.
4. Clementi E, Nisoli E. Nitric oxide and mitochondrial biogenesis: a key to long-term regulation of cellular metabolism. Comp Biochem Physiol A Mol Integr Physiol. 2005;142(2):102-10.
5. Dai Z, Wu Z, Yang Y, Wang J, Satterfield MC, Meininger CJ, et al. Nitric oxide and energy metabolism in mammals. Biofactors. 2013;39(4):383-91.
6. Nisoli E, Falcone S, Tonello C, Cozzi V, Palomba L, Fiorani M, et al. Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. Proc Natl Acad Sci USA. 2004;101(47):16507-12.
7. El-Hattab AW, Emrick LT, Hsu JW, Chanprasert S, Almannai M, Craigen WJ, et al. Impaired nitric oxide production in children with MELAS syndrome and the effect of arginine and citrulline supplementation. Mol Genet Metab. 2016;117(4):407-12.
8. El-Hattab AW, Hsu JW, Emrick LT, Wong LJ, Craigen WJ, Jahoor F, et al. Restoration of impaired nitric oxide production in MELAS syndrome with citrulline and arginine supplementation. Mol Genet Metab. 2012;105(4):607-14.
9. Koga Y, Akita Y, Junko N, Yatsuga S, Povalko N, Fukiyama R, et al. Endothelial dysfunction in MELAS improved by l-arginine supplementation. Neurology. 2006;66(11):1766-9.
10. Ganetzky RD, Falk MJ. 8-year retrospective analysis of intravenous arginine therapy for acute metabolic strokes in pediatric mitochondrial disease. Mol Genet Metab. 2018;123(3):301-8.
11. Koenig MK, Emrick L, Karaa A, Korson M, Scaglia F, Parikh S, et al. Recommendations for the management of stroke-like episodes in patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes. JAMA Neurol. 2016;73(5):591-4.
12. Parikh S, Goldstein A, Karaa A, Koenig MK, Anselm I, Brunel-Guitton C, et al. Patient care standards for primary mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med. 2017;19(12).

13. Posner K, Melvin GA, Stanley B, Oquendo MA, Gould M. Factors in the assessment of suicidality in youth. CNS spectrums. 2007;12(2):156-62.
14. Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011;168(12):1266-77.
15. Highlights of Prescribing Information - ADEMPAS (riciguat) tablets, for oral use FDA; 2018.

APPENDIX 1. CONCOMITANT MEDICATIONS AND SUPPLEMENTS

Note: Also see Section 6.3.2.

Use of the following is <u>prohibited</u> from the Screening Visit through the Follow-up Visit, unless noted otherwise below:

- Specific inhibitors of PDE5, including sildenafil, tadalafil, vardenafil
- Nonspecific inhibitors of PDE5, including dipyridamole, theophylline
- Any supplement for the treatment of erectile dysfunction
- Other sGC stimulators, including riociguat and vericiguat
- Nitrates such as nitroglycerin, isosorbide mononitrate, isosorbide dinitrate, sodium nitroprusside, amyl nitrate
  Note: Participants taking arginine or citrulline for the treatment of mitochondrial disease will be allowed to continue these therapies.
- Aspirin ≥325 mg/day
- Any anticoagulant medication
- Any P2Y12 inhibitor, including cangrelor, clopidogrel, prasugrel, ticagrelor, ticlopidine
- Use of any "illicit drug" is not permitted beginning 1 month before Screening through the Follow-up Visit
  Note: Use of cannabis and cannabidiol products for medical purposes is permitted, except for 24 hours before the Screening, Day 1, and EOT visits, and for 4 hours prior to all other study visits.
- [redacted]

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