STATISTICAL ANALYSIS PLAN

(Double Blind Part)

Final Version 3.0 dated 09-Jul 2024

A Randomized, Double-blind, Placebo-controlled Phase 2 Study to Assess the Efficacy, Safety and Tolerability of ASP0367 in Participants with Primary Mitochondrial Myopathy

ISN: 0367-CL-1201
IND number: 146773

Astellas Pharma Inc. (API)

This document contains confidential information which is the intellectual property of Astellas. By accepting or reviewing this document, you agree to hold this information in confidence and not copy or disclose it to others or use it for unauthorized purposes except (1) as otherwise agreed to in writing; (2) where required by applicable law; (3) where disclosure is directly related to the care and safety of the research participant; and (4) where disclosure of such information is made to a member of the investigator's team who agrees to hold this information in confidence.

Table of Contents

I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
5XSTS	5 times sit to stand
6MWT	6-minute walk test
AE	adverse event
AESI	adverse events of special interest
AIDMC	Astellas Internal Data Monitoring Committee
ALDOA	aldolase A
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
$C_{ave, ss}$	average plasma concentration at steady state
CI	confidence interval
CK	creatine kinase
CPEO	Chronic Progressive Externa Ophthalmoplegia
CSR	clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
cTnI	cardiac troponin I
cTnT	cardiac troponin T
C_{trough}	concentration immediately prior to dosing at multiple dosing
CYC	cytochrome c
ECG	electrocardiogram
ECHO	echocardiogram
EQ-5D	European Quality of Life 5-dimension questionnaire
EQ-5D-5L	European Quality of Life 5-dimension, 5-level questionnaire
EQ-VAS	EuroQol visual analogue scale
FAS	full analysis set
FLNC	filamin-C
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	investigational new drug
IP	investigational product
IRT	interactive response technology
ISN	international study number
KSS	Kearns Sayre Syndrome
LS	least-squares
MedDRA	medical dictionary for regulatory activities
MEGDEL	3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like disease
MELAS	Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes
MERRF	Myoclonic Epilepsy with Ragged Red Fibers
MFIS	modified fatigue impact scale
MIDD	Maternally Inherited Diabetes Deafness
MMRM	mixed model for repeated measures
Neuro-QoL	Quality of Life in Neurological Disorders
OLE	open-label extension

Abbreviations	Description of abbreviations
PD	protocol deviation
PGIC	patient global impression of change
PGIS	patient global impression of severity
PMM	primary mitochondrial myopathy
POLG	polymerase-gamma
PPARδ	peroxisome proliferator-activated receptor delta
QTcF	QT interval using Fridericia's correction
SAF	safety analysis set
SAP	statistical analysis plan
SD	standard deviation
SE	standard errors
TEAE	treatment emergent adverse event
TLF	tables, listings and figures
ULN	upper limit of normal

List of Key Terms

Terms	Definition of terms
Baseline	Assessments of participants as they enter a study before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study.
Enroll	To register or enter a participant into a study. Note: Once a participant has received the investigational product or placebo, the protocol applies to the participant.
Randomization	The process of assigning participants to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential participants for enrollment in a study.
Screen failure	Potential participant who signed the informed consent form but did not meet one or more criteria required for participation in the study and was not enrolled.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

This SAP contains technical and detailed elaboration of the principal features of the analysis described in the protocol and includes procedures for executing the statistical analysis to fulfil the objectives of the study.

The study was designed to be, and executed as, a double-blinded with an OLE. The OLE was removed from protocol version 9.0 and so, the use of the present tense is no longer accurate. In addition to the objectives and endpoints defined in Study 0367-CL-1201 protocol (core protocol), some additional objectives and endpoints specific to the Mayo Clinic (Minnesota) had been defined in Study 0367-CL-1201 [MAYO] Protocol. However, as there have been no participants recruited to the protocol from the Mayo sites, therefore there will be no SAP created. Until protocol version 7.0, the double-blinded period was 52 weeks and open label extension period was 24 weeks. However, from protocol version 8.0 onwards, the double-blinded period was revised to 24 weeks and in protocol version 9.0 the OLE period was removed. Since no participants have been evaluated under protocol version 8.0 or later, two SAPs will be prepared: one for analyses up to 52 weeks of treatment (the double-blind part of the study), and the other one for analyses at the end of 76 weeks of treatment (the double-blind part and the OLE part of the study). In analysis for the double-blind part and the OLE part of the study, participants assigned ASP0367 in the double-blinded period will be analyzed for through the double-blinded part and the OLE part of the study including follow-up visit, while participants switched from Placebo to ASP0367 in the OLE part will be analyzed in the OLE part only including follow-up visit.

This document is an analysis plan for 52 weeks (double-blinded part) including follow-up visit which correspond to protocol v7.0. However, inferential analyses for efficacy endpoints will be conducted at week 24 aligned with protocol v9.0.

Analyses for pharmacokinetic and pharmacodynamic (target gene expression and biochemical markers) endpoints, including exposure-response assessment, will be described in the separate analysis plan and this SAP only focus on efficacy and safety endpoints.

This document will be finalized prior to the database lock at the end of study. If there are any changes from the planned analyses in the final version of the SAP that impact the statistical analyses, then it will be documented in the CSR.

2 STUDY OBJECTIVES AND DESIGN

2.1 Study Objectives, Endpoints and Estimands

Objectives	Endpoints
Primary	
● To assess the dose response of ASP0367 on functional improvement relative to placebo	● Change from baseline in distance walked on the 6MWT at week 24
● To assess the safety and tolerability of ASP0367 relative to placebo	● Safety and Tolerability through week 24 and end of study: o Nature, frequency and severity of TEAEs o Vital signs

	○ Weight ○ 12-lead ECG ○ Clinical laboratory tests (hematology, biochemistry [including serum cardiac troponin I] and urinalysis) ○ C-SSRS
Secondary	
● To assess the dose response of ASP0367 on functional improvement and fatigue relative to placebo	● Change from baseline in Neuro-QoL Short Form Fatigue and Lower Extremity Function (Mobility) scores at week 24 ● Change from baseline in time spent on the 5XSTS at week 24 ● Change from baseline in MFIS at week 24
● To assess the effect of ASP0367 in overall participant functioning relative to placebo	● PGIC scores at week 24 ● Change from baseline in PGIS scores at week 24
Exploratory	
● To assess the pharmacokinetics of ASP0367	● Plasma ASP0367: C_{trough} at weeks 12 and 24 and population pharmacokinetics
● To assess the exposure-response relationship of ASP0367	● Relationship between measured- and model-based pharmacokinetic exposure parameters (e.g., C_{trough}, $C_{ave, ss}$) of ASP0367 and endpoints of efficacy, safety and pharmacodynamic biomarkers, as appropriate
● To assess the effect of ASP0367 on lower extremity function relative to placebo	● Change from baseline in distance walked on the 6MWT at weeks 4 and 12 ● Change from baseline in minute-by-minute analyses (i.e., 6^{th} vs 1^{st}) of 6MWT at weeks 4, 12 and 24 ● Change from baseline in time spent on 5XSTS at weeks 4 and 12 ● Change from baseline in Neuro-QoL Short Form Fatigue score at weeks 4 and 12 ● Change from baseline in Neuro-QoL Short Form Lower Extremity Function (Mobility) score at weeks 4 and 12 ● Change from baseline in MFIS score at weeks 4 and 12
● To assess the effects of ASP0367 on quality of movement and patient perception of change	● PGIC scores at weeks 4 and 12 ● Change from baseline in PGIS scores at weeks 4 and 12 ● Change from baseline in EQ-VAS and EQ-5D-5L index at weeks 4, 12 and 24
● To assess the relationship between functional improvement and biochemical markers, as well as gene expression-based pharmacodynamic biomarkers	● Change from baseline in the levels of serum and urinary biomarkers (serum FLNC, CYC, ALDOA and urinary TITIN) at weeks 12 and 24 ● Relative change from baseline of whole blood PPARδ target gene expression levels at weeks 12 and 24[†]

Note: Placebo comparison will be up to 24 weeks and will not include open-label extension.
5XSTS: 5 times sit to stand; 6MWT: 6-minute walk test; ALDOA: aldolase A; $C_{ave, ss}$: average plasma concentration at steady state; C-SSRS: Columbia-Suicide Severity Rating Scale; C_{trough}: concentration immediately prior to dosing at multiple dosing; CYC: cytochrome c; ECG: electrocardiogram; EQ-5D-5L:

European Quality of Life 5-dimension, 5-level questionnaire; EQ-VAS: EuroQol visual analogue scale; FLNC: filamin-C; MFIS: modified fatigue impact scale; Neuro-QoL: Quality of Life in Neurological Disorders; PGIC: patient global impression of change; PGIS: patient global impression of severity; PPARδ: peroxisome proliferator-activated receptor delta; TEAE: treatment emergent adverse event.
†Blood samples will be collected and stored appropriately over the course of the study and then analyzed.

Estimands

Primary estimand will be defined by the following 5 attributes:

- Treatment: ASP0367 or placebo
- Population: Participants with PMM, as defined by the inclusion/exclusion criteria of the study.
- Endpoint: Change from baseline in distance walked on the 6MWT at week 24.
- Intercurrent events and their corresponding strategies (see table below).
- Population level summary: Difference in change from baseline in distance walked on the 6MWT at week 24 between ASP0367 group and placebo group.

Intercurrent Events	Strategies to Handle Intercurrent Events
Treatment Discontinuation Due to Any Reason	For those participants who discontinued treatment, all observations after treatment discontinuation (Date of Last Study Drug Taken + 7 days) will be ignored. Analysis will be conducted assuming those participants remained assigned treatment and the condition that was observed before treatment discontinuation will continue, i.e., hypothetical strategy.
Study Discontinuation	For those participants who discontinued study, all observations after treatment discontinuation (Date of Last Study Drug Taken + 7 days) will be ignored. Analysis will be conducted assuming those participants remained assigned treatment and the condition that was observed before treatment discontinuation will continue, i.e., hypothetical strategy.
Prohibited Concomitant Treatment Use	For those participants who used the prohibited concomitant medication treatment, all observations will be included in the analysis, i.e., treatment policy strategy.

2.2 Study Design

This is a randomized, double-blind, placebo-controlled, oral dose, phase 2 study to evaluate the dose response of ASP0367 on functional improvement relative to placebo, safety and tolerability in participants with PMM. Efficacy (i.e., functional improvement) will be assessed by a functional motor test, 6MWT. The study consists of the following portions: screening (4 weeks); double-blind treatment period with 2 doses of ASP0367 vs matching placebo (24 weeks); and follow-up (4 weeks).

A 24-week treatment analysis of efficacy and safety will be the primary focus of this study. Since participants enrolled and participated in the study prior to implementation of protocol version 9.0, which removes the 52-week OLE period, they may have a treatment duration of up to 76 weeks, a separate analysis will be conducted up to 76 weeks and follow-up as per available data.

2.3 Randomization

Approximately 66 participants will be enrolled. At randomization, participants will be randomly placed into 1 of 3 arms (30 mg ASP0367, 75 mg ASP0367 or placebo; n = 22 for each arm) at a ratio of 1:1:1. Stratified randomization by site group (2 level: Mayo clinic_Minnesota; Non-Mayo clinic_Minnesota) will be applied if there are any participants available from the Mayo site group.

3 SAMPLE SIZE

The sample size of this study was determined from feasibility and statistical perspectives. From the statistical perspective, 57 participants (19 participants in each group) will provide > 80% power to detect a difference of 45 meters in 6MWT between ASP0367 and placebo at a 2-sided significance level of 0.10 assuming a common standard deviation of 55.0 meters.

The 45-meter difference in 6MWT under the 24-week treatment is assumed based on preliminary results that were observed in cohort 1 of the MMPOWER-2 phase 2 randomized controlled crossover study of elamipretide [Cohen et al, 2018].

Considering the interim analysis, 60 participants will be required to maintain > 80% power in the efficacy analysis, and assuming an approximately 10% study discontinuation (drop out) rate during the double-blind treatment period, 66 participants are required to be randomized. This sample size supports an evaluation of proof of concept.

4 ANALYSIS SETS

In accordance with ICH recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

The determination of whether participants are included or excluded from the safety and efficacy analysis sets will be made prior to database lock for the primary study report and prior to unblinding.

The following populations are defined for the 52-week treatment analysis:

Population	Description
FAS	All participants who receive at least 1 dose of ASP0367 or placebo for the double-blind treatment period and have at least 1 post baseline efficacy measurement. The randomized treatment for each participant will be used for summaries by treatment group based on the FAS, even if a participant erroneously received a different treatment. The FAS will be used for all summaries of efficacy data.
SAF	All participants who receive at least 1 dose of ASP0367 or placebo for the double-blind treatment period. If a participant erroneously received the wrong treatment during the double-blind treatment period, then the actual treatment received (i.e. the treatment received for longest time in the double-blind treatment period) will be used for summaries based on the SAF. The SAF will be used for all summaries of the safety data.

FAS: full analysis set; SAF: safety analysis set.

5 EFFICACY AND SAFETY ENDPOINTS

5.1 Primary Efficacy Endpoint (6-minute Walk Test)

The 6MWT will be performed indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom traveled. The total distance walked by a participant, as well as distance per minute will be calculated by rounding to the nearest meter and recorded. The 6MWT will be video recorded for quality purposes.

Participants will be asked to wear comfortable clothing and appropriate shoes for walking. A light meal is acceptable before early morning or early afternoon tests. Participants will be recommended not to have exercised within 2 hours prior to beginning of the test. A "warm up" period before the test will not be performed. Participants will sit at rest in a chair located near the starting position for at least 10 minutes before the test starts. The test will be performed at weeks 0, 4, 12, 24, 36 and 52.

5.2 Secondary Efficacy Endpoints

5.2.1 5 Times Sit To Stand (5XSTS)

For the 5XSTS, the participant will be instructed to sit with arms folded across their chest and with back against the chair and will be asked to stand up and sit down 5 times in a row, as quickly as he/she can. The instructor will make sure that the participant will stand up fully and try not to let his/her back touch the chair between each repetition. The duration from the time instructor indicates "Go" until the time participant's body touches the chair following the fifth repetition will be recorded. If participant is unable to complete the first sit to stand independently, without use of arms, the test will be terminated. The test will be performed at weeks 0, 4, 12, 24, 36 and 52.

5.2.2 Quality of Life in Neurological Disorders (Neuro-QoL)

The Neuro-QoL is a measurement system that evaluates and monitors the physical, mental and social effects experienced by adults and children living with neurological conditions. Participants will report Neuro-QoL Item Bank v1.0 Fatigue Short Form and Lower Extremity Function (Mobility) Short Form, which consists of 8 items each. Neuro-QoL will be performed at weeks 0, 4, 12, 24, 36 and 52.

5.2.3 Modified Fatigue Impact Scale (MFIS)

The MFIS is a modified form of the Fatigue Impact Scale. The questionnaire specifically measures how fatigue impacts the lives of those affected by fatigue-like symptoms.

There are 21 items in the scale measuring 3 domains of fatigue including physical, cognitive and psychosocial functioning. MFIS will be performed at weeks 0, 4, 12, 24, 36 and 52.

5.2.4 Patient Global Impression of Change (PGIC)

The PGIC scale evaluates the participant's most bothersome symptom and assesses if there has been an improvement or decline in clinical status. The participant will rate his/her change on a 7-point scale. The PGIC score will be evaluated at weeks 4, 12, 24, 36 and 52.

5.2.5 Patient Global Impression of Severity (PGIS)

The PGIS is a questionnaire designed to assess patient's impression of disease severity. The questionnaire asks the participant to best describe the severity of the participant's most bothersome pre-defined symptom over the past week. The PGIS score will be assessed at weeks 0, 4, 12, 24, 36 and 52.

5.3 Exploratory Endpoints

5.3.1 European Quality of Life 5-dimension, 5-level questionnaire (EQ-5D-5L)

The EQ-5D-5L is a brief, generic health-related quality of life assessment that can also be used to incorporate participant preferences into health economic evaluations. The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 response levels: no problems, slight problems, moderate problems, severe problems and extreme problems/unable to perform the activity. In addition, it does record the patient's self-rated health on a vertical visual analogue scale with response options ranging from 0 (worst imaginable health) to 100 (best imaginable health). The EQ-5D-5L will be assessed at weeks 0, 4, 12, 24, 36 and 52.

5.4 Primary Safety Endpoints

5.4.1 Treatment Emergent Adverse Events (TEAEs)

AE collection begins after the ICF has been signed and continues until the follow up visit or 28 days after the final IP administration or when the participant is determined to be a screen failure. A TEAE is defined as an AE observed after starting administration of the IP to 28 days after the last dose of IP for double-blind treatment period of the study or moving to the OLE part, whichever comes first.

An IP-related TEAE is defined as any TEAE with a causal relationship of "yes" by the investigator.

5.4.2 Vital signs

Vital signs including blood pressure, pulse rate and body temperature will be assessed at each visit scheduled in the protocol.

5.4.3 12-lead Electrocardiogram (ECG)

- A standard 12-lead ECG will be conducted using both a central ECG reading laboratory and local ECG reading. ECGs at weeks 2 and 8 will be performed at the participant's home by a qualified healthcare provider from the home healthcare vendor. In case the home healthcare visit is not feasible, the visit can be conducted at the study site.
- ECGs will be recorded in triplicate (3 separate ECGs, 5 minutes resting prior to first ECG and at least 1 minute apart per time point), prior to blood draw and transmitted electronically for central reading. The mean of the triplicate ECG from central and local read should be used for treatment decisions and AE reporting.
- If the mean triplicate QTcF is > 500 msec at any time point, one follow-up triplicate ECG may be performed on the same day. If the follow-up mean QTcF is ≤ 500 msec, the

participant may proceed with IP. If the follow-up mean QTcF > 500 msec is confirmed, then the participant must discontinue IP.

- An ECG will also be performed within 24 hours of any of these events: an elevation of cTnI above the ULN (see Appendix 10.9 in the protocol for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 in the protocol for reference range) or above the participant's baseline value if it was elevated, or when a participant has any signs or symptoms reflecting cardiac involvement, inclusive of new onset shortness of breath. If the ECG is within normal limits, a repeat of the abnormal troponin value (cTnI or cTnT) should be obtained. For any of the situations above, it may be required to interrupt or discontinue further administration of IP. Participants with persistent cTnI or cTnT elevations, abnormal ECG or abnormal echocardiogram should undergo cardiology follow up.

The requirement to perform an ECG after elevations observed in Troponin T was not added to the protocol until version 9.0. Therefore, this ECG requirement related to cTnT elevation was not implemented as all participants enrolled under and participated in the protocol version 7.0 or earlier.

5.4.4 Echocardiogram (ECHO)

A local echocardiogram is to be performed within 24 hours of any of these events: an elevation of cTnI above the ULN (see Appendix 10.9 in the protocol for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 in the protocol for reference range) or above the participant's baseline value if it was elevated, or when a participant has any signs or symptoms reflecting cardiac involvement, inclusive of new onset shortness of breath. If the echocardiogram is within normal limits, a repeat of the abnormal troponin value (cTnI or cTnT) should be obtained. For any of the situations above, it may be required to interrupt or discontinue further administration of IP. Participants with persistent cTnI or cTnT elevations, abnormal ECG or abnormal echocardiogram should undergo cardiology follow up.

The requirement to perform an ECHO after elevations observed in Troponin T was not added to the protocol until version 9.0. Therefore, this ECHO requirement related to cTnT elevation was not implemented as all participants enrolled under and participated in the protocol version 7.0 or earlier.

5.4.5 Body Weight

Body weight is planned to be collected at each visit scheduled in the protocol.

5.4.6 Laboratory test (hematology, biochemistry and urinalysis)

Safety laboratory tests will be done by a central laboratory from the screening to the end of the study. The scheduled visit and the list of clinical laboratory tests are described in the protocol.

5.4.7 Columbia-Suicide Severity Rating Scale (C-SSRS)

The C-SSRS will be performed by trained site staff via interview at screening, randomization, and all subsequent study visits. At screening, the "Screening" version is to be

used to determine eligibility. During all subsequent visits, the "Since Last Visit" version is used to monitor on study suicidal ideation and behavior after the initial assessment. If possible, continuity of raters should be maintained across visits for each patient. The following five questions regarding suicidal ideation, six questions regarding suicidal behavior and question for non-suicidal self-injurious behavior will be used for analysis.

- Suicidal Ideation ('Yes' to any one of the below 5 questions for suicidal ideation)
  - Wish to be Dead (No/Yes)
  - Non-Specific Active Suicidal Thoughts (No/Yes)
  - Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act (No/Yes)
  - Active Suicidal Ideation with Some Intent to Act, without Specific Plan (No/Yes)
  - Active Suicidal Ideation with Specific Plan and Intent (No/Yes)
- Suicidal Behavior ('Yes' to any one of the below 5 questions for suicidal behavior)
- Preparatory Acts or Behavior (No/Yes)
  - Aborted Attempt (No/Yes)
  - Interrupted Attempt (No/Yes)
  - Actual Attempt (No/Yes)
  - Completed Suicide (No/Yes)
- Suicidal Ideation or Behavior ('Yes' to any one of the above 10 questions for suicidal ideation and behavior)
- Self-injurious Behavior Without Suicidal Intent (No/Yes)

5.5 Other Endpoints

5.5.1 Pharmacokinetics Endpoints

Plasma ASP0367: C_{trough} at week 12 and 24.

Relationship between measured- and model-based pharmacokinetic exposure parameters (e.g., C_{trough}, $C_{ave, ss}$) of ASP0367 and endpoints of efficacy, safety and pharmacodynamic biomarkers, as appropriate.

5.5.2 Pharmacodynamics Endpoints

Blood samples for PPARδ target gene expression assay

5.5.3 Biomarkers

Urine samples for TITIN

Blood samples for serum target proteins (ALDOA, CYC and FLNC)

6 STATISTICAL METHODOLOGY

6.1 General Considerations

Continuous data will be summarized descriptively including the number of participants (n), mean, standard deviation (SD), median, minimum and maximum. Categorical data will be summarized by frequencies and percentages. Percentages by categories will be based on the

number of participants with no missing data, i.e. the percentages for the non-missing categories will add up to 100%.

There are 4 different groups defined based in the randomization group in double-blind treatment period of the study:

- ASP0367 30 mg: Participants randomly assigned to 30 mg of ASP0367 during double-blind treatment period.
- ASP0367 75 mg: Participants randomly assigned to 75 mg of ASP0367 during double-blind treatment period.
- ASP0367 any dose: Participants randomly assigned to 30mg or 75 mg of ASP0367 during double-blind treatment period.
- Placebo group: Participants randomly assigned to placebo during double-blind treatment period.

Summaries based on FAS (e.g. efficacy data) will be presented by analysis group (ASP0367 30 mg group, ASP0367 75 mg group or placebo group), unless specifically stated otherwise. Statistical model and inference based on FAS will be performed and obtained by analysis group. To control the family-wise type I error under the nominal significance level of 10% 2-sided, the hierarchical testing procedure in the following order (Comparison between ASP0367 75 mg and placebo; Comparison between ASP0367 30 mg and placebo) will be applied in the primary analysis.

Safety analysis and other summaries based on SAF will be presented by analysis group (ASP0367 30 mg group, ASP0367 75 mg group or placebo group), unless specifically stated otherwise.

All data summarization and analyses will be performed using SAS® Version 9.4 or higher on Linux. Specifications for table, figures, and data listing formats can be found in the TLF specifications.

For continuous variables that are recorded as “<X”, “<=X”, “>X”, or “>=X”, the value of “X” will be used in the calculation of summary statistics if otherwise noted. This approach may result in a summary that reflects the presence of an analyte (such as troponin) when in fact the analyte was not detected but report as less than the lower limit of detection by the assay.

6.2 Study Population

6.2.1 Disposition of Participants

The following participant data will be presented. Unique participants are defined as rescreened participants for whom the data from the last enrollment are used, unless stated otherwise.

Analysis population: All participants with informed consent, Analysis group: Total

- Number of participants with informed consent and who were discontinued before randomization. For any participant who was screened multiple times, the participant will be counted for each screening attempt,
- Number of unique participants with informed consent and who were discontinued before randomization. For any participant who was screened multiple times, the participant will be only counted once,
- Number of participants with re-screening. For any participant who was re-screened multiple times, the participant will be counted for each re-screening attempt,
- Number of participants who were randomized,
- Number and percentage of participants who discontinued before randomization, by primary reason for study discontinuation.

Analysis population: All randomized participants, Analysis group: 30, 75 mg, any dose of ASP0367, placebo group and Total

- Number and percentage of participants who were randomized in each analysis set, administered at least one dose of study drug,
- Number and percentage of participants who completed and those who discontinued the treatment during double-blind treatment period, by primary reason for treatment discontinuation,
- Number and percentage of participants who completed and those who discontinued the study during double-blind treatment period, by primary reason for study discontinuation.

6.2.2 Protocol Deviations

Analysis population: All randomized participants, Analysis group: 30, 75 mg, any dose of ASP0367, placebo group and Total

The number and percentage of participants with the following deviation criteria (defined as an important protocol deviation) will be summarized for each criterion and overall, by treatment group and total as well as by investigative site. Participants deviating from a criterion more than once will be counted only once for the corresponding criterion.

The unique identifiers will be as follows:

PD1 - Inclusion/Exclusion,

PD2 - Withdrawal Criteria,

PD3 - Study Intervention,

PD4 - Excluded Concomitant Medications,

PD5 - Informed Consent,

PD6 - Safety Reporting, and

PD7 - Procedures/Tests

6.2.3 Demographic and Other Baseline Characteristics

Analysis population: All randomized participants, SAF and FAS, Analysis group: 30, 75 mg, any dose of ASP0367, placebo group and Total

- Demographic and other baseline characteristics (age, sex, race and ethnicity, body weight, height and BMI), as well as genetic abnormality for PMM will be summarized by descriptive statistics and frequency tabulations [Table 1].

Table 1 Demographic and baseline characteristics

Item	Classification
Sex	1: Male 2: Female
Age (Years) [Informed Consent]	Measurement value
Age	1: >=18 to <65 2: >=65 to <=80
Ethnicity	1: Not Hispanic or Latino 2: Hispanic or Latino
Race	1: White 2: Black or African American 3: Asian 4: American Indian or Alaska Native 5: Native Hawaiian or Other Pacific Islander 6: Other
Height (cm) [Screening]	Measurement value
Weight (kg) [Screening]	Measurement value
BMI (kg/m^2) [Screening]	Measurement value
BMI	1: < 25 2: >=25
Site Group	1: Mayo clinic_Minnesota 2: Non-Mayo clinic Minnesota
Walking aid use [Week 0]	0: No 1: Yes
Duration of disease† (Years)	Measurement value
Treated with medication [Screening]	0: No 1: Yes
Syndrome	1: Kearns Sayre Syndrome (KSS) 2: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) 3: Maternally Inherited Diabetes Deafness (MIDD) 4: Myoclonic Epilepsy with Ragged Red Fibers (MERRF) 5: Chronic Progressive Externa Ophthalmoplegia (CPEO) 6: 3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like disease (MEGDEL) 7: Leigh Syndrome 8: Multisystem mitochondrial disorder 9: Mitochondrial Myopathy 10: POLG-related disorders 11: Others
Genetic abnormality	1: Mitochondrial DNA 2: Nuclear DNA
Mitochondrial DNA mutant heteroplasmy information is available	0: No 1: Yes
Level of heteroplasmy (%)	Measurement value

BMI: body mass index; CPEO: Chronic Progressive Externa Ophthalmoplegia; KSS: Kearns Sayre Syndrome; MEGDEL: 3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like disease; MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes; MERRF: Myoclonic Epilepsy with Ragged Red Fibers; MIDD: Maternally Inherited Diabetes Deafness; POLG: polymerase-gamma.

†: Duration of disease will be calculated as follows:

("Date of First Study Drug Taken"-"Date of Diagnosis" + 1)/365.25.

If Date of Diagnosis is missing or incomplete, then impute the date following the rule stated in [Section 6.8.2].

6.3 Study Drugs Exposure and Compliance

Analysis population: SAF, Analysis group: ASP0367 30 mg group, ASP0367 75 mg group, ASP0367 any dose group or placebo group

- The duration of study drug exposure for each participant in double blind part will be summarized by descriptive statistics and frequency tabulations. The following categories of the duration of exposure (days) will be defined:
  - < 29 (4 weeks)
  - >= 29 (4 weeks) to < 85 (12 weeks)
  - >= 85 (12 weeks) to < 169 (24 weeks)
  - >= 169 (24 weeks) to < 253 (36 weeks)
  - >= 253 (36 weeks) to < 365 (52 weeks)
  - >= 365 (52 weeks)

  The duration of study drug exposure in double blind part will be calculated as follows: {"Date of Last Study Drug Taken in double blind part" – "Date of First Study Drug Taken"} + 1.

  Date of Last Study Drug Taken in double blind part is defined as the latest date of ["Date of Last Study Drug Taken (Phase 2 Treatment Period)" or "Date of Last Study Drug Taken (Dose selection Period)" or "Date of Last Study Drug Taken (Phase 3 Treatment Period)"].

- The compliance of study drug exposure for each participant in double blind part will be summarized by descriptive statistics and frequency tabulations. The following categories of the study drug compliance (%) will be defined:
  - < 50
  - >= 50 to < 75
  - >= 75 to < 90
  - >= 90
  - Unknown

  The compliance of study drug exposure in double blind part will be calculated as follows:

$$\frac{\text{Total number of dispensed tablets - Total number of returned tablets in double blind part}}{\text{The number of tablets to be taken in double blind part}} \times 100$$

  The number of tablets to be taken in double blind part is defined as follows:

  [{"Date of Last Study Drug Taken in double blind part" – "Date of First Study Drug Taken"} + 1] x 3.

6.4 Analysis of Efficacy

6.4.1 Analysis of Primary Efficacy Endpoint(s)

The primary efficacy endpoint is change from baseline to week 24 in distance walked on the 6MWT.

6.4.1.1 Primary Analysis for Primary Efficacy Endpoint

Analysis population: FAS, Analysis group: ASP0367 30 mg group, ASP0367 75 mg group or placebo group

The primary efficacy endpoint will be analyzed on FAS for primary analysis. In order to compare the change from baseline in distance walked on the 6MWT between ASP0367 and placebo group, the null and alternative hypothesis will be constructed:

H_0: The change from baseline in distance walked on the 6MWT at week 24 for each dose of ASP0367 and placebo are the same.

H_1: The change from baseline in distance walked on the 6MWT at week 24 for each dose of ASP0367 and placebo are not the same.

To control the family-wise type I error rate (i.e., the probability of incorrectly rejecting at least one true null hypothesis), the hierarchical testing procedure in the following order will be applied:

Comparison between ASP0367 75 mg and placebo
Comparison between ASP0367 30 mg and placebo

Comparison to placebo will be conducted using a MMRM with treatment group (3 level: ASP0367 75 mg, ASP0367 30 mg, and placebo group), week (weeks 4, 12 and 24) as factors, with baseline measurement as a covariate, as well as an interaction of treatment by week and an interaction of baseline measurement by week. An unstructured covariance structure will be used to model the within-participant correlation. If the unstructured covariance structure fails to converge, then compound symmetry will be used. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom and adjust standard errors.

Contrasts will be constructed to compare each dose group of ASP0367 to the placebo group at week 24. The LS mean change from baseline in distance walked on the 6MWT with associated SE will be displayed for each treatment group. Estimated treatment differences between each dose group of ASP0367 and placebo group along with corresponding 2-sided 90% CI and p-values will also be presented.

6.4.2 Analysis of Secondary Efficacy Endpoints

Analysis population: FAS, Analysis group: ASP0367 30 mg group, ASP0367 75 mg group or placebo group

The change from baseline to week 24 in 5XSTS and MFIS will be analyzed using the same MMRM model as with the primary analysis for primary endpoint.

The change from baseline to week 24 in T-scores for Neuro-QoL Short Form Fatigue and Lower Extremity Function (Mobility) scores will be analyzed using the same MMRM model as with the primary analysis for primary endpoint.

For PGIC score at week 24, responder analysis will be used. Participants with scores of 1 (Very Much Improved), 2 (Much Improved) and 3 (Minimally Improved) will be considered

as responders, whereas participants with scores of 4 (No Change), 5 (Minimally Worse), 6 (Much Worse) and 7 (Very Much Worse) will be treated as nonresponders.

For PGIS score, responder analysis will be used for post baseline visits. Participants who have a lower severity score than baseline will be considered as responders, otherwise will be treated as non-responders. The participants who have 0 (None) as baseline score will be excluded from this responder analysis.

For the secondary endpoints, the multiplicity of statistical comparison will not be adjusted.

The change from baseline in 6MWT, 5XSTS, MFIS and Neuro-QoL Short Form Fatigue and Lower Extremity Function (Mobility) scores will be summarized by treatment group and time point (other than week 24).

PGIC score and PGIS score will also be summarized by treatment group and time point (other than week 24).

6.5 Analysis of Safety

Analysis population: SAF, Analysis group: 30, 75 mg of ASP0367, ASP0367 any dose group or placebo group

6.5.1 Adverse Events

AEs will be coded using the MedDRA.

An overview table will include the following:

- Number of TEAEs,
- Number and percentage of participants with TEAEs,
- Number of drug related TEAEs,
- Number and percentage of participants with drug related TEAEs,
- Number of serious TEAEs,
- Number and percentage of participants with serious TEAEs,
- Number of serious drug related TEAEs,
- Number and percentage of participants with serious drug related TEAEs,
- Number of TEAEs leading to death,
- Number and percentage of participants with TEAEs leading to death,
- Number of drug related TEAEs leading to death,
- Number and percentage of participants with drug related TEAEs leading to death,
- Number of TEAEs leading to withdrawal of study drug,
- Number and percentage of participants with TEAEs leading to withdrawal of study drug,
- Number of drug related TEAEs leading to withdrawal of study drug,
- Number and percentage of participants with drug related TEAEs leading to withdrawal of study drug, and
- Number of deaths.

Number of deaths is counted all reported deaths after starting administration of the IP. For participants who move to the OLE, all reported deaths after starting administration of the IP to moving to the OLE.

The number and percentage of participants with TEAEs, as classified by SOC, and PT will be summarized by analysis group. Summaries will be provided for the following:

- TEAEs,
- drug related TEAEs,
- serious TEAEs,
- drug related serious TEAEs,
- TEAEs leading to withdrawal of study drug,
- drug related TEAEs leading to withdrawal of study drug,
- TEAEs excluding Serious AEs that equal to or exceed a threshold of 5.0% in any analysis group.

The number of TEAEs and the number and percentage of participants with TEAEs, as classified by SOC and PT will be summarized by severity and by relationship to study drug. In the participant count, if a participant has multiple TEAEs with the same SOC or PT, but with differing severity or relationship, then the participant will be counted once with the worst severity and highest degree of relationship. If severity or relationship is missing for all episodes of the event, the participant will be counted under missing severity or relationship.

The following AESIs are defined:

- Cardiac tissue injury defined by cTnI above ULN or if the cTnT is above ULN or baseline if the participant's baseline value was above ULN and a TEAE as pre-defined by a MedDRA search strategy for elevated troponin.
- Cardiac tissue injury with any signs or symptoms defined by an echocardiogram or electrocardiogram interpretation is reported as abnormal, clinically significant and a TEAE as pre-defined by a MedDRA search strategy for cardiac complaints.
- Skeletal muscle injury defined by CK increase >= 5 times the value of the ULN or the individual's baseline value, whichever is higher and a TEAE as pre-defined by a MedDRA search strategy for skeletal muscle injury.

AESIs prior to version 9.0 of the protocol did not include elevations in cTnT in the definition of an AESI but instead only included elevations of cTnI. All participants participated in protocol version 7.0 or earlier; therefore participants for whom elevations in cTnT were observed may not have been identified as having experienced an AESIs during study conduct and for this reason may not have had additional ECG and echocardiographic evaluations performed.

The number and percentage of participants who report AESIs will be summarized by analysis group. In addition, any (serious) AEs that are considered abuse of the study drug will also be collected and followed. The number and percentage of AEs of special interest related to abuse, as classified by SOC and PT will be summarized by analysis group.

6.5.2 Clinical Laboratory Evaluation

Quantitative values including hematology, biochemistry, and urinalysis will be summarized using mean, standard deviation, minimum, maximum and median by analysis group at each analysis visit. Additionally, a within-participant change will be calculated as the post-baseline measurement minus the baseline measurement and summarized in the same way.

Frequency tabulations of qualitative clinical laboratory variables (urinalysis) will be presented for each analysis group at each visit.

Additionally, amylase, lipase and troponin elevations above the ULN and 2xULN will be summarized by analysis group. The denominator for each criterion will be the number of participants who have at least one value after the day of first IP taken to 28 days after the last dose of IP for the double-blind treatment period of the study or moving to the OLE, whichever comes first.

The number and percentage of participants meeting the criteria during the defined period above will be summarized by analysis group.

6.5.2.1 Liver Safety Assessment

The liver safety assessments will be summarized by the categories below based on the measurements from ALP, ALT, total bilirubin, AST and their combination. These parameters will be based on measurements from a local laboratory. If the baseline value is above ULN then use the baseline value instead of ULN.

- ALT > ULN, > 2xULN, > 3xULN, > 5xULN, > 10xULN, > 20xULN
- AST > ULN, > 2xULN, > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALT or AST > ULN, > 2xULN, > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALP > 1.5xULN, 2xULN, 2.5xULN
- Total Bilirubin > 2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN
- (ALT or AST > 3xULN) and ALP < 2xULN and Total Bilirubin > 2xULN

The last 2 criteria where 2 or more parameters are evaluated will be with the measurements on the same day or up to 1 day apart.

The denominator for each criterion will be the number of participants who have at least one value after the day of first dose taken to 28 days after the last dose of IP for the 52 weeks or moving to the OLE, whichever comes first. The number and percentage of participants meeting the criteria by 28 days after the last dose of IP for the 52 weeks or moving to the OLE will be summarized by analysis group.

6.5.3 Vital Signs

Vital signs (systolic blood pressure, diastolic blood pressure, and pulse rate) will be summarized using mean, standard deviation, minimum, maximum and median by analysis group and visit. Additionally, a within-participant change will be calculated per visit as the

post-baseline measurement minus the baseline measurement and summarized by analysis group and visit.

6.5.4 Electrocardiograms

6.5.4.1 Routine 12-lead Electrocardiograms

The number and percent of participants with normal, not clinically significant abnormal, and clinically significant abnormal results as assessed by site principal investigator for the 12 lead ECG will be tabulated by analysis group and time point. Continuous 12-lead Electrocardiogram

For all analyses, replicates at each visit will be averaged for each continuous ECG parameter.

ECG parameters [Heart Rate (bpm), PR Interval (ms), QRS Interval (ms) and QTcF (ms)] read by the central laboratory will be summarized using mean, standard deviation, minimum, maximum and median for each analysis group at each visit, including changes from baseline.

The QTcF interval will be summarized using frequency tables for each visit for values of clinical importance using the range criteria below.

	QTcF Interval Criteria Value (msec)
Normal	≤ 450
Borderline	> 450
Prolonged	> 480
Clinically significant	> 500

QTcF: QT interval using Fridericia's correction.

The QTcF interval will also be summarized by the frequencies of participants with an increase from baseline of clinical importance using the criteria identified below. These summaries will be provided for each visit.

Variable	Change from Baseline
QTcF Interval (msec)	≤ 30 > 30 > 60

QTcF: QT interval using Fridericia's correction.

6.5.5 Other Safety-Related Assessment

6.5.5.1 Body Weight

Body weight will be summarized using mean, standard deviation, minimum, maximum and median by analysis group and visit. Additionally, a within-subject change will be calculated per visit as the post-baseline measurement minus the baseline measurement (screening visit) and summarized by analysis group.

6.5.5.2 C-SSRS

The results of five questions regarding suicidal ideation, six questions regarding suicidal behavior, suicidal ideation or behavior and self-injurious behavior without suicidal intent will be summarized using number and percent of participants by analysis group at each visit. For

all analysis visits, the data “by the last dose + 7 days” and the data before the first dose date of open label extension part will be included.

6.6 Other Analyses

Not applicable in this study.

6.7 Interim Analysis

6.7.1 Interim Analysis of Efficacy

The analysis of futility and of efficacy was performed when the first 33 participants completed the efficacy evaluation at week 24 or discontinued as of date the last participant had the week 24 visit, 20-Nov-2023. The change from baseline in distance walked on the 6MWT at week 24 was analyzed using the same MMRM as with the primary endpoint excluding the site group from the model to decide whether the study will continue.

Analysis of Futility

For the futility stop, the study may be stopped if the conditional probability of achieving the criteria below at the final analysis is less than 10%.

Criteria: Lower limit of the 90% CI for the difference is greater than 0 m AND the point estimate of the difference between the ASP0367 75 mg group and the placebo group is greater than 30 m.

In addition to 6MWT at week 24, outputs for participant disposition, demographics and the secondary endpoints 5XSTS, Neuro-QoL Short Form Fatigue score, Neuro-QoL Short Form Lower Extremity Function (Mobility) score, MFIS, PGIC, and PGIS at week 24 were analyzed at the interim analysis.

Analysis of Efficacy

For the efficacy stop, a two-stage group sequential design with Lan-DeMets alpha-spending function determined by means of the O'Brien-Fleming approach was used to preserve the overall two-sided type I error rate of 0.10 between this single interim analysis and the final analysis. If the p-value of the comparison between ASP0367 75 mg and placebo groups at interim analysis is lower than the boundary determined by O'Brien-Fleming approach, efficacy would be considered to be achieved at week 24.

The interim analysis outputs were prepared by an external statistician and presented to the AIDMC who made recommendations about the ongoing conduct of the study. After the AIDMC reviewed the interim analysis results, the AIDMC recommended the study to be stopped because the study met results of the interim analysis the criteria for futility. Details of the analysis were defined in the interim analysis plan.

6.8 Additional Conventions

6.8.1 Analysis Windows

Day 1 and Day -1 are defined as the day of start taking the IP and its prior day, respectively.

The following analysis window will be used.

The data summary by visits will be done following the analysis windows specified in the table below:

Analysis Visits	**Scheduled Day in Protocol**	**Analysis Windows (day)**
Screening	Day -28	-35 to -14
Week 0 (Baseline)	Day -2 to 1	-2 to 1
Week 1	Day 8	5 to 13
Week 2	Day 14	14 to 18
Week 3	Day 22	19 to 25
Week 4	Day 29	26 to 42
Week 8	Day 57	43 to 70
Week 12	Day 85	71 to 98
Week 16	Day 113	99 to 126
Week 20	Day 141	127 to 154
Week 24	Day 169	155 to 182
Week 28	Day 197	183 to 210
Week 32	Day 225	211 to 238
Week 36	Day 253	239 to 266
Week 40	Day 281	267 to 294
Week 44	Day 309	295 to 322
Week 48	Day 337	323 to 350
Week 52	Day 365	351 to 378
End of Double Blind Part	Day 365	2 to 378
Follow-up	The last dose + 28 days	The last dose + 28 days to the last dose + 35 days

For the analyses of safety and efficacy, for all analysis visits excluding follow-up visit, the data “by the last dose + 7 days” and the data before the first dose date of open label extension part will be included.

If more than one observation exists within the analysis window, the observation closest to the scheduled visit day will be selected for that visit. If there are two observations that have the same distance from the scheduled day, the value that is after the scheduled day will be selected in the analysis. If more than one observation is made on the same day, an average value if continuous or the worst value if categorical will be included in the analysis.

6.8.2 Imputation Rules for Incomplete Dates

For AEs, a missing or incomplete onset date will be imputed according to the following conventions.

If an onset date is missing, the imputed onset date will be the date of first dose of study drug.

If only the year is known for the AE onset date, the imputed onset date will be the latest of the following non-missing dates:

- Date of first dose of study drug
- January 1 of the year of AE onset date

If only the month and year is known for the onset date, set the surrogate onset date to the first day of that month and then apply the following rules.

- If the month and year of the onset date is prior to the month and year of the first dose of study drug, then the surrogate onset date will be the imputed onset date.
- If the month and year of the onset date is on or after the month and year of the first dose of study drug, then the imputed onset date will be the **latest** of the following non-missing dates:
- Date of first dose of study drug
- Surrogate onset date
- If the imputed onset date is after the AE end date, the imputed onset date will be the same as the AE end date.

For duration of disease of primary diagnosis, a missing or incomplete diagnosis date will be imputed according to the following conventions.

If a diagnosis date is missing, the diagnosis date will not be imputed. The duration of disease in baseline analysis will be treated as missing data.

If only the year is known for the diagnosis date, the imputed diagnosis date will be January 1 of the year of the diagnosis date.

If only the month and year is known for the diagnosis date, set the imputed diagnosis date will be the first day of that month.

6.8.3 Outliers

All values will be included in the analyses.

7 REVISION AND RATIONALE

Version	Date	Changes	Comment/rationale for change
1.0	16-Jul-2020		Document finalized
2.0	5-Aug-2020	Section 2.3: • Removed description of stratified randomization by site group. Section 6.4.1.1, 6.4.2: • Removed site group factor from analysis model. Section 6.4.1.4: • Removed site from grouping variables.	Updated due to Protocol amendment 2
3.0	9-Jul-2024	• Revised study design and statistical methodology related sections based on substantial protocol amendments 7 and 8. • Removed several statistical analyses due to the futility stop. • Updated the description of analysis sets • Updated due to change in the duration of the study periods for the DB and OLE and the addition of an interim analysis in substantial protocol amendment 7 and the removal of the OLE period and the addition of an AESI of elevated cardiac troponin T in substantial protocol amendment 8.	Updated due to changes in study design and some protocol details added in substantial protocol amendments 7 and 8. Adjusted some descriptions to align the data and/or TOC Clarified the detail definition of analysis sets

8 REFERENCES

Cohen B, Karaa A, Haas R, Goldstein A, Vockley G. Effects of Elamipretide in Adults with Primary Mitochondrial Myopathy: a Phase 2 Double-Blind, Randomized, Placebo-Controlled Crossover Trial (MMPOWER-2). Neurology. 2018;90(Suppl 15):S5.003.

ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

ICH Harmonized Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

9 APPENDICES

9.1 Appendix 1: Neuro-QoL

9.1.1 Neuro-QoL Item Bank v1.0 Fatigue Short Form and Lower Extremity Function (Mobility) Short Form

- Missing Items: A score can be approximated if a participant skips a question. If items are missing, first check how many items were answered. For short forms with 8 items, confirm that 4 items have been answered. After confirming that enough responses were provided, sum the response scores from the items that were answered. Multiply this sum by the total number of items in the short form. Finally, divide by the number of items that were answered. If the result is a fraction, round up to the nearest whole number.
- The total raw score equals sum of the values of the response to each question. For fatigue short forms with 8 items, the total raw score ranges from 8 to 40.
- T-score distributions rescale raw scores into standardized scores with a mean of 50 and a SD of 10.
- Neuro-QoL Item Bank v1.0 Fatigue Short Form consists of the following eight items:
- In the past 7 days.
    - Q1 I felt exhausted.
    - Q2 I felt that I had no energy.
    - Q3 I felt fatigued.
    - Q4 I was too tired to do my household chores.
    - Q5 I was too tired to leave the house.
    - Q6 I was frustrated by being too tired to do the things I wanted to do.
    - Q7 I felt tired.
    - Q8 I had to limit my social activity because I was tired.
- Neuro-QoL Item Bank v1.0 Fatigue Short Form items employ five response options: Never (1); Rarely (2); Sometimes (3); Often (4); Always (5).
- Neuro-QoL Lower Extremity Function (Mobility) Short Form consists of the following eight items:
    - Q1 Are you able to get on and off the toilet?
    - Q2 Are you able to step up and down curbs?
    - Q3 Are you able to get in and out of a car?
    - Q4 Are you able to get out of bed into a chair?
    - Q5 Are you able to push open a heavy door?
    - Q6 Are you able to run errands and shop?
    - Q7 Are you able to get up off the floor from lying on your back without help?
    - Q8 Are you able to go for a walk of at least 15 minutes?

Neuro-QoL Lower Extremity Function (Mobility) Short Form items employ five response options: Unable to do (1); With much difficulty (2); With some difficulty (3); With a little difficulty (4); Without any difficulty (5).

9.1.2 Scoring Transformation Table for Neuro-QoL Item Bank v1.0 Fatigue Short Form

Neuro-QoL Item Bank 1.0 Fatigue Short Form (Adult)								
Raw Score	T-Score	SE	Raw Score	T-score	SE	Raw Score	T-score	SE
8	29.5	4.4	19	46.5	1.7	30	57.6	1.8
9	34.1	2.7	20	47.4	1.7	31	58.8	1.8
10	36.5	2.2	21	48.4	1.7	32	59.9	1.8
11	38.2	2.0	22	49.3	1.7	33	61.1	1.8
12	39.5	1.9	23	50.3	1.7	34	62.3	1.8
13	40.7	1.8	24	51.3	1.8	35	63.5	1.8
14	41.8	1.7	25	52.3	1.7	36	64.8	1.9
15	42.8	1.7	26	53.3	1.7	37	66.2	2.0
16	43.8	1.7	27	54.4	1.7	38	67.9	2.2
17	44.7	1.7	28	55.4	1.7	39	70.1	2.7
18	45.6	1.7	29	56.5	1.8	40	74.1	4.0

Neuro-QoL: Quality of Life in Neurological Disorders; SE: standard errors.

The scoring transformation table is only accurate when all questions on the short form have been answered. Approximations can be estimated by prorating the raw sum score based upon those questions that are answered, however this diminishes the advantages of IRT scoring.

9.1.3 Scoring Transformation Table for Neuro-QoL Lower Extremity Function (Mobility) Short Form

Neuro-QoL Lower Extremity Function (Mobility) Short Form (Adult)								
Raw Score	T-Score	SE	Raw Score	T-score	SE	Raw Score	T-score	SE
8	16.5	3.0	19	30.7	2.1	30	39.1	2.2
9	19.2	2.8	20	31.5	2.1	31	39.9	2.2
10	21.1	2.6	21	32.2	2.1	32	40.8	2.3
11	22.6	2.4	22	33.0	2.1	33	41.7	2.4
12	23.9	2.3	23	33.7	2.0	34	42.8	2.5
13	25.1	2.3	24	34.5	2.1	35	43.9	2.6
14	26.2	2.2	25	35.2	2.1	36	45.2	2.9
15	27.2	2.2	26	36.0	2.1	37	46.7	3.1
16	28.1	2.1	27	36.7	2.1	38	48.6	3.3
17	29.0	2.1	28	37.5	2.1	39	51.2	3.8
18	29.9	2.1	29	38.3	2.1	40	58.6	6.4

Neuro-QoL: Quality of Life in Neurological Disorders; SE: standard error.

The scoring transformation table is only accurate when all questions on the short form have been answered. Approximations can be estimated by prorating the raw sum score based upon those questions that are answered, however this diminishes the advantages of IRT scoring.

9.2 Appendix 2: MFIS

Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. But people who have medical conditions like multiple sclerosis experience stronger feelings of fatigue more often and with greater impact than others.

Following is a list of statements that describe the effects of fatigue. Please read each statement carefully, the circle the one number that best indicates how often fatigue has affected you in this way during the past 4 weeks. (If you need help in marking your responses, tell the interviewer the number of the best response.) Please answer every question. If you are not sure which answer to select choose the one answer that comes closest to describing you. Ask the interviewer to explain any words or phrases that you do not understand.

Because of my fatigue during the past 4 weeks

		Never	Rarely	Sometimes	Often	Almost Always
1.	I have been less alert.	0	1	2	3	4
2.	I have had difficulty paying attention for long periods of time.	0	1	2	3	4
3.	I have been unable to think clearly.	0	1	2	3	4
4.	I have been clumsy and uncoordinated.	0	1	2	3	4
5.	I have been forgetful.	0	1	2	3	4
6.	I have had to pace myself in my physical activities.	0	1	2	3	4
7.	I have been less motivated to do anything that requires physical effort.	0	1	2	3	4
8.	I have been less motivated to participate in social activities.	0	1	2	3	4
9.	I have been limited in my ability to do things away from home.	0	1	2	3	4
10.	I have trouble maintaining physical effort for long periods.	0	1	2	3	4
11.	I have had difficulty making decisions.	0	1	2	3	4
12.	I have been less motivated to do anything that requires thinking.	0	1	2	3	4
13.	My muscles have felt weak.	0	1	2	3	4
14.	I have been physically uncomfortable.	0	1	2	3	4
15.	I have had trouble finishing tasks that require thinking.	0	1	2	3	4
16.	I have had difficulty organizing my thoughts when doing things at home or at work.	0	1	2	3	4
17.	I have been less able to complete tasks that require physical effort.	0	1	2	3	4
18.	My thinking has been slowed down.	0	1	2	3	4

19.	I have had trouble concentrating.	0	1	2	3	4
20.	I have limited my physical activities.	0	1	2	3	4
21.	I have needed to rest more often or for longer periods.	0	1	2	3	4

Instructions for Scoring the MFIS

Items on the MFIS can be aggregated into three subscales (physical, cognitive, and psychosocial), as well as into a total MFIS score. All items are scaled so that higher scores indicate a greater impact of fatigue on a person's activities. If there is the assessment with the missing data, then the score for that assessment will not be calculated and will be excluded from the analysis.

Physical Subscale

This scale can range from 0 to 36. It is computed by adding raw scores on the following items: 4+6+7+10+13+14+17+20+21.

Cognitive Subscale

This scale can range from 0 to 40. It is computed by adding raw scores on the following items: 1+2+3+5+11+12+15+16+18+19.

Psychosocial Subscale

This scale can range from 0 to 8. It is computed by adding raw scores on the following items: 8+9.

Total MFIS Score

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

9.3 Appendix 3: Author and Approver Signatures

(E-signatures are attached at the end of document.)

Prepared by: *E-signatures are attached at end of document* Date: _____

PPD

Data Science, Astellas Pharma Inc.

Date (DD Mmm YYYY)

Approved by: *E-signatures are attached at end of document* Date: _____

PPD

Data Science, Astellas Pharma Inc.

Date (DD Mmm YYYY)

Approved by: *E-signatures are attached at end of document* Date: _____

PPD

BioPharma Development,
Astellas Pharma Global Development, Inc.

Date (DD Mmm YYYY)

STATISTICAL ANALYSIS PLAN
(Double Blind Part And Open-Label Extension Part)

Final Version 1.0 dated 09-Jul 2024

A Randomized, Double-blind, Placebo-controlled Phase 2 Study to Assess the Efficacy, Safety and Tolerability of ASP0367 in Participants with Primary Mitochondrial Myopathy

ISN: 0367-CL-1201
IND number: 146773

Astellas Pharma Inc. (API)

This document contains confidential information which is the intellectual property of Astellas. By accepting or reviewing this document, you agree to hold this information in confidence and not copy or disclose it to others or use it for unauthorized purposes except (1) as otherwise agreed to in writing; (2) where required by applicable law; (3) where disclosure is directly related to the care and safety of the research participant; and (4) where disclosure of such information is made to a member of the investigator's team who agrees to hold this information in confidence.

Table of Contents

I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
5XSTS	5 times sit to stand
6MWT	6-minute walk test
AE	adverse event
AESI	adverse events of special interest
AIDMC	Astellas Internal Data Monitoring Committee
ALDOA	Aldolase A
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
$C_{ave, ss}$	average plasma concentration at steady state
CI	confidence interval
CK	creatine kinase
CK-MB	creatine kinase isoenzymes found in heart muscle
CK-MM	creatine kinase isoenzymes found in skeletal and heart muscle
CPEO	Chronic Progressive Externa Ophthalmoplegia
CSR	clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
cTnI	cardiac troponin I
cTnT	cardiac troponin T
C_{trough}	concentration immediately prior to dosing at multiple dosing
CYC	Cytochrome c
ECG	electrocardiogram
ECHO	echocardiogram
EQ-5D	European Quality of Life 5-dimension questionnaire
EQ-5D-5L	European Quality of Life 5-dimension, 5-level questionnaire
EQ-VAS	EuroQol visual analogue scale
FLNC	Filamin-C
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IP	investigational product
ISN	international study number
KSS	Kearns Sayre Syndrome
MedDRA	medical dictionary for regulatory activities
MEGDEL	3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like disease
MELAS	Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes
MERRF	Myoclonic Epilepsy with Ragged Red Fibers
MIDD	Maternally Inherited Diabetes Deafness
MMRM	mixed effect models for repeated measures
Neuro-QoL	Quality of Life in Neurological Disorders
OLE	open-label extension
PD	protocol deviation
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity

Abbreviations	Description of abbreviations
PMM	primary mitochondrial myopathy
POLG	polymerase-gamma
PPARδ	peroxisome proliferator-activated receptor delta
QTcF	QT interval using Fridericia's correction
SAF	safety analysis set
SAP	statistical analysis plan
SD	standard deviation
TEAE	treatment emergent adverse event
TLF	tables, listings and figures
ULN	upper limit of normal

List of Key Terms

Terms	Definition of terms
Baseline	Assessments of participants as they enter a study before they receive ASP0367.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study.
Enroll	To register or enter a participant into a study. Note: Once a participant has received the investigational product or placebo, the protocol applies to the participant.
Randomization	The process of assigning participants to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential participants for enrollment in a study.
Screen failure	Potential participant who signed the informed consent form but did not meet one or more criteria required for participation in the study and was not enrolled.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

This SAP contains technical and detailed elaboration of the principal features of the analysis described in the protocol and includes procedures for executing the statistical analysis to fulfil the objectives of the study.

The study was designed to be, and executed as, a double-blinded with an OLE. The OLE was removed from protocol version 9.0 and so the use of the present tense is no longer accurate. In addition to the objectives and endpoints defined in Study 0367-CL-1201 protocol (core protocol), some additional objectives and endpoints specific to the Mayo Clinic (Minnesota) had been defined in Study 0367-CL-1201 [MAYO] Protocol. However, as there have been no participants recruited to the protocol from the Mayo sites, therefore there will be no SAP created. Until protocol version 7.0, the double-blinded period was 52 weeks and open label extension period was 24 weeks. However, from protocol version 8.0 onwards, the double-blinded period was revised to 24 weeks and in protocol version 9.0 the OLE period was removed. Since no participants have been evaluated under protocol version 8.0 or later, two SAPs will be prepared: one for analyses up to 52 weeks of treatment (the double-blind part of the study), and the other one for analyses at the end of 76 weeks of treatment (the double-blind part and the OLE part of the study). In this analysis, participants assigned ASP0367 in the double-blinded period will be analyzed for through the double-blinded part and the OLE part of the study including follow-up visit, while participants switched from Placebo to ASP0367 in the OLE part will be analyzed in the OLE part only including follow-up visit. This document is an analysis plan for the up to 76 weeks (double-blinded part and OLE part of the study) including follow-up visit.

Analyses for pharmacokinetic and pharmacodynamic (target gene expression and biochemical markers) endpoints, including exposure-response assessment, will be described in the separate analysis plan and this SAP only focus on safety endpoints.

This document will be finalized prior to the database lock at the end of study. If there are any changes from the planned analyses in the final version of the SAP that impact the statistical analyses, then it will be documented in the CSR.

2 STUDY OBJECTIVES AND DESIGN

2.1 Study Objectives, Endpoints and Estimands

Objectives	Endpoints
Primary	
● To assess the dose response of ASP0367 on functional improvement relative to placebo	● Change from baseline in distance walked on the 6MWT at week 24
● To assess the safety and tolerability of ASP0367 relative to placebo	● Safety and Tolerability through week 24 and end of study: o Nature, frequency and severity of TEAEs o Vital signs o Weight o 12-lead ECG

	○ Clinical laboratory tests (hematology, biochemistry [including serum cardiac troponin I] and urinalysis) ○ C-SSRS
Secondary	
● To assess the dose response of ASP0367 on functional improvement and fatigue relative to placebo	● Change from baseline in Neuro-QoL Short Form Fatigue and Lower Extremity Function (Mobility) scores at week 24 ● Change from baseline in time spent on the 5XSTS at week 24 ● Change from baseline in MFIS at week 24
● To assess the effect of ASP0367 in overall participant functioning relative to placebo	● PGIC scores at week 24 ● Change from baseline in PGIS scores at week 24
Exploratory	
● To assess the pharmacokinetics of ASP0367	● Plasma ASP0367: C_{trough} at weeks 12 and 24 and population pharmacokinetics
● To assess the exposure-response relationship of ASP0367	● Relationship between measured- and model-based pharmacokinetic exposure parameters (e.g., C_{trough}, $C_{ave, ss}$) of ASP0367 and endpoints of efficacy, safety and pharmacodynamic biomarkers, as appropriate
● To assess the effect of ASP0367 on lower extremity function relative to placebo	● Change from baseline in distance walked on the 6MWT at weeks 4 and 12 ● Change from baseline in minute-by-minute analyses (i.e., 6^{th} vs 1^{st}) of 6MWT at weeks 4, 12 and 24 ● Change from baseline in time spent on 5XSTS at weeks 4 and 12 ● Change from baseline in Neuro-QoL Short Form Fatigue score at weeks 4 and 12 ● Change from baseline in Neuro-QoL Short Form Lower Extremity Function (Mobility) score at weeks 4 and 12 ● Change from baseline in MFIS score at weeks 4 and 12
● To assess the effects of ASP0367 on quality of movement and patient perception of change	● PGIC scores at weeks 4 and 12 ● Change from baseline in PGIS scores at weeks 4 and 12 ● Change from baseline in EQ-VAS and EQ-5D-5L index at weeks 4, 12 and 24
● To assess the relationship between functional improvement and biochemical markers, as well as gene expression-based pharmacodynamic biomarkers	● Change from baseline in the levels of serum and urinary biomarkers (serum FLNC, CYC, ALDOA and urinary TITIN) at weeks 12 and 24 ● Relative change from baseline of whole blood PPARδ target gene expression levels at weeks 12 and 24[†]

Note: Placebo comparison will be up to 24 weeks and will not include open-label extension.

5XSTS: 5 Times Sit to Stand; 6MWT: 6-minute walk test; ALDOA: aldolase A; $C_{ave, ss}$: average plasma concentration at steady state; C-SSRS: Columbia-Suicide Severity Rating Scale; C_{trough}: concentration immediately prior to dosing at multiple dosing; CYC: cytochrome c; ECG: electrocardiogram; EQ-5D-5L: European Quality of Life 5-dimension, 5-level questionnaire; EQ-VAS: EuroQol visual analogue scale; FLNC:

filamin-C; MFIS: Modified Fatigue Impact Scale; Neuro-QoL: Quality of Life in Neurological Disorders; PGIC: Patient Global Impression of Change; PGIS: Patient Global Impression of Severity; PPARδ: peroxisome proliferator-activated receptor delta; TEAE: treatment emergent adverse event.

†Blood samples will be collected and stored appropriately over the course of the study and then analyzed.

Estimands

Primary estimand will be defined by the following 5 attributes:

- Treatment: ASP0367 or placebo
- Population: Participants with PMM, as defined by the inclusion/exclusion criteria of the study.
- Endpoint: Change from baseline in distance walked on the 6MWT at week 24.
- Intercurrent events and their corresponding strategies (see table below).
- Population level summary: Difference in change from baseline in distance walked on the 6MWT at week 24 between ASP0367 group and placebo group.

Intercurrent Events	Strategies to Handle Intercurrent Events
Treatment Discontinuation Due to Any Reason	For those participants who discontinued treatment, all observations after treatment discontinuation (Date of Last Study Drug Taken + 7 days) will be ignored. Analysis will be conducted assuming those participants remained assigned treatment and the condition that was observed before treatment discontinuation will continue, i.e., hypothetical strategy.
Study Discontinuation	For those participants who discontinued study, all observations after treatment discontinuation (Date of Last Study Drug Taken + 7 days) will be ignored. Analysis will be conducted assuming those participants remained assigned treatment and the condition that was observed before treatment discontinuation will continue, i.e., hypothetical strategy.
Prohibited Concomitant Treatment Use	For those participants who used the prohibited concomitant medication treatment, all observations will be included in the analysis, i.e., treatment policy strategy.

2.2 Study Design

This is a randomized, double-blind, placebo-controlled, oral dose, phase 2 study to evaluate the dose response of ASP0367 on functional improvement relative to placebo, safety and tolerability in participants with PMM. Efficacy (i.e., functional improvement) will be assessed by a functional motor test, 6MWT. The study consists of the following portions: screening (4 weeks); double-blind treatment period with 2 doses of ASP0367 vs matching placebo (24 weeks); and follow-up (4 weeks).

A 24-week treatment analysis of efficacy and safety will be the primary focus of this study. Since participants enrolled and participated in the study prior to implementation of protocol version 9.0, which removes the 52-week OLE period, they may have a treatment duration of up to 76 weeks, a separate analysis will be conducted up to 76 weeks and follow-up as per available data.

2.3 Randomization

Approximately 66 participants will be enrolled. At randomization, participants will be randomly placed into 1 of 3 arms (30 mg ASP0367, 75 mg ASP0367 or placebo; n = 22 for each arm) at a ratio of 1:1:1. Stratified randomization by site group (2 level: Mayo clinic_Minnesota; Non-Mayo clinic_Minnesota) will be applied, if there are any participants from in the Mayo site group.

3 SAMPLE SIZE

The sample size of this study was determined from feasibility and statistical perspectives. From the statistical perspective, 57 participants (19 participants in each group) will provide > 80% power to detect a difference of 45 meters in 6MWT between ASP0367 and placebo at a 2-sided significance level of 0.10 assuming a common standard deviation of 55.0 meters.

The 45-meter difference in 6MWT under the 24-week treatment is assumed based on the results of the MMPOWER-2 study of elamipretide [Cohen et al, 2018].

Considering the interim analysis, 60 participants will be required to maintain > 80% power in the efficacy analysis, and assuming an approximately 10% study discontinuation (drop out) rate during the double-blind treatment period, 66 participants are required to be randomized. This sample size supports an evaluation of proof of concept.

4 ANALYSIS SETS

In accordance with ICH recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

4.1 Safety Analysis Set

The SAF consists of all participants who receive at least 1 dose of ASP0367. The SAF only includes participants who were randomly assigned to ASP0367 in DB part or participants who were randomly assigned to placebo in the DB part and continued into the OLE part. Participants who were randomly assigned to placebo and discontinued the study in the DB part are not included in the SAF, even if they erroneously received ASP0367 during the DB part.

The SAF will be used for all summaries of the safety data.

5 EFFICACY AND SAFETY ENDPOINTS

5.1 Exploratory Endpoints

5.1.1 6-minute Walk Test

The 6MWT will be performed indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom traveled. The total distance walked by a participant, as well as distance per minute will be calculated by rounding to the nearest meter and recorded. The 6MWT will be video recorded for quality purposes.

Participants will be asked to wear comfortable clothing and appropriate shoes for walking. A light meal is acceptable before early morning or early afternoon tests. Participants will be recommended not to have exercised within 2 hours prior to beginning of the test. A "warm up" period before the test will not be performed. Participants will sit at rest in a chair located near the starting position for at least 10 minutes before the test starts. The test will be performed at weeks 0, 4, 12, 24, 36, 52, 64 and 76.

5.1.2 5 Times Sit To Stand (5XSTS)

For the 5XSTS, the participant will be instructed to sit with arms folded across their chest and with back against the chair and will be asked to stand up and sit down 5 times in a row, as quickly as he/she can. The instructor will make sure that the participant will stand up fully and try not to let his/her back touch the chair between each repetition. The duration from the time instructor indicates "Go" until the time participant's body touches the chair following the fifth repetition will be recorded. If participant is unable to complete the first sit to stand independently, without use of arms, the test will be terminated. The test will be performed at weeks 0, 4, 12, 24, 36, 52, 64 and 76.

5.1.3 Quality of Life in Neurological Disorders (Neuro-QoL)

The Neuro-QoL is a measurement system that evaluates and monitors the physical, mental and social effects experienced by adults and children living with neurological conditions. Participants will report Neuro-QoL Item Bank v1.0 Fatigue Short Form and Lower Extremity Function (Mobility) Short Form, which consists of 8 items each. Neuro-QoL will be performed at weeks 0, 4, 12, 24, 36, 52, 64 and 76.

5.1.4 Modified Fatigue Impact Scale (MFIS)

The Modified Fatigue Impact Scale (MFIS) is a modified form of the Fatigue Impact Scale. The questionnaire specifically measures how fatigue impacts the lives of those affected by fatigue-like symptoms.

There are 21 items in the scale measuring 3 domains of fatigue including physical, cognitive and psychosocial functioning. MFIS will be performed at weeks 0, 4, 12, 24, 36, 52, 64 and 76.

5.1.5 Patient Global Impression of Change (PGIC)

The Patient Global Impression of Change (PGIC) scale evaluates the participant's most bothersome symptom and assesses if there has been an improvement or decline in clinical status. The participant will rate his/her change on a 7-point scale. The PGIC score will be evaluated at weeks 4, 12, 24, 36, 52, 64 and 76.

5.1.6 The Patient Global Impression of Severity (PGIS)

The Patient Global Impression of Severity (PGIS) is a questionnaire designed to assess patient's impression of disease severity. The questionnaire asks the participant to best describe the severity of the participant's most bothersome pre-defined symptom over the past week. The PGIS score will be assessed at weeks 0, 4, 12, 24, 36, 52, 64 and 76.

5.1.7 European Quality of Life 5-dimension, 5-level questionnaire (EQ-5D-5L)

The EQ-5D-5L is a brief, generic health-related quality of life assessment that can also be used to incorporate participant preferences into health economic evaluations. The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 response levels: no problems, slight problems, moderate problems, severe problems and extreme problems/unable to perform the activity. In addition, it does record the patient's self-rated health on a vertical visual analogue scale with response options ranging from 0 (worst imaginable health) to 100 (best imaginable health). The EQ-5D-5L will be assessed at weeks 0, 4, 12, 24, 36, 52, 64 and 76.

5.2 Safety Endpoints

5.2.1 Treatment Emergent Adverse Events (TEAEs)

AE collection begins after the ICF has been signed and continues until the follow up visit or 28 days after the final IP administration or when the participant is determined to be a screen failure. A TEAE is defined as an AE observed after starting administration of the ASP0367 to 28 days after the last dose of ASP0367.

An IP-related TEAE is defined as any TEAE with a causal relationship of "yes" by the investigator.

5.2.2 Vital signs

Vital signs including blood pressure, pulse rate and body temperature will be assessed at each visit scheduled in the protocol.

5.2.3 12-lead Electrocardiogram (ECG)

- A standard 12-lead ECG will be conducted using both a central ECG reading laboratory and local ECG reading. ECGs at weeks 2 and 8 will be performed at the participant's home by a qualified healthcare provider from the home healthcare vendor. In case the home healthcare visit is not feasible, the visit can be conducted at the study site.
- ECGs will be recorded in triplicate (3 separate ECGs, 5 minutes resting prior to first ECG and at least 1 minute apart per time point), prior to blood draw and transmitted electronically for central reading. The mean of the triplicate ECG from central and local read should be used for treatment decisions and AE reporting.
- If the mean triplicate QTcF is > 500 msec at any time point, one follow-up triplicate ECG may be performed on the same day. If the follow-up mean QTcF is ≤ 500 msec, the participant may proceed with IP. If the follow-up mean QTcF > 500 msec is confirmed, then the participant must discontinue IP.
- An ECG will also be performed within 24 hours of any of these events: an elevation of cTnI above the ULN (see Appendix 10.9 in the protocol for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 in the protocol for reference range) or above the participant's baseline value if it was elevated, or when a participant has any signs or symptoms reflecting cardiac involvement, inclusive of new onset shortness of breath. If the ECG is within normal limits, a repeat of the abnormal troponin value (cTnI or cTnT) should be obtained. For any of the situations above, it

may be required to interrupt or discontinue further administration of IP. Participants with persistent cTnI or cTnT elevations, abnormal ECG or abnormal echocardiogram should undergo cardiology follow up.

The requirement to perform an ECG after elevations observed in Troponin T was not added to the protocol until version 9.0. Therefore, this ECG requirement related to cTnT elevation was not implemented as all participants enrolled under and participated in the protocol version 7.0 or earlier.

5.2.4 Echocardiogram (ECHO)

A local echocardiogram is to be performed within 24 hours of any of these events: an elevation of cTnI above the ULN (see Appendix 10.9 in the protocol for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 in the protocol for reference range) or above the participant's baseline value if it was elevated, or when a participant has any signs or symptoms reflecting cardiac involvement, inclusive of new onset shortness of breath. If the echocardiogram is within normal limits, a repeat of the abnormal troponin value (cTnI or cTnT) should be obtained. For any of the situations above, it may be required to interrupt or discontinue further administration of IP. Participants with persistent cTnI or cTnT elevations, abnormal ECG or abnormal echocardiogram should undergo cardiology follow up.

The requirement to perform an ECHO after elevations observed in Troponin T was not added to the protocol until version 9.0. Therefore, this ECHO requirement related to cTnT elevation was not implemented as all participants enrolled under and participated in the protocol version 7.0 or earlier.

5.2.5 Body Weight

Body weight is planned to be collected at each visit scheduled in the protocol.

5.2.6 Laboratory test (hematology, biochemistry and urinalysis)

Safety laboratory tests will be done by a central laboratory from the screening to the end of the study. The scheduled visit and the list of clinical laboratory tests are described in the protocol.

5.2.7 Columbia-Suicide Severity Rating Scale (C-SSRS)

The C-SSRS will be performed by trained site staff via interview at screening, randomization, and all subsequent study visits. At screening, the "Screening" version is to be used to determine eligibility. During all subsequent visits, the "Since Last Visit" version is used to monitor on study suicidal ideation and behavior after the initial assessment. If possible, continuity of raters should be maintained across visits for each patient. The following five questions regarding suicidal ideation, six questions regarding suicidal behavior and question for non-suicidal self-injurious behavior will be used for analysis.

- Suicidal Ideation ('Yes' to any one of the below 5 questions for suicidal ideation)
  - Wish to be Dead (No/Yes)
  - Non-Specific Active Suicidal Thoughts (No/Yes)

  - Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act (No/Yes)
  - Active Suicidal Ideation with Some Intent to Act, without Specific Plan (No/Yes)
  - Active Suicidal Ideation with Specific Plan and Intent (No/Yes)
- Suicidal Behavior ('Yes' to any one of the below 5 questions for suicidal behavior)
- Preparatory Acts or Behavior (No/Yes)
  - Aborted Attempt (No/Yes)
  - Interrupted Attempt (No/Yes)
  - Actual Attempt (No/Yes)
  - Completed Suicide (No/Yes)
- Suicidal Ideation or Behavior ('Yes' to any one of the above 10 questions for suicidal ideation and behavior)
- Self-injurious Behavior Without Suicidal Intent (No/Yes)

5.3 Other Endpoints

5.3.1 Pharmacokinetics Endpoints

Relationship between measured- and model-based pharmacokinetic exposure parameters (e.g., C_{trough}, $C_{ave, ss}$) of ASP0367 and endpoints of efficacy, safety and pharmacodynamic biomarkers, as appropriate.

5.3.2 Pharmacodynamics Endpoints

Blood samples for PPARδ target gene expression assay

5.3.3 Biomarkers

Urine samples for TITIN

Blood samples for serum target proteins (ALDOA, CYC and FLNC)

6 STATISTICAL METHODOLOGY

6.1 General Considerations

Continuous data will be summarized descriptively including the number of participants (n), mean, standard deviation (SD), median, minimum and maximum. Categorical data will be summarized by frequencies and percentages. Percentages by categories will be based on the number of participants with no missing data, i.e. the percentages for the non-missing categories will add up to 100%.

There are 2 different groups defined based in the randomization group:

- ASP0367 any dose group: Participants randomly assigned to ASP0367.
- Placebo to ASP0367 group: Participants randomly assigned to placebo and who continue into the OLE and receive ASP0367.

Safety analysis and other summaries based on SAF will be presented by analysis group (ASP0367 any dose group or Placebo to ASP0367 group), unless specifically stated otherwise.

All data summarization and analyses will be performed using SAS® Version 9.4 or higher on Linux. Specifications for table, figures, and data listing formats can be found in the TLF specifications.

For continuous variables that are recorded as "<X", "<=X", ">X", or ">=X", the value of "X" will be used in the calculation of summary statistics if otherwise noted. This approach may result in a summary that reflects the presence of an analyte (such as troponin) when in fact the analyte was not detected but reported as less than the lower limit of detection by the assay.

6.2 Study Population

6.2.1 Disposition of Participants

The following participant data will be presented:

Analysis population: All participants who complete double blind part, Analysis group: ASP0367 any dose group, Placebo to ASP0367 group and Total

- Number and percentage of participants who were administered at least one dose of study drug during the OLE part,
- Number and percentage of participants who completed and those who discontinued the treatment during the OLE part, by primary reason for treatment discontinuation,
- Number and percentage of participants who completed and those who discontinued the follow-up period, by primary reason for follow-up discontinuation.

6.2.2 Protocol Deviations

Analysis population: All randomized participants who took ASP0367, Analysis group: ASP0367 any dose group, Placebo to ASP0367 group and Total

The number and percentage of participants with the following protocol deviation criteria (defined as an important protocol deviation) will be summarized for each criterion and overall, by treatment group and total as well as by investigative site. Participants deviating from a criterion more than once will be counted only once for the corresponding criterion.

The unique identifiers will be as follows:

PD1 - Inclusion/Exclusion,

PD2 - Withdrawal Criteria,

PD3 - Study Intervention,

PD4 - Excluded Concomitant Medications,

PD5 - Informed Consent,

PD6 - Safety Reporting, and

PD7 - Procedures/Tests

6.2.3 Demographic and Other Baseline Characteristics

Analysis population: SAF, Analysis group: ASP0367 any dose group, Placebo to ASP0367 group and Total

- Demographic and other baseline characteristics (age, sex, race and ethnicity, body weight, height and BMI) will be summarized by descriptive statistics and frequency tabulations [Table 1].

Table 1 Demographic and baseline characteristics

Item	Classification
Sex	1: Male 2: Female
Age (Years) [Informed Consent]	Measurement value
Age	1: >=18 to <65 2: >=65 to <=80
Ethnicity	1: Not Hispanic or Latino 2: Hispanic or Latino
Race	1: White 2: Black or African American 3: Asian 4: American Indian or Alaska Native 5: Native Hawaiian or Other Pacific Islander 6: Other
Height (cm) [Screening]	Measurement value
Weight (kg) [Screening]	Measurement value
BMI (kg/m^2) [Screening]	Measurement value
BMI	1: < 25 2: >=25
Site Group	1: Mayo clinic_Minnesota 2: Non-Mayo clinic Minnesota
Walking aid use [Baseline†]	0: No 1: Yes
Duration of disease (Years)‡	Measurement value
Treated with medication [Screening]	0: No 1: Yes
Syndrome	1: Kearns Sayre Syndrome (KSS) 2: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) 3: Maternally Inherited Diabetes Deafness (MIDD) 4: Myoclonic Epilepsy with Ragged Red Fibers (MERRF) 5: Chronic Progressive Externa Ophthalmoplegia (CPEO) 6: 3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like disease (MEGDEL) 7: Leigh Syndrome 8: Multisystem mitochondrial disorder 9: Mitochondrial Myopathy 10: POLG-related disorders 11: Others
Genetic abnormality	1: Mitochondrial DNA 2: Nuclear DNA
Mitochondrial DNA mutant heteroplasmy information is available	0: No 1: Yes
Level of heteroplasmy (%)	Measurement value

BMI: body mass index; CPEO: Chronic Progressive Externa Ophthalmoplegia; KSS: Kearns Sayre Syndrome;

MEGDEL: 3-methylglutaconic aciduria, Deafness, Encephalopathy, and Leigh-like disease; MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes; MERRF: Myoclonic Epilepsy with Ragged Red Fibers; MIDD: Maternally Inherited Diabetes Deafness; POLG: polymerase-gamma.

†: For participants received ASP0367 on double blind part, the measurement at week 0 will be used as baseline, and for participants received placebo on double blind part, the measurement at week 52 will be used as baseline.

‡: Duration of disease will be calculated as follows:

[("Date of First Study Drug Taken" – "Date of Diagnosis") + 1] / 365.25.

If Date of Diagnosis is missing or incomplete, then impute the date following the rule stated in [Section 6.8.2].

6.3 Study Drugs Exposure and Compliance

Analysis population: SAF, Analysis group: ASP0367 any dose group or Placebo to ASP0367 group

- The duration of ASP0367 exposure for each participant will be summarized by descriptive statistics and frequency tabulations. The following categories of the duration of exposure (days) will be defined:
  - < 29 (4 weeks)
  - >= 29 (4 weeks) to < 85 (12 weeks)
  - >= 85 (12 weeks) to < 169 (24 weeks)
  - >= 169 (24 weeks) to < 253 (36 weeks)
  - >= 253 (36 weeks) to < 365 (52 weeks)
  - >= 365 (52 weeks) to < 449 (64 weeks)
  - >=449 (64 weeks) to <533 (76 weeks)
  - >=533 (76 weeks)

The duration of ASP0367 exposure will be calculated as follows: {"Date of Last Study Drug Taken" – "Date of First ASP0367 Taken*"} + 1.

Date of Last Study Drug Taken is defined as the latest date of ["Date of Last Study Drug Taken (Phase 2 Treatment Period)", "Date of Last Study Drug Taken (Dose selection Period)", or "Date of Last Study Drug Taken (Open-Label Extension Part)"].

*: For participants received ASP0367 on double blind part, "Date of First Study Drug Taken" will be used. For participants received placebo on double blind part, "Date of First Study Drug Taken (Open-Label Extension Part)" will be used.

- The compliance of ASP0367 exposure for each participant will be summarized by descriptive statistics and frequency tabulations. The following categories of the study drug compliance (%) will be defined:
  - < 50
  - >= 50 to < 75
  - >= 75 to < 90
  - >= 90
  - Unknown

The compliance of study drug exposure for ASP0367 any dose group will be calculated as follows:

$$\frac{\text{Total number of dispensed tablets of ASP0367 - Total number of returned tablets of ASP0367}}{\text{The number of tablets of ASP0367 to be taken}} \times 100$$

The number of tablets to be taken is defined as follows;

[{"Date of Last Study Drug Taken" – "Date of First Study Drug Taken"} + 1] x the number of tablets per day.

The compliance of study drug exposure for Placebo to ASP0367 arm will be calculated as follows:

$$\frac{\text{Total number of dispensed tablets in OLE part - Total number of returned tablets in OLE part}}{\text{The number of tablets to be taken in OLE part}} \times 100$$

The number of tablets to be taken in OLE part is defined as follows;

["Date of Last Study Drug Taken (Open-Label Extension Part)" – "Date of First Study Drug Taken (Open-Label Extension Part)" + 1] x the number of tablets per day.

6.4 Analysis of Efficacy

No efficacy analyses will be conducted in this OLE SAP.

6.5 Analysis of Safety

Analysis population: SAF, Analysis group: ASP0367 any dose group or Placebo to ASP0367 group

6.5.1 Adverse Events

AEs will be coded using the MedDRA.

An overview table will include the following:

- Number of TEAEs,
- Number and percentage of participants with TEAEs,
- Number of drug related TEAEs,
- Number and percentage of participants with drug related TEAEs,
- Number of serious TEAEs,
- Number and percentage of participants with serious TEAEs,
- Number of serious drug related TEAEs,
- Number and percentage of participants with serious drug related TEAEs,
- Number of TEAEs leading to death,
- Number and percentage of participants with TEAEs leading to death,
- Number of drug related TEAEs leading to death,
- Number and percentage of participants with drug related TEAEs leading to death,
- Number of TEAEs leading to withdrawal of study drug,
- Number and percentage of participants with TEAEs leading to withdrawal of study drug,
- Number of drug related TEAEs leading to withdrawal of study drug,
- Number and percentage of participants with drug related TEAEs leading to withdrawal of study drug, and

- Number of deaths.

The number and percentage of participants with TEAEs, as classified by SOC, and PT will be summarized by analysis group. Summaries will be provided for the following:

- TEAEs,
- drug related TEAEs,
- serious TEAEs,
- drug related serious TEAEs,
- TEAEs leading to withdrawal of study drug,
- drug related TEAEs leading to withdrawal of study drug,
- TEAEs excluding Serious AEs that equal to or exceed a threshold of 5.0% in any analysis group.

The number of TEAEs and the number and percentage of participants with TEAEs, as classified by SOC and PT will be summarized by severity and by relationship to study drug. In the participant count, if a participant has multiple TEAEs with the same SOC or PT, but with differing severity or relationship, then the participant will be counted once with the worst severity and highest degree of relationship. If severity or relationship is missing for all episodes of the event, the participant will be counted under missing severity or relationship.

The following AESIs are defined:

- Cardiac tissue injury defined by cTnI above ULN or if the cTnT is above ULN or baseline if the participant's baseline value was above ULN and a TEAE as pre-defined by a MedDRA search strategy for elevated troponin.
- Cardiac tissue injury with any signs or symptoms defined by an echocardiogram or electrocardiogram interpretation is reported as abnormal, clinically significant and a TEAE as pre-defined by a MedDRA search strategy for cardiac complaints.
- Skeletal muscle injury defined by CK increase >= 5 times the value of the ULN or the individual's baseline value, whichever is higher and a TEAE as pre-defined by a MedDRA search strategy for skeletal muscle injury.

AESIs prior to version 9.0 of the protocol did not include elevations in cTnT in the definition of an AESI but instead only included elevations of cTnI. All participants participated in protocol version 7.0 or earlier; therefore participants for whom elevations in cTnT were observed may not have been identified as having experienced an AESIs during study conduct and for this reason may not have had additional ECG and echocardiographic evaluations performed.

The number and percentage of participants who report AESIs will be summarized by analysis group. In addition, any (serious) AEs that are considered abuse of the study drug will also be collected and followed. The number and percentage of AEs of special interest related to abuse, as classified by SOC and PT will be summarized by analysis group.

6.5.2 Clinical Laboratory Evaluation

Quantitative values including hematology, biochemistry, and urinalysis will be summarized using mean, standard deviation, minimum, maximum and median by analysis group at each analysis visit. Additionally, a within-participant change will be calculated as the post-baseline measurement minus the baseline measurement and summarized in the same way. Each laboratory result will be classified as low (L), normal (N), or high (H) at each visit according to the site supplied reference ranges. For the Placebo and ASP0367 groups, baseline values for CK-MM and CK-MB will be defined as the last non-missing observation prior to the first administration of ASP0367.

Frequency tabulations of qualitative clinical laboratory variables (urinalysis) will be presented for each analysis group at each visit.

Additionally, amylase, lipase and troponin elevations above the ULN and 2xULN will be summarized by analysis group. The denominator for each criterion will be the number of participants who have at least one value after the day of first ASP0367 taken to 28 days after the last dose of ASP0367. The number and percentage of participants meeting the criteria by 28 days after the last dose of ASP0367 will be summarized by analysis group.

6.5.2.1 Liver Safety Assessment

The liver safety assessments will be summarized by the categories below based on the measurements from ALP, ALT, total bilirubin, AST and their combination. These parameters will be based on measurements from a local laboratory. If the baseline value is above ULN then use the baseline value instead of ULN.

- ALT > ULN, > 2xULN, > 3xULN, > 5xULN, > 10xULN, > 20xULN
- AST > ULN, > 2xULN, > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALT or AST > ULN, > 2xULN, > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALP > 1.5xULN, 2xULN, 2.5xULN
- Total Bilirubin > 2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN
- (ALT or AST > 3xULN) and ALP < 2xULN and Total Bilirubin > 2xULN

The last 2 criteria where 2 or more parameters are evaluated will be with the measurements on the same day or up to 1 day apart.

The denominator for each criterion will be the number of participants who have at least one value after the day of first ASP0367 taken to 28 days after the last dose of ASP0367. The number and percentage of participants meeting the criteria by 28 days after the last dose of ASP0367 will be summarized by analysis group.

6.5.3 Vital Signs

Vital signs (systolic blood pressure, diastolic blood pressure, and pulse rate) will be summarized using mean, standard deviation, minimum, maximum and median by analysis group and visit. Additionally, a within- participant change will be calculated per visit as the

post-baseline measurement minus the baseline measurement and summarized by analysis group and visit.

6.5.4 Electrocardiograms

6.5.4.1 Routine 12-lead Electrocardiograms

The number and percent of participants with normal, not clinically significant abnormal, and clinically significant abnormal results as assessed by site principal investigator for the 12 lead ECG will be tabulated by analysis group and time point. Continuous 12-lead Electrocardiogram.

For all analyses, replicates at each visit will be averaged for each continuous ECG parameter.

ECG parameters [Heart Rate (bpm), PR Interval (ms), QRS Interval (ms) and QTcF (ms)] read by the central laboratory will be summarized using mean, standard deviation, minimum, maximum and median for each analysis group at each visit, including changes from baseline.

The QTcF interval will be summarized using frequency tables for each visit for values of clinical importance using the range criteria below.

	QTcF Interval Criteria Value (msec)
Normal	≤ 450
Borderline	> 450
Prolonged	> 480
Clinically significant	> 500

QTcF: QT interval using Fridericia's correction.

The QTcF interval will also be summarized by the frequencies of participants with an increase from baseline of clinical importance using the criteria identified below. These summaries will be provided for each visit.

Variable	Change from Baseline
QTcF Interval (msec)	≤ 30
	> 30
	> 60

QTcF: QT interval using Fridericia's correction.

6.5.5 Other Safety-Related Assessment

6.5.5.1 Body Weight

Body weight will be summarized using mean, standard deviation, minimum, maximum and median by analysis group and visit. Additionally, a within-subject change will be calculated per visit as the post-baseline measurement minus the baseline measurement and summarized by analysis group. For Placebo to ASP0367 group, the measurement at week 52 is used as baseline, and for ASP0367 Any Dose, the measurement at Screening is used as baseline.

6.5.5.2 C-SSRS

The results of five questions regarding suicidal ideation, six questions regarding suicidal behavior, suicidal ideation or behavior and self-injurious behavior without suicidal intent will be summarized using number and percent of participants by analysis group at each visit.

6.6 Other Analyses

No analyses will be conducted.

6.7 Interim Analysis

6.7.1 Interim Analysis of Efficacy

The analysis of futility and of efficacy was performed when the first 33 participants completed the efficacy evaluation at week 24 or discontinued as of date the last participant had the week 24 visit, 20-Nov-2023. The change from baseline in distance walked on the 6MWT at week 24 was analyzed using the same MMRM as with the primary endpoint excluding the site group from the model to decide whether the study will continue.

Analysis of Futility

For the futility stop, the study may be stopped if the conditional probability of achieving the criteria below at the final analysis is less than 10%.

Criteria: Lower limit of the 90% CI for the difference is greater than 0 m AND the point estimate of the difference between the ASP0367 75 mg group and the placebo group is greater than 30 m.

In addition to 6MWT at week 24, outputs for participant disposition, demographics and the secondary endpoints 5XSTS, Neuro-QoL Short Form Fatigue score, Neuro-QoL Short Form Lower Extremity Function (Mobility) score, MFIS, PGIC, and PGIS at week 24 were analyzed at the interim analysis.

Analysis of Efficacy

For the efficacy stop, a two-stage group sequential design with Lan-DeMets alpha-spending function determined by means of the O'Brien-Fleming approach was used to preserve the overall two-sided type I error rate of 0.10 between this single interim analysis and the final analysis. If the p-value of the comparison between ASP0367 75 mg and placebo groups at interim analysis is lower than the boundary determined by O'Brien-Fleming approach, efficacy would be considered to be achieved at week 24.

The interim analysis outputs were prepared by an external statistician and presented to the AIDMC who made recommendations about the ongoing conduct of the study. After the AIDMC reviewed the interim analysis results, the AIDMC recommended the study to be stopped because the results of the interim analysis met the criteria for futility. Details of the analysis were defined in the interim analysis plan.

6.8 Additional Conventions

6.8.1 Analysis Windows

Day 1 and Day -1 are defined as the day of start taking the IP and its prior day, respectively. The following analysis window will be used. The data summary by visits will be done following the analysis windows specified in the table below:

Analysis Visits	Scheduled Day in Protocol	Analysis Windows (day)
Screening	Day -28	-35 to -14
Week 0	Day -2 to 1	-2 to 1
Week 1	Day 8	5 to 13
Week 2	Day 14	14 to 18
Week 3	Day 22	19 to 25
Week 4	Day 29	26 to 42
Week 8	Day 57	43 to 70
Week 12	Day 85	71 to 98
Week 16	Day 113	99 to 126
Week 20	Day 141	127 to 154
Week 24	Day 169	155 to 182
Week 28	Day 197	183 to 210
Week 32	Day 225	211 to 238
Week 36	Day 253	239 to 266
Week 40	Day 281	267 to 294
Week 44	Day 309	295 to 322
Week 48	Day 337	323 to 350
Week 52	Day 365	351 to 378
Week 56	Day 393	379 to 406
Week 60	Day 421	407 to 434
Week 64	Day 449	435 to 462
Week 68	Day 477	463 to 490
Week 72	Day 505	491 to 518
Week 76	Day 533	519 to 560
End of Treatment	Day 533	2 to 560
Follow-up	The last dose + 28 days	The last dose + 28 days to the last dose + 35 days

For all analysis visits excluding follow-up visit, the data “by the last dose + 7 days” will be included.

For participants who received ASP0367 on the double blind part, all analysis visits will be used. For participants received placebo on the double blind part, baseline and analysis visit at and after the first ASP0367 taken will be used.

If more than one observation exists within the analysis window, the observation closest to the scheduled visit day will be selected for that visit. If there are two observations that have the same distance from the scheduled day, the value that is after the scheduled day will be selected in the analysis. If more than one observation is made on the same day, an average value if continuous or the worst value if categorical will be included in the analysis.

For Placebo to ASP0367 group, the measurement at week 52 is used as baseline, and for ASP0367 groups, the measurement at week 0 is used as baseline, unless otherwise specified.

6.8.2 Imputation Rules for Incomplete Dates

For AEs, a missing or incomplete onset date will be imputed according to the following conventions.

If an onset date is missing, the imputed onset date will be the date of first dose of study drug.

If only the year is known for the AE onset date, the imputed onset date will be the latest of the following non-missing dates:

- Date of first dose of study drug
- January 1 of the year of AE onset date

If only the month and year is known for the onset date, set the surrogate onset date to the first day of that month and then apply the following rules.

- If the month and year of the onset date is prior to the month and year of the first dose of study drug, then the surrogate onset date will be the imputed onset date.
- If the month and year of the onset date is on or after the month and year of the first dose of study drug, then the imputed onset date will be the **latest** of the following non-missing dates:
- Date of first dose of study drug
- Surrogate onset date
- If the imputed onset date is after the AE end date, the imputed onset date will be the same as the AE end date.

For duration of disease of primary diagnosis, a missing or incomplete diagnosis date will be imputed according to the following conventions.

If a diagnosis date is missing, the diagnosis date will not be imputed. The duration of disease in baseline analysis will be treated as missing data.

If only the year is known for the diagnosis date, the imputed diagnosis date will be January 1 of the year of the diagnosis date.

If only the month and year is known for the diagnosis date, set the imputed diagnosis date will be the first day of that month.

6.8.3 Outliers

All values will be included in the analyses.

7 REVISION AND RATIONALE

Version	Date	Changes	Comment/rationale for change
1.0	09-JUL-2024	NA	Document finalized

8 REFERENCES

Cohen B, Karaa A, Haas R, Goldstein A, Vockley G. Effects of Elamipretide in Adults with Primary Mitochondrial Myopathy: a Phase 2 Double-Blind, Randomized, Placebo-Controlled Crossover Trial (MMPOWER-2). Neurology. 2018;90(Suppl 15):S5.003.

ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

ICH Harmonized Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

9 APPENDICES

9.1 Appendix 1: Author and Approver Signatures

(E-signatures are attached at the end of document.)

Prepared by:	*E-signatures are attached at end of document* PPD Data Science, Astellas Pharma Inc.	Date: Date (DD Mmm YYYY)
Approved by:	*E-signatures are attached at end of document* PPD Data Science, Astellas Pharma Inc.	Date: Date (DD Mmm YYYY)
Approved by:	*E-signatures are attached at end of document* PPD Data Science, Astellas Pharma Inc.	Date: Date (DD Mmm YYYY)
Approved by:	E-signatures are attached at end of document PPD BioPharma Development, Astellas Pharma Global Development, Inc.	Date: Date (DD Mmm YYYY)