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

MOUNTAINSIDE

ISN/Protocol 0367-CL-1201

Version 9.0

Incorporating Substantial Amendment 8

24 Jan 2024

IND 146773

Sponsor:

Astellas Pharma Inc.
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Protocol History:
Version 1.0 [05 May 2020]
Version 1.1 Incorporating Nonsubstantial Amendment 1 [13 Jul 2020]
Version 1.2 Incorporating Nonsubstantial Amendment 2 [30 Jul 2020]
Version 2.0 Incorporating Substantial Amendment 1 [21 Sep 2020]
Version 2.1 Incorporating Nonsubstantial Amendment 3 [28 Sep 2020]
Version 2.2 Incorporating Nonsubstantial Amendment 4 [01 Oct 2020]
Version 3.0 Incorporating Substantial Amendment 2 [02 Dec 2020]
Version 3.1 Incorporating Nonsubstantial Amendment 5 [02 Mar 2021]
Version 4.0 Incorporating Substantial Amendment 3 [07 May 2021]
Version 5.0 Incorporating Substantial Amendment 4 [05 Nov 2021]
Version 6.0 Incorporating Substantial Amendment 5 [17 Mar 2022]
Version 7.0 Incorporating Substantial Amendment 6 [10 Nov 2022]
Version 8.0 Incorporating Substantial Amendment 7 [24 Apr 2023]
Version 8.1 Incorporating Nonsubstantial Amendment 6 [06 Jul 2023]

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SIGNATURES

1. SPONSOR'S SIGNATURES

Required signatures (e.g., protocol authors and contributors, etc.) are located in [Section 13 Sponsor's Signatures].

2. INVESTIGATOR'S SIGNATURE

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/Protocol 0367-CL-1201

Version 9.0, Substantial Amendment 8

24 Jan 2024

I have read all pages of this protocol for which Astellas is the sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations. I will also ensure that subinvestigator(s) and other relevant members of my personnel have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: .....

Date (DD-MMM-YYYY)

Printed Name: .....

Address of trial site: .....

.....

CONTACT DETAILS OF SPONSOR'S KEY PERSONNEL

24-hour Contact for Serious Adverse Events See [Section 10.3.6 Reporting Procedures for Serious Adverse Events]	**Please fax or email the serious adverse events/special situations worksheet to:** Astellas Pharma Global Development Inc. Global/US Pharmacovigilance **North America fax number: +1-888-396-3750** **North America alternate fax number: +1-847-317-1241** **International fax number: +44-800-471-5263** **Email: safety-us@astellas.com**
Medical Monitor	PPD
Study Physician	PPD Astellas Pharma Global Development, Inc. PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES

DOCUMENT HISTORY	
Document	**Date**
Nonsubstantial Amendment 6	06 Jul 2023
Substantial Amendment 7	24 Apr 2023
Substantial Amendment 6	10 Nov 2022
Substantial Amendment 5	17 Mar 2022
Substantial Amendment 4	05 Nov 2021
Substantial Amendment 3	07 May 2021
Nonsubstantial Amendment 5	02 Mar 2021
Substantial Amendment 2	02 Dec 2020
Nonsubstantial Amendment 4	01 Oct 2020
Nonsubstantial Amendment 3	28 Sep 2020
Substantial Amendment 1	21 Sep 2020
Nonsubstantial Amendment 2	30 Jul 2020
Nonsubstantial Amendment 1	13 Jul 2020
Original Protocol	05 May 2020

Amendment 8 [Substantial] Date 24 Jan 2024

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and EU Clinical Trial Regulation.

Overall Rationale for the Amendment:

The primary rationale for this amendment is to align with the guidance from the FDA to limit the dosing period to no longer than 6 months while the nonclinical carcinogenicity study results are under review by the FDA. The double-blind treatment period will be shortened from 52 weeks to 24 weeks and the open-label extension period has been removed.

Summary of Changes

Substantial Changes

Section Number	Description of Change	Brief Rationale
Title page, Investigator's signature page, 1.1, 1.2 (Figure 1), 1.3 (Table 1), 1.3.1 (Table 2), 2.1, 2.2.3.2, 2.3.2, 3, 4.1, 4.2.1, 4.3, 5.1, 5.3, 6.1, 6.3.1, 6.5, 7.2.1, 7.2.4, 7.2.8 (Table 6), 7.11 (Table 7), 9, 9.1, 9.3, 9.3.2, 9.4, 9.4.1, 9.4.2, 9.4.3, 9.4.5.1, 9.4.5.2, 9.6, 12	The double-blind treatment period of the study is shortened from 52 weeks to 24 weeks and the open-label extension period has been removed.	[redacted] CCI To evaluate a proof-of-concept declaration.
1.1, 1.3 (Table 1), 3, 7.1.3, 7.1.4, 7.1.5, 7.1.6, 7.1.7, 7.2.8 (Table 6), 7.9, 7.9.1, 7.9.2, 7.9.3, 7.9.4, 7.9.6, 9.4.2.3, 9.4.2.4, 9.6.1, 10.8 (Table 11)	Reinstate the following efficacy assessments: Neuro-QoL Lower Extremity Function (Mobility) Short Form, Modified Fatigue Impact Scale (MFIS), Patient Global Impression of Change (PGIC) scale, Patient Global Impression of Severity (PGIS), and European Quality of Life 5-dimension, 5-level questionnaire (EQ-5D-5L).	To maintain collection of efficacy assessments to further inform the effect of ASP0367.
1.1, 3	Update the study objectives, endpoints and estimands.	To reflect changes in study design to evaluate a proof-of-concept declaration.
2.2.3.1	Update text with results from an ASP0367 study in rats of prenatal and postnatal development and maternal function. Also, update text with results from in vitro studies suggesting that the risk of DDI is considered to be low.	To add new ASP0367 nonclinical study results to further inform the safety profile of ASP0367.

Section Number	Description of Change	Brief Rationale
2.2.3.1, 2.3.1.1	Update ASP0367 nonclinical development and risk assessment with carcinogenicity study results.	To add results from histopathological examination in a 2-year carcinogenicity study in rats.
2.3.1.3	Update the following sentences: "Only participants with a cTnI level below upper limit (~~or above upper limit and as assessed as clinically insignificant by the investigator~~) and a mean QT interval using Fridericia's correction (QTcF) of ≤ 450 msec for male participants and ≤ 480 msec for female participants will be allowed into the study." "Participant**s** who experience **any signs or symptoms potentially reflecting cardiac involvement**~~cardiac complaints~~ will undergo ECG, echocardiogram ~~(ECHO)~~, and have a cTnI drawn for further evaluation prior to continuing study drug."	To be consistent with the change to Exclusion Criterion #4 to provide clear exclusion to subjects who have an elevation in cTnI that may indicate the possibility of cardiac injury.
2.3.3	Update the overall benefit-risk conclusion.	To clarify that the sponsor considers the overall benefit-risk to participants with PMM taking part in Study 0367-CL-1201 to be favorable.

Section Number	Description of Change	Brief Rationale
5.1	• Update Inclusion Criterion #4a to the following: "a. Molecular genetic abnormality (i.e., nuclear or mitochondrial) known to be associated with ~~causing~~ mitochondrial dysfunction (such as, but not limited to, mtDNA single~~, variable~~ deletions in CPEO and KSS; mtDNA m.3243 A > G ~~common mutation in MELAS~~; pathogenic nuclear or mitochondrial genome variants demonstrated to cause primary mitochondrial disease), and…" • Update Inclusion Criterion #5 to the following: "Participant has been on stable dose regimen of coenzyme Q10 (CoQ10), carnitine, creatine or other mitochondrial disease-focused vitamins or supplemental therapies **for the treatment of symptoms of the mitochondrial disease** for **at least** 3 months prior to randomization and intends to stay on a stable dose for duration of study period ~~(for participants who take any above-mentioned medications or supplements)~~."	To clarify terminology regarding molecular genetic abnormalities associated with mitochondrial dysfunction and to clarify that a participant must have been on stable dose regimens for the treatment of symptoms of mitochondrial disease to be included in the study.

5.2	• Update Exclusion Criterion #1 to the following: "Participant has additional signs and/or symptoms due to non-myopathic process (e.g., cerebellar dysfunctions, movement disorder, peripheral neuropathy, stroke or other) or a gait problem not attributed to the myopathy that would interfere~~/may in addition to the myopathy affect~~**with** the participant's performance during 6MWT or 5 times sit to stand (5XSTS), in the opinion of the investigator." • Update Exclusion Criterion #4 to the following: "Participant has cTnI > ULN at screening ~~and is assessed as clinically significant by the investigator~~." • Update Exclusion Criterion #5 to the following: "Participant has estimated glomerular filtration rate (eGFR) calculated by the Chronic Kidney Disease Epidemiology Collaboration equation < 60 mL/min/1.73 m^2 at screening **or a history of chronic kidney disease stage 3 or greater**.*" • Update Exclusion Criterion #6 to the following: "Participant has at screening**: total bilirubin (TBL) > ULN or transaminase(s) (aspartate aminotransferase [AST] or alanine aminotransferase [ALT]) > ULN in the absence of elevations in CK. Participants who have a slightly elevated TBL and/or ALT and/or AST and are suitable candidates for the study~~, per investigator's opinion, can~~ **may** be enrolled **after discussion of the case with the medical monitor and completion of**~~in the study as long as the investigator can rule out any underlying~~	To clarify several criteria for excluding participants from participation in the study.

	~~liver dysfunction by running additional tests** and after~~ **further evaluation as warranted**~~discussing the case with the medical monitor~~." • Update Exclusion Criterion #14 to the following: "ECG evidence of acute ischemia, atrial fibrillation or active conduction system abnormalities**. The following conduction system abnormalities may be** ~~with the exception~~ **permitted per the investigator's discretion, only after discussing the case with the medical monitor**~~of any of the following~~: …" • Update Exclusion Criterion #15 to the following: "Participant requires any ventilatory support**, inclusive of any respiratory device to support breathing such as home ventilators and any form of non-invasive positive pressure ventilation (including continuous positive airway pressure [CPAP], bilevel positive airway pressure [BiPAP], and average volume-assured pressure support [AVAPS]). Participants who require oxygen therapy (even by low-flow nasal cannula [LFNC]) are not candidates for this study**." • Update Exclusion Criterion #25 to the following: "Participant has initiated the use of CoQ10, carnitine, creatine or other mitochondrial disease-focused supplements **for the treatment of symptoms of the mitochondrial disease** within 3 months prior to study randomization." • Update Exclusion Criterion #30 to the following: "Participant has **signs or symptoms of** bulbar	

Section Number	Description of Change	Brief Rationale
	weakness**, such as dysphagia, dysphonia, hoarseness or drooling/sialorrhea,** due to either neuropathy or myopathy." Also, added the following reference to footnote *: "**https://www.kidney.org/atoz/content/stages-chronic-kidney-disease-ckd**"	
7.2.4, 7.2.5	Update text specifying that ECG and echocardiogram will be performed within 24 hours of any of these events: an elevation of cTnI above the ULN (see Appendix 10.9 for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 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 or 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.	To [redacted] to implement additional safety monitoring for all participants who experience troponin elevations.

Section Number	Description of Change	Brief Rationale
7.3.6	Update the first 2 bullets describing (S)AEs of special interest: ● "Cardiac tissue injury, indicated by cTnI above ULN **(see Appendix 10.9 for reference range)** or **if the cTnT is** above **ULN (see Appendix 10.9 for reference range) or** baseline if the participant's baseline value of **cTnT** was above ULN" ● "Cardiac tissue injury with **any signs or symptoms reflecting cardiac involvement, inclusive of new onset shortness of breath** ~~cardiac complaints~~ confirmed by ECG ~~and/~~or ~~ECHO~~**echocardiogram**"	To clarify the AEs of Special Interest and [redacted] to implement additional safety monitoring for all participants who experience troponin elevations.

8.1	Update the following bullets describing how an individual participant may be required to discontinue further administration of IP if any of the following occurs, which is considered to be clinically significant and related to IP: • "Participant who experiences a cTnI elevation (i.e., > ULN [**see Appendix 10.9 for reference range]** ~~or > his/her baseline value if the baseline value is > ULN~~) **or a cTnT elevation above the ULN (see Appendix 10.9 for reference range) or above the participant's baseline value if it was elevated** should ~~interrupt IP and~~ undergo ECG and ~~ECHO~~**echocardiogram** within 24 hours. **The investigator should determine whether it is appropriate to continue administration of IP to the participant while the evaluations are being completed.** If the ECG and ~~ECHO~~**echocardiogram** are within normal limits, a repeat **of the abnormal value (**cTnI **or cTnT)** should be obtained. Participants with persistent cTnI **or cTnT** elevations, abnormal ECG or ~~ECHO~~**echocardiogram** should **interrupt IP and** undergo cardiology follow up." • "Participant who develops **any signs or symptoms potentially reflecting** cardiac **involvement, inclusive of** ~~complaints (which should include~~ new **onset** shortness of breath~~,~~) should interrupt IP and undergo ECG, ~~ECHO~~**echocardiogram**, and have a cTnI drawn to be evaluated prior to continuing IP."	To clarify several criteria for discontinuation of individual participants from further administration of IP.

Section Number	Description of Change	Brief Rationale
	● "Participant who newly develops **signs or symptoms of** bulbar weakness**, such as dysphagia, dysphonia, hoarseness or drooling/sialorrhea,** should discontinue IP~~ after a discussion with the medical monitor~~." ● "Participant with **a** new **requirement for oxygen therapy or for ventilatory support, inclusive of any respiratory device to support breathing, such as home ventilators and any form of non-invasive positive pressure ventilation (including CPAP, BiPAP, and AVAPS),** ~~ventilatory requirements~~ should discontinue IP~~ after a discussion with the medical monitor~~."	
9, 9.1, 9.2, 9.3, 9.4, 9.4.1, 9.4.2, 9.4.2.1, 9.4.2.2, 9.4.2.3, 9.4.2.4	Update the analysis of efficacy to clarify that a 24-week treatment analysis will be the primary focus. Available data collected after the 24-week double-blind period for participants who may have a treatment duration of up to 76 weeks if enrolled in the study prior to implementation of Protocol Version 9.0 (Substantial Amendment 8) will be summarized and details provided in a separate statistical analysis plan.	To best derive evidence of efficacy for ASP0367 to support a proof-of-concept declaration.

Nonsubstantial Changes

Section Number	Description of Change	Brief Rationale
1.1	Update text to state that there will be up to approximately 25 sites in the United States.	To clarify the planned location of the study sites.
1.1, 4.1	Update text to clarify that an independent Data and Safety Monitoring Board (DSMB) will review safety data as currently described in the protocol and a separate Data Monitoring Committee (DMC) for interim analysis, comprised of Astellas internal members, will assess the futility and efficacy of the study treatments and make recommendations about the ongoing conduct of the study. The previous detailed description of the DMC is removed, and reference links are provided in the text to details regarding the structure and role of each committee. An interim analysis of pharmacokinetic and pharmacodynamic data will also be reviewed by the DMC.	To clarify that the study will utilize a DSMB to review safety data and a separate DMC for the interim analysis.
1.3 (Table 1)	Update the Schedule of Assessments.	To align with the revised study design and assess relevant parameters for efficacy and safety monitoring.
1.3 (Table 1), 7.2.4	Remove the ECG performed at the participant's home at week 4 and add ECGs at the study site visits at week 4 and week 28 (follow-up period).	To align with the revised study design and optimize the ECG schedule during the study.
1.1, 1.3 (Table 1), 1.3.1 (Table 2), 3 (Table 3), 7.2.8 (Table 6), 7.11 (Table 7)	Update the pharmacokinetic (PK) sample collection schedule.	To align with the revised study design and to optimize the PK sampling schedule.

Section Number	Description of Change	Brief Rationale
1.1, 9, 9.1, 9.2, 9.6, 9.6.1, 9.6.2	Add text specifying that an interim analysis will occur after 24 weeks of double-blind treatment to evaluate futility and efficacy of the primary endpoint and update the interim analysis section by adding a subsection for "Interim Analysis of Efficacy" and a subsection for "Interim Analysis of Pharmacokinetics and Pharmacodynamics."	To specify the interim analyses that will be performed to decide if the study should continue based on efficacy data and the interim analysis of Pharmacokinetics and Pharmacodynamics data to help understand the results of the interim analysis for futility and efficacy.
2.2.3.2, 2.3.1.2	Update text with additional completed clinical studies.	To add results from 3 completed clinical studies.
4.3, 6.3.3	Update text to clarify that an independent DSMB will be utilized to review safety and tolerability data.	To clarify that the DSMB for safety will assess overall safety and tolerability.
6.3.3	Add text to clarify that the DMC will be provided with access to the treatment assignment for review of the interim analysis data, and that its operation will be documented in the DMC Charter.	To clarify that the DMC will be provided with access to the treatment assignment for review of the interim analysis data.
6.8.3	Update text to be consistent with the revision to Exclusion Criterion #25.	To clarify restricted concomitant treatments.
7.1, 7.9	Reorder the efficacy assessment and electronic clinical outcome assessment subheadings.	To clarify the description of the efficacy and electronic clinical outcome assessments.
7.11 (Table 7)	Update with a revised number of samples and approximate total volume.	To reflect changes in study design.
9.4.2.3	Text is updated to categorize participants with a PGIC score of 3 (Minimally Improved) as responders.	To clarify that minimal improvement in PGIC score is also considered a response.

Section Number	Description of Change	Brief Rationale
9.4.3.1	Remove the heading for "24-week Treatment Analysis" and replace with "Adverse Events" and update the definition of treatment-emergent adverse event (TEAE) to account for participants who consented for this study in Protocol Version 7.0 or former version and did not re-consent to Protocol Version 8.0 or later version by week 24, or who consented or re-consented to this study in Protocol Version 8.0 or later version by week 24. Also, remove the section describing the "76-week Treatment Analysis."	To clarify the TEAE definition and to align safety analysis text with the revised study design.
9.4.4	Revised text summarizing the analysis of pharmacokinetics and added text referencing the statistical analysis plans (SAPs) for details regarding the calculation of the pharmacokinetic parameters.	To clarify the analysis of pharmacokinetics.
9.4.5	Added text describing that the analysis of pharmacodynamics data for participants enrolled prior to implementation of Protocol Version 9.0 (Substantial Amendment 8) will differ from analysis of data for participants enrolled starting with Protocol Version 9.0 (Substantial Amendment 8).	To clarify the analysis of pharmacodynamics.
9.4.5.1	Update the section heading to "Analysis of Treatment-emergent Gene Expression" and replace Section 9.4.5.2 with "Estimation of Pharmacodynamic Parameters."	To clarify the analysis of pharmacodynamics.
9.4.6.1, 9.4.6.2, 9.4.6.3	Update text to clarify that biomarker data and polymorphisms of genes may be summarized graphically **and**/or descriptively. Update text to clarify exposure-response analysis.	To clarify analyses of biomarkers, genotyping and exposure-response.

Section Number	Description of Change	Brief Rationale
10.1.5.1, 10.1.5.1.1, 10.1.5.1.2	Add text specifying that an independent DSMB will be established to review safety data and a separate DMC for the interim analysis. Details regarding the structure and role of each committee are included in Sections 10.1.5.1.1 and 10.1.5.1.2, respectively.	To clarify that the study will utilize a DSMB to review safety data and a separate DMC for the interim analysis, and to provide details regarding the operation of each committee.
10.8 (Table 11)	Add Neuro-QoL Short Form Lower Extremity Function (Mobility), MFIS, PGIC, and PGIS to the list of critical assessments in the alternative schedule of assessments in response to a crisis.	To include the continuation of assessment for these efficacy endpoints.
10.9	Added Appendix 9 List of Normal Ranges for Selected Laboratory Assessments.	To [redacted] to implement additional safety monitoring for all participants who experience troponin elevations.
Throughout	Minor administrative-type changes (e.g., typos, format, abbreviations, numbering and consistency throughout the protocol) and updates to abbreviations in tables and list of abbreviations.	To provide clarifications to the protocol and to ensure complete understanding of study procedures.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title of Study:

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

Planned Study Period:

From approximately 2Q2021 to 2Q2026

Study Objectives, Endpoints and Estimands:

The study objectives and endpoints are outlined in the table below.

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 o Clinical laboratory tests (hematology, biochemistry [including serum cardiac troponin I] and urinalysis) o 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., 6th vs 1st) 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.

[redacted]

Estimands

Primary estimand will be defined by the following 5 attributes:

- Treatment: ASP0367 or placebo
- Population: Participants with Primary Mitochondrial Myopathy (PMM), as defined by the inclusion/exclusion criteria of the study.
- Endpoint: Change from baseline in distance walked on the 6-minute walk test (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.

Planned Total Number of Study Sites and Location(s):

Up to approximately 25 sites in the United States.

Study Population:

Male and female participants (age 18 to < 65 years old) with PMM diagnosed by mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) alterations, which are known to be associated with mitochondrial dysfunction, and by reported symptoms including muscle weakness, fatigue and exercise intolerance.

Number of Participants:

Approximately 66 randomized participants.

Study Design Overview:

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. Since participants enrolled in the study prior to implementation of Protocol Version 9.0 (Substantial Amendment 8), which removes the 52-week OLE period, may have a treatment duration of up to 76 weeks, a

separate treatment analysis will also be conducted at later timepoints up to 76 weeks as per available data. An unblinded, independent Data and Safety Monitoring Board (DSMB) will assess the overall safety and tolerability of ASP0367 throughout the course of the study.

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. [redacted].

At week 24 while enrollment continues, data from the first approximately 30 evaluable participants will be reviewed in an interim analysis to determine if the futility or efficacy stopping criteria are met while the study is ongoing. An Astellas internal multidisciplinary Data Monitoring Committee (DMC) for interim analysis will assess the futility and efficacy of the study treatments and make recommendations about the ongoing conduct of the study. An interim analysis of pharmacokinetic and pharmacodynamic data will also be reviewed by the DMC.

Treatment Groups and Duration

Arm/IP Name	ASP0367	Placebo
Use	Test product	Matching placebo for ASP0367
Dose	75 mg (3 × 25 mg tablet) 30 mg (3 × 10 mg tablet)	NA (3 × tablet)
Frequency	Once daily	Once daily
Route	Oral	Oral
Duration	24 weeks	24 weeks

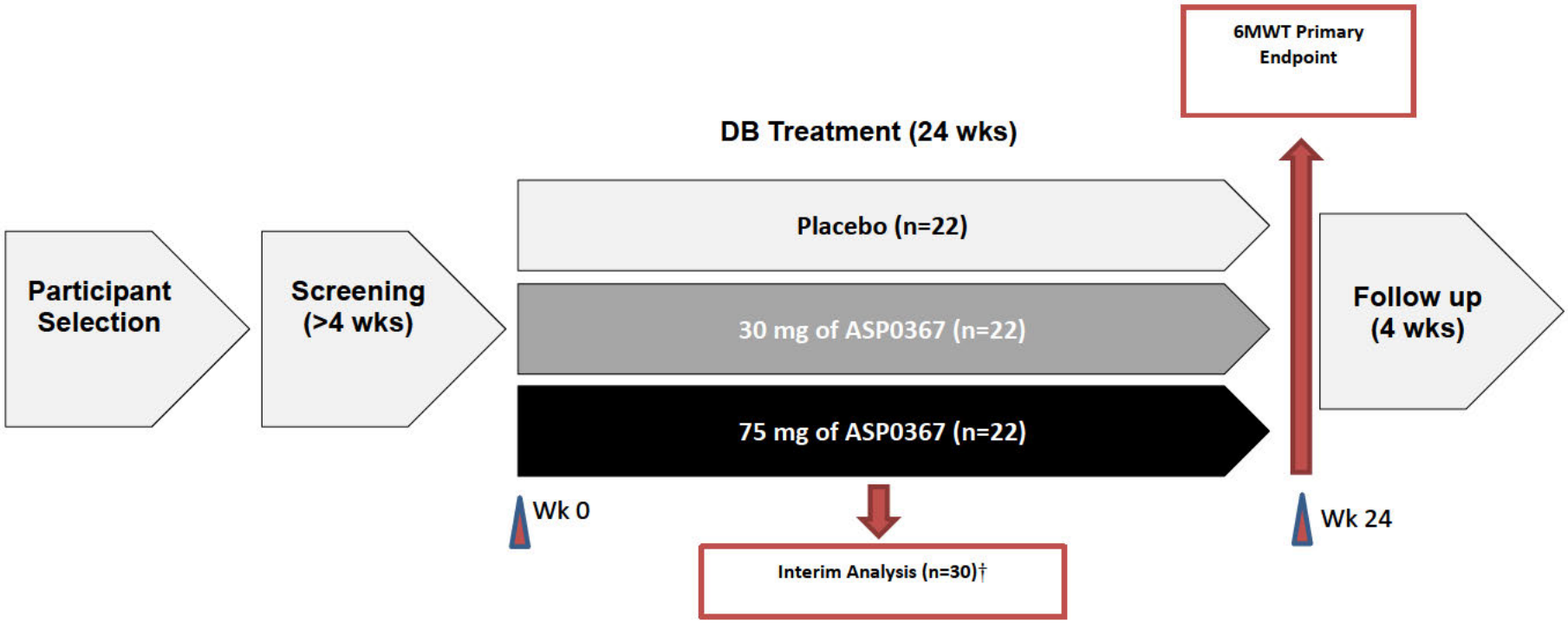
IP: investigational product; NA: not applicable

The anticipated duration of the study for each participant, including screening and follow-up, is approximately 32 weeks.

Participants should be instructed to take the investigational product (IP) in the morning at the same time each day as close as possible. Crushing of tablets is not allowed. IP will be administered orally with or without food.

1.2 Study Schema

Figure 1 Study Level Schema



6MWT: 6-minute walk test; DB: double-blind.

† At week 24 while enrollment continues, data from the first approximately 30 evaluable participants will be reviewed in an interim analysis to determine if the futility or efficacy stopping criteria are met while the study is ongoing.

1.3 Schedule of Assessments

Table 1 Schedule of Assessments

Assessments	Screening Period	Treatment Period										Follow-up Period
		Double-blind Treatment (24 weeks)										
Visit Number	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
Week	**-4**	**0**	**1**	**2**	**3**	**4**	**8**	**12**	**16**	**20**	**24**	**28**
Day	**-28**	**-2 to 1[c]**	**8**	**14**	**22**	**29**	**57**	**85**	**113**	**141**	**169/EOT_P2[d]**	**197/EOS[d]**
Visit window (days)	**-7 to +14**	**-**	**± 3**	**-2 to + 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**+ 7**
Study site visit[a]	X	X				X		X			X	X
Follow-up phone call[b]			X	X	X		X		X	X		
Informed consent	X											
Medical history	X											
Demographics	X											
On-site training for all endpoint assessments	X											
Verify inclusion/ exclusion criteria	X	X[e]										
Randomization		X										
Physical examination	X	X				X		X			X	X
Height	X											
Body weight	X										X	X
Vital signs[f]	X	X[e]				X		X			X	X
Hematology, biochemistry, urinalysis[g]	X	X				X		X			X	X
Abbreviated biochemistry and hematology[g]			X	X	X		X		X	X		
Serology (hepatitis screening)	X											
Pregnancy test	X[h]	X[e,i]				X[i]		X[i]			X[i]	

Table continued on next page

Footnotes

Assessments	Screening Period	Treatment Period										Follow-up Period
		Double-blind Treatment (24 weeks)										
Visit Number	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
Week	**-4**	**0**	**1**	**2**	**3**	**4**	**8**	**12**	**16**	**20**	**24**	**28**
Day	**-28**	**-2 to 1[c]**	**8**	**14**	**22**	**29**	**57**	**85**	**113**	**141**	**169/EOT_P2[d]**	**197/ EOS[d]**
Visit window (days)	**-7 to +14**	**-**	**± 3**	**-2 to + 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**+ 7**
Study site visit[a]	X	X				X		X			X	X
Follow-up phone call[b]			X	X	X		X		X	X		
Urine drug and alcohol test	X											
12-lead ECG[j]	X	X[e]		X		X	X	X			X	X
C-SSRS[k]	X	X				X		X			X	X
6MWT[l]		X				X		X			X	
5XSTS		X				X		X			X	
Blood for plasma pharmaco-kinetics[m]		X						X			X	
Serum and urinary biomarkers[n]		X						X			X	
Blood for pharmacodynamics (PPARδ target gene expression)[o]		X						X			X	
Blood sample collection for genotyping		X										
Blood sample collection for pharmaco-genomics (banking)		X										
Patient reported outcomes[p]		X				X		X			X	
Dispense/ Collect IP		X				X		X				
IP dosing		X	→	→	→	→	→	→	→	→		
Previous and concomitant treatment	X	X	X	X	X	X	X	X	X	X	X	X
Exercise status[q]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse event assessment	X	X	X	X	X	X	X	X	X	X	X	X

5XSTS: 5 time sit to stand; 6MWT: 6-minute walk test; ALDOA: aldolase A; CK: creatine kinase; C-SSRS: Columbia-Suicide Severity Rating Scale; CYC: cytochrome c; ECG: electrocardiogram; EOS: end of study; EOT_P2: end of treatment during double-blind treatment period; EQ-5D-5L: European Quality of Life 5-dimension, 5-level

questionnaire; FLNC: filamin-C; IP: investigational product; MB: isoenzymes found in heart muscle; MFIS: Modified Fatigue Impact Scale; MM: isoenzymes found in skeletal and heart muscle; 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; QTcF: QT interval using Fridericia's correction; WOCBP: women of childbearing potential

a. All assessments at the study site visit may be performed over 2 to 3 days. Assessments scheduled for each study site visit should be completed within visit window.

b. The site will perform follow-up phone call visits. Blood sample collection and ECG will be performed by qualified healthcare provider from the home healthcare vendor (ECG will be performed at weeks 2 and 8 only). In case the home healthcare visit is not feasible, the visit can be conducted at the study site.

c. IP administration should be started after the completion of assessments at week 0, except for postdose blood sampling for pharmacokinetics. The day participants start taking the IP is considered as day 1.

d. Participants who discontinue the study treatment will visit the site and perform EOT_P2 assessments. Participants will be encouraged to visit the study site for EOT_P2 assessments as soon as the discontinuation of the study treatment. Participants who discontinue the study will be followed until the EOS visit. Participants who remain in the study for efficacy and safety evaluations will continue to follow the schedule of assessments without IP dosing.

e. To be performed prior to randomization.

f. Vital signs will be taken prior to blood draw. Blood pressure and pulse will be measured in the sitting or supine position after the participant has rested for at least 5 minutes.

g. Safety laboratory will be done by a central laboratory from the screening to EOS. Blood collection for hematology and biochemistry; muscle biomarkers include CK, CK MM, CK-MB and cardiac troponins I and T. Urinary creatinine should be analyzed quantitatively like blood creatinine analysis that will be used for correcting urinary TITIN. Biochemistry and hematology samples will be taken prior to study assessment, except for ECG and vital signs. At follow-up phone call visits, abbreviated biochemistry (including cardiac troponin I) will be collected at the participant's home (abbreviated hematology will only be collected at week 2) 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.

h. Serum pregnancy tests will be performed for WOCBP.

i. Urine pregnancy tests will be performed for WOCBP.

j. ECGs should be performed prior to blood draw. ECGs will be recorded in triplicate (3 separate ECGs with 5-minute resting prior to first ECG and at least 1 minute apart per time point). In addition, ad hoc triplicate ECG may be required to follow up on an elevation of cTnI above the ULN (see Appendix 10.9 for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 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 QTcF > 450 msec in male participants and > 480 msec in female participants at screening or randomization, one additional triplicate ECG can be performed on the same day in order to determine the participant's eligibility. 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.

k. 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.

l. Following randomization, participants will be encouraged to perform all 6MWTs within a 2-hour window of the performance time recorded during visit 2.

Footnotes continued on next page

m. A blood sample will be collected predose at week 12 (-90 minutes to 0 minutes before dosing) and predose at week 24. A blood sample will be collected postdose at week 0 (Day 1) (0.5 to 4 hours after dosing) and postdose at week 12 (0.5 to 4 hours after dosing). For visits with postdose sample collections, site staff should document the type (high fat defined as ≥ 55 g fat, non-high fat defined as < 55 g fat or unknown) and completion time of the meal immediately prior to on-site dosing. Meal type and completion time are not collected at visits with only predose sample collection. A blood draw will be taken prior to study assessments, except for ECG and vital signs. The actual date and time of each blood sample collection and the actual date and time of IP administration on the site visit day and day before pharmacokinetics sampling visits will be collected.

n. Serum biomarkers include FLNC, CYC and ALDOA. Urinary biomarker includes urinary TITIN. These samples will be taken prior to study assessment, except for ECG and vital signs.

o. Blood samples for pharmacodynamics will be collected prior to dosing at weeks 0, 12 (-90 minutes to 0 minutes before dosing) and 24. A blood draw will be taken prior to study assessments, except for ECG and vital signs. Date and time of the last dose of IP before sampling (except for week 0) and the date and time of the dose of IP on day of sampling will be collected.

p. Neuro-QoL measures of fatigue score and mobility score, MFIS score, PGIS and EQ-5D-5L will be assessed at weeks 0, 4, 12 and 24 at the study site. PGIC will be assessed at weeks 4, 12 and 24 at the study site.

q. For the participants who are on exercise regimen, status of exercise regimen will be collected at each visit (on site or phone call).

1.3.1 Sample Collection Schedule

Table 2 Sample Collection Schedule

Week	Time Point	Plasma pharmacokinetics	Pharmacodynamics (PPARδ target gene expression)	Genotyping	PGx (banking)
0 (Day 1)	Predose		X	X	X
	Postdose (0.5 to 4 h)	X			
12	Predose (-90 min to 0 min)	X	X		
	Postdose (0.5 to 4 h)	X			
24	Predose	X	X		

h: hour; PGx: pharmacogenomics; PPARδ: peroxisome proliferator-activated receptor delta

2 INTRODUCTION

2.1 Study Rationale

Primary Mitochondrial Myopathies (PMMs) comprise a large heterogeneous group of disorders resulting from alterations in genes that affect mitochondrial function and lead to muscle disease. These diseases may be characterized by dysfunction in additional organ systems and extensive variability in clinical presentation. Currently, there is no approved treatment for mitochondrial myopathies.

In skeletal and cardiac muscle, mitochondrial dysfunction contributes to poor energy production, increased lactate, decreased muscle repair, and increased inflammation. Peroxisome proliferator-activated receptor delta (PPARδ) is a nuclear receptor that, when activated, induces a transcriptional program that increases a cell's capacity to transport and oxidize fatty acids, which can preserve glucose and decrease inflammation and fibrosis.

[redacted]

Nonclinical data indicate that ASP0367: a) increases mitochondrial fatty acid oxidation (FAO) in normal and mitochondrial disease patient-derived fibroblasts; b) increases muscle function/distance run-in X-linked mutation of the murine dystrophin gene (*mdx*) mutant mice; c) improves locomotor activity impairment during the dark cycle in mouse model of *mdx* and diet-induced obesity; d) prevents cardiac function decline in aged *mdx* mice; and e) decreases running fatigue in aged, diet-induced obese (DIO) mice. ASP0367 was well-tolerated by healthy adult participants at doses up to 75 mg once daily for 14 days with an acceptable safety profile. Based on the nonclinical pharmacology data, as well as safety and tolerability profile observed in both nonclinical and phase 1 studies in humans, ASP0367 warrants further evaluation in PMM participants.

2.2 Background

2.2.1 Mitochondria Diseases and Primary Mitochondrial Myopathy

Mitochondrial diseases are metabolic disorders mainly caused by pathogenic alterations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). The estimated prevalence of mitochondrial diseases is 1 in 4300 [Gorman et al, 2015]. Mitochondrial diseases affect multiple organ systems such as the nervous, muscular, cardiac and endocrine systems [Pfeffer & Chinnery, 2013]. Myopathy is not only one of the most prevalent manifestations but is also a very debilitating feature of these diseases because of weakness and exercise intolerance that impair mobility, as well as the capability of the patient to perform daily activities. In fact, a recent survey study performed on 2 independent cohorts of patients with PMM showed that among the symptoms experienced by patients, the principal symptoms motivating patients to participate in a clinical study were exercise intolerance, chronic fatigue and muscle weakness [Zolkipli-Cunningham et al, 2018].

Several primary mitochondrial syndromes such as chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), and myoclonic epilepsy with

ragged-red fibers (MERRF) are well described and involve muscles and manifest with myopathy [de Barcelos et al, 2019]. Common clinical manifestations other than myopathy include diabetes, sensorineural hearing loss, optic atrophy, peripheral neuropathy, cardiomyopathy, nephropathy, hepatopathy, stroke-like episodes, seizures and dementia [Mancuso et al, 2017].

2.2.2 Unmet Medical Need

There are currently no approved disease-modifying therapies available for patients with PMM. Current treatment options aim at improving symptoms and typically include combinations of vitamin and supplements (typically referred to as mito-cocktail) and specific therapies that may have particular benefit for some genetic etiologies and/or shared phenotypes (e.g., arginine for metabolic strokes, creatine for metabolic myopathy); however, there is no clinical evidence for effectiveness of these treatments in patients with PMM [Montano et al, 2021; Barcelos et al, 2020; Ahmed et al, 2018; Mancuso et al, 2017].

2.2.3 ASP0367 Development

Refer to the Investigator's Brochure for complete details of ASP0367 nonclinical and clinical studies conducted to date.

Modulating PPARδ in patients with PMM is hypothesized to both benefit muscle metabolism and protect muscles from cell death. Through the targeting of FAO genes, ASP0367 is expected to increase the ability of patient muscle to utilize fatty acids thereby protecting it from exercise intolerance and fatigue. In addition, muscle myofiber loss may be reduced through improved mitochondrial function and turnover, improving the state of cellular stress that persists in PMM. Finally, ASP0367 is expected to reduce inflammation through various mechanisms such as release of the anti-inflammatory corepressor B-cell lymphoma 6, inhibition of nuclear factor kappa B, extracellular receptor kinase 1/2 and mitogen-activated protein kinase and induction of anti-inflammatory and antioxidant genes [Bishop-Bailey & Bystrom, 2009].

2.2.3.1 Nonclinical

In nonclinical studies, ASP0367 increased mRNA expression of PPARδ target genes in muscle cells and activated FAO in mouse myoblast C2C12 cells and in patient-derived fibroblasts with mitochondrial mutation including Leber hereditary optic neuropathy and Leigh syndrome, MELAS, KSS, and MERRF (additional details in the Investigator's Brochure).

ASP0367 was further tested in the DIO mouse model. Aged DIO mice were shown to present with reduced mitochondrial FAO and mitochondrial dysfunction in muscle and decreased exercise tolerance compared to wild type mice. DIO mice treated with ASP0367 had decreased exercise induced fatigue compared to vehicle-treated animals.

Repeat-dose, Good Laboratory Practice (GLP), toxicity studies have been conducted up to 11 weeks in juvenile rats, up to 26 weeks in adult Wistar Han rats (see 26-week adult rat study [doses up to 50 mg/kg per day] in the Investigator's Brochure) and up to 52 weeks

(doses up to 500 mg/kg per day) in cynomolgus monkeys. In adult rats treated with ASP0367 for 26 weeks, no tumors were observed. However, the incidence of hyperplasia of the limiting ridge of the non-glandular stomach and the incidence of hypertrophy/hyperplasia of the follicular cells in the thyroid gland were observed and the incidence increased in a dose-dependent manner. In the long-term toxicology study in monkeys treated with ASP0367 for 52 weeks, no tumors or proliferative lesions were observed. Key toxicity findings with associated monitoring plans are summarized in [Section 2.3 Risk/Benefit Assessment].

In the 2-year carcinogenicity study in rats, incidence of benign vaginal polyps and benign endometrial stromal polyps in the uterus were increased in females at 50 and ≥ 25 mg/kg per day, respectively. The incidence of benign vaginal polyps was not increased at 25 mg/kg per day. The increased incidence of benign endometrial stromal polyps at 25 mg/kg per day was significantly different compared with water control in 5 statistical tests but was not significantly different compared to vehicle control group in 4 of 5 statistical tests. The incidence of benign endometrial stromal polyps in the water control was below historical background control values. The incidence of benign endometrial stromal polyps at 25 mg/kg per day was within the range of historical background control values. Endometrial adenocarcinoma was observed in both control groups and all dose groups, however, there was no statistically significant difference between the controls and dose groups. Because the incidence of benign vaginal polyps at 25 mg/kg per day was not increased, and the incidence of benign endometrial stromal polyps at 25 mg/kg per day was within the range of historical background control values, the conclusion of this study is that this is the dose level at which no increased incidence of polyps occurred. The AUC_{24} at 25 mg/kg per day in rats was 16-fold higher than the human AUC_{24} at 75 mg/day. For these reasons, the risk of human carcinogenicity is considered low at human doses up to 75 mg/day.

ASP0367 showed no potential to induce genotoxicity or phototoxicity.

With respect to cardiovascular function, ASP0367 caused a concentration-related inhibition of hERG channel-mediated potassium current in vitro in protein-free buffer, albeit with an IC_{50} of 44.4 µM (20400 ng/mL), which is 137 times the steady-state C_{max} in human participants given ASP0367 at 75 mg/day in Study 0367-CL-0001.

Daily oral administration of ASP0367 to rats did not affect fertility in either sex. In addition, no findings were reported indicative of toxicity to male or female reproductive organs in repeat-dose toxicity studies in rats or monkeys.

In studies in pregnant rats and rabbits dosed during the period of major organogenesis, ASP0367 was not teratogenic but did affect fetal growth and/or survival at higher dose levels.

In a study of prenatal and postnatal development, including maternal function in rats, reduction in body weight gain/growth rate and lower survival were observed in F1 pups during the lactation period, which resolved after weaning. A few pups that died or were euthanized during the lactation period had bilateral lens opacity or a small caudate process in the liver. There were no detectable long-term effects, in particular on nervous or reproductive

system development, in F1 pups at any of the dose levels examined because ASP0367-related effects on F1 pups resolved when ASP0367 exposure ended at weaning.

No human-specific ASP0367 metabolites were formed in vitro by hepatocytes. Unchanged ASP0367 and 5 metabolites (including oxidative metabolites and acyl glucuronide conjugates of ASP0367) were detected in human plasma samples collected after repeated oral administration of ASP0367 to healthy adult participants. The major metabolites of ASP0367 in human clinical plasma samples are glucuronides. Based on in vitro studies, multiple cytochrome P450 (CYP) and uridine diphosphate-glucuronosyltransferase (UGT) isoforms were suggested to be involved in the metabolism of ASP0367. CYP3A is the primary CYP-mediated metabolism pathway.

Based on results from in vitro studies, the risk of drug-drug interactions (DDI) with CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 substrates is considered to be low. ASP0367 showed a time-dependent inhibition on CYP3A, but not on the other CYP isozymes examined. The anticipated concentrations with the ASP0367 doses in this study relative to the in vitro CYP3A inhibitory concentrations are low. Therefore, the risk of a DDI with concomitant administration of CYP3A substrates is considered to be low.

In vitro, ASP0367 exhibited inhibitory effects on the transport of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporting polypeptide (OATP)1B1, OATP1B3, organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2 and multidrug and toxin extrusion (MATE)1 substrates; MATE2-K transport was not inhibited. Due to the anticipated concentrations in this study relative to the in vitro inhibitory concentrations, the likelihood of DDI with OAT1, OCT1, OCT2, MATE1 and MATE2-K is considered to be low.

2.2.3.2 Clinical

ASP0367 has been assessed in 4 completed phase 1 studies including first-in-human single ascending dose (SAD) and multiple ascending dose (MAD) (0367-CL-0001), absorption, metabolism and excretion (AME) (0367-CL-1103), hepatic impairment (0367-CL-1102) and renal impairment (0367-CL-1111) studies. Details and results from these studies are summarized in the Investigator's Brochure. Overall, a total of 124 participants were exposed to ASP0367 in the completed phase 1 studies and ASP0367 was well-tolerated in all participants. No deaths or SAEs or TEAEs leading to withdrawal of treatment were reported across the completed phase 1 studies.

ASP0367 has also been assessed in 1 completed phase 1b clinical study (0367-CL-0102) in late ambulatory and early non-ambulant participants with Duchenne Muscular Dystrophy (DMD). This phase 1b study included a 24-week double-blind (DB) part and a 12-week open-label extension (OLE) part. A total of 8 participants were exposed to ASP0367 in Study 0367-CL-0102. No deaths, SAEs or TEAEs leading to withdrawal of treatment were reported.

Relevant clinical findings with associated monitoring plans are summarized in [Section 2.3 Risk/Benefit Assessment].

ASP0367 showed roughly dose-proportional and time-invariant pharmacokinetics. After oral administration under fasted condition, ASP0367 was rapidly absorbed with median t_{max} reached by 2 hours postdose and eliminated with mean terminal elimination half-life of 14.1 to 17.5 hours. Steady-state ASP0367 concentrations were achieved after approximately 4 days of administration with negligible accumulation, following once-daily dosing. The exposure (AUC_{inf}) of ASP0367 was not affected by high-fat meal. C_{max} of ASP0367 was decreased by food consumption, as the C_{max} geometric least squares mean fed/fasted ratio was 73.2%, with delayed t_{max} of approximately 1.7 hours compared to that observed when administered in the fasted state.

In the AME study in healthy participants, the majority of the [^{14}C]-radioactivity was recovered in feces (78.7%), with 13.8% recovered in urine. Only 0.0170% of the [^{14}C]-radioactivity was excreted in urine as parent ASP0367, suggesting that the majority of urinary excretion was metabolites. ASP0367 and metabolites, AS3566736 (glucuronide metabolite) and AS3566737 (amidine metabolite), accounted for 64.4% of the total [^{14}C]-radioactivity in plasma.

In participants with mild or moderate hepatic impairment, mild impairment increased C_{max} and moderate impairment increased AUC_{last}, AUC_{inf} and C_{max} of ASP0367 and its metabolite AS3566736, compared to the respective normal to match groups.

In participants with severe renal impairment, severe impairment did not substantially affect the C_{max} and AUC_{inf} of ASP0367 or its metabolite AS3566736 (glucuronide metabolite); AS3566737 (amidine metabolite) AUC_{inf} increased by approximately double in participants with severe renal impairment compared to matched healthy participants with normal renal function.

Gene expression analysis in blood samples from healthy volunteers showed a similar pattern in PPARδ target gene upregulation as was observed in DIO mice and cells that harbor mutations in mtDNA. ASP0367, at doses of 10 mg or higher, showed consistent treatment- and dose-dependent up-regulation of the FAO-related PPARδ target genes (ABCA1, ACAA2, ACADVL, CPT1a, PDK4 and SLC25A20). The gene up-regulation was rapid after the administration of a single dose of ASP0367 and appeared to persist throughout the 24-hour sampling period after the administration of multiple doses of ASP0367. Based on visual inspection of the results, the target gene up-regulation by ASP0367 on day 1 at doses of 10 mg or higher showed consistent, treatment- and dose-dependent up-regulation and was suggestive of saturated dose-responses at higher dose levels and no major increments from 60 mg or 75 mg to 120 mg. In addition, multiple doses of 10 mg ASP0367 or higher (40 mg and 75 mg) appeared to have a dose-related effect on PPARδ target genes.

The current study will be a randomized double-blind, placebo-controlled phase 2 study to evaluate the safety, tolerability, pharmacokinetics and efficacy of ASP0367 in participants with PMM 18 to < 65 years old.

2.3 Risk/Benefit Assessment

2.3.1 Risk Assessment

The safety profile of ASP0367 is based on the results of nonclinical studies and 2 clinical studies in healthy adult participants and participants with mild or moderate hepatic impairment. Please refer to the current Investigator's Brochure for the most recent safety data.

2.3.1.1 Nonclinical

The toxicity profile of ASP0367 was similar in juvenile and adult rats, with effects targeting the heart; specifically, an increase in the incidence and/or the average grade of myocardial inflammation/necrosis in males, sometimes accompanied by an increased serum cardiac troponin I (cTnI) concentration. Myocardial inflammation/necrosis is a common, age-related background finding in male rats. The greater incidence and/or severity produced by administration of ASP0367 was not observed after dosing was stopped, suggesting the reversibility of these findings.

In juvenile rats, there were no ASP0367-related effects on the development of organ systems that undergo postnatal development, such as the nervous, skeletal and reproductive systems. No microscopic kidney findings similar to those seen in the euthanized cynomolgus monkey (in the 13-week study) were observed in any animals (rats or monkeys) up to 13 weeks of daily dosing, and renal function safety markers were normal in all other animals.

Nonclinical studies have identified the target organs of toxicity as the heart and skeletal muscle. In this phase 2 study in participants with PMM, cardiac (including cTnI, cardiac troponin T [cTnT] and creatine kinase [CK] isoenzymes found in heart muscle [CK-MB]) and skeletal muscle (including total CK and CK isoenzymes found in skeletal and heart muscle [CK-MM]) biomarkers indicative of damage will be measured frequently.

The risk of carcinogenicity has not yet been fully evaluated. Histopathological examination from the carcinogenicity study in rats has been completed and another carcinogenicity study in mice is planned. During the longer-term toxicology studies in adult Wistar Han rats (up to 26-weeks and doses up to 50 mg/kg per day) and in cynomolgus monkeys (up to 52-weeks and doses up to 500 mg/kg per day) no development of tumors was observed. However, in the 26-week rat study a dose-dependent increase in the incidence of hyperplasia of the limiting ridge of the non-glandular stomach and the incidence of hypertrophy/hyperplasia of the follicular cells in the thyroid gland were observed. In the monkey toxicology study, no proliferative lesions were observed during the course of this 52-week study.

In the 2-year carcinogenicity study, Wistar Han (RccHan®: WIST) rats were treated with ASP0367 at dose levels of 0 (water), 0 (vehicle), 10, 25 and 50 mg/kg/day for 105 weeks. The histopathological data from this 2-year study comprised neoplastic observations, revealing statistically significant increases in benign vaginal polyps and benign endometrial stromal polyps, and statistically non-significant occurrences of endometrial adenocarcinoma.

- Benign vaginal polyps were observed in the water control and 50 mg/kg/day groups. The increased incidence of benign vaginal polyps in the 50 mg/kg/day group was statistically significant compared to the water and vehicle control groups.
- Benign endometrial stromal polyps were observed at all dose levels, including the water and vehicle controls. The increased incidence of benign endometrial stromal polyps in the 25 and 50 mg/kg/day groups differed in statistical significance depending on the analysis. Of note, the overall rate of benign endometrial stromal polyps was within the range of historical control groups at the study sites for the 25 mg/kg/day group and slightly higher than historical control groups for the 50 mg/kg/day group.
- Endometrial adenocarcinomas were observed at all dose levels, including water and vehicle controls. There were no statistically significant differences between ASP0367 dose groups and the water or vehicle control groups.
- No neoplastic findings were observed in the stomach or thyroid gland.

There have been no reports of tumors in the female reproductive tract in the clinical study program to date. The safety margins for exposure at the dose levels administered to humans up to 75 mg/day compared to the exposure at the doses administered to rats are considered to be adequate in the ongoing Study 0367-CL-1201. Given the risk of carcinogenicity associated with the use of PPAR agonists and the neoplastic observations from the draft report for the 2-year carcinogenicity rat study, administration of ASP0367 is currently limited to no longer than 6 months.

In nonclinical safety pharmacology studies, ASP0367 revealed no effects on central nervous system or respiratory system function in rats at doses up to 300 mg/kg. Although ASP0367 inhibited the hERG channel current in hERG transfected HEK293 cells, no QT prolongation or other effect on the cardiovascular system was discernable in cynomolgus monkeys at doses up to 1000 mg/kg. There was no evidence of genotoxic potential with ASP0367 based on the findings from a standard battery of genotoxicity assays. ASP0367 showed no phototoxic potential in an in vitro phototoxicity study in cultured Balb/c 3T3 cells.

ASP0367 is considered unlikely to affect fertility in humans as daily oral administration of ASP0367 to rats did not affect fertility in either sex. Studies in pregnant rats and rabbits dosed during the period of major organogenesis suggest that ASP0367 is not teratogenic but may have the potential to affect fetal growth and/or survival in humans. Any potential embryofetal risks will be mitigated by enrolling participants under instruction of intensive control of pregnancy risk over the study duration.

2.3.1.2 Clinical

ASP0367 has been assessed in 4 completed phase 1 clinical studies (0367-CL-0001, 0367-CL-1102, 0367-CL-1103 and 0367-CL-1111). ASP0367 was well tolerated in the healthy adult participants and participants with mild or moderate hepatic and severe renal impairment. There were no deaths and no serious adverse events (SAEs) reported. Also, there were no discontinuations due to treatment-emergent adverse events (TEAEs). All adverse events (AEs) were transient and mild or moderate in severity. No drug-related cardiac or skeletal muscle AEs were reported.

In the first-in-human Study 0367-CL-0001, one participant at the single dose of 60 mg had cTnI values above the upper limit of normal (ULN; 0.056 ng/ml and 0.047 ng/mL above the ULN of 0.045 ng/mL) at the end of study visit (8 days after a single dose) and repeat assessment poststudy on day 21, respectively, with no clinical signs associated and deemed not clinically relevant. There was no test article-related elevation in serum cTnT; CK-MB stayed within the normal range, and no participants were observed with ischemic changes or prolonged QT. Amylase and lipase elevations above the laboratory reference range were observed for a number of participants following administration of ASP0367 or placebo. There were no trends for treatment or dose, or day of onset-related trends in the frequency or magnitude of these elevations. The participants with amylase or lipase elevations remained asymptomatic and the elevations resolved without treatment. None of the amylase or lipase elevations were reported as TEAEs. There were no other clinically significant findings in laboratory tests (specifically in skeletal and other cardiac muscle markers [cTnT, CK-MB]), vital signs or electrocardiograms (ECGs; changes related to ischemia or QT interval changes).

In Studies 0367-CL-1102 (mild and moderate hepatic impaired participants and healthy matched participants) and 0367-CL-1111 (severe renal impaired participants and healthy matched participants), the incidence of TEAEs reported during study conduct was low. In each study, 2 TEAEs were reported that were considered by the investigator to be mild and related to study intervention. In the hepatic impairment study, 1 participant with mild hepatic impairment experienced nausea and 1 participant with mild hepatic impairment experienced fatigue. In the renal impairment study, 1 participant with severe renal impairment had a TEAE of troponin I increased (single episode with onset on day 2; value of 0.09 ng/mL [normal range: not reported to 0.015 ng/mL]) and 1 participant with severe renal impairment had a TEAE of lipase increased (values above ULN of 92 U/L on day 5, 142 U/L at EOS visit and 85 U/L during a follow-up unscheduled visit [normal range: 11 to 82 U/L]). None of the participants in either study required concomitant medications for the treatment of these TEAEs. There were no other clinical laboratory (hematology, biochemistry and urinalysis) results, vital signs measurements or routine 12-lead ECG measurements reported as TEAEs during the conduct of these studies. None of the participants had potentially clinically significant values or increases in liver enzymes or TBL after study intervention administration.

In the AME study 0367-CL-1103, 1 participant experienced a TEAE (diarrhea) that was considered by the investigator to be possibly related to study intervention. There were no clinical laboratory (hematology, biochemistry and urinalysis) results, vital sign measurements or routine 12-lead ECG measurements reported as TEAEs during the conduct of this study. No participant had potentially clinically significant increases in liver enzymes or TBL after study intervention administration.

ASP0367 has also been assessed in 1 completed phase 1b clinical study (0367-CL-0102) in late ambulatory and early non-ambulant patients with DMD. No SAEs, drug-related TEAEs, TEAEs leading to death or TEAEs leading to withdrawal of treatment were observed in the

12-week DB or OLE parts of the study. Similar TEAE frequencies were reported across the ASP0367 and placebo treatment groups in the 12-week DB part. These events were consistent with the underlying disease of DMD. Overall, the mean changes in clinical laboratory values and vital signs were similar between treatment groups. No significant safety issues were observed in subjects who received ASP0367.

2.3.1.3 Cardiac and Skeletal Muscle

Based on the nonclinical findings observed with ASP0367, toxicity of the heart and skeletal muscle have been identified as important potential risks, although they have been observed only in rats but not in monkeys.

Baseline cTnI values in patients with PMM are not well established. Since mitochondrial oxidative phosphorylation is the main source of energy in the heart, responsible over 95% of adenosine triphosphate synthesis [Zdończyk et al, 2020], cardiac involvement in mitochondrial diseases is common with an estimated prevalence of more than 20% in adult mitochondrial disease patients [Limongelli et al, 2010]. A single cTnI level at a fixed time point in patients with PMM is insufficient to determine clinical significance, particularly with biological variability (diurnal variation) and bioanalytic (assay variation) variability.

In addition to routine standard monitoring of safety in the proposed study (0367-CL-1201), the sponsor proposes the following screening activities and repeated sequential monitoring to address the cardiac and skeletal muscle toxicity monitoring:

All participants will undergo screening including cardiac biomarkers and ECG prior to enrollment in the study. This study appropriately excludes PMM patients with clinically significant and unstable cardiovascular disease as described in Exclusion Criteria #12 through 14. As such, the investigator will clinically correlate the cTnI level to assess eligibility. Only participants with a cTnI level below upper limit and a mean QT interval using Fridericia's correction (QTcF) of $\leq$ 450 msec for male participants and $\leq$ 480 msec for female participants will be allowed into the study.

Sequential cardiac muscle monitoring will include assessment of serum cardiac enzymes, including cTnI, cTnT, CK-MB and ECGs at protocol-specified intervals. Participants who experience any signs or symptoms potentially reflecting cardiac involvement will undergo ECG, echocardiogram, and have a cTnI drawn for further evaluation prior to continuing study drug. Participants who develop an increase in cardiac enzymes levels (i.e., increased cTnI) will interrupt investigational product (IP) and will undergo more intensive assessments of cardiac function such as ECG and echocardiogram for further evaluation.

Sequential monitoring for skeletal muscle injury will include assessment of CK, transaminases and lactate dehydrogenase at protocol-specific intervals. An individual participant may be required to discontinue treatment if they develop an elevation in CK levels $\geq$ 5 times the value of the ULN or the individual's baseline value, whichever is higher and it is judged that this elevation is not related to an explainable increase including excessive movement, fall or disease state.

2.3.2 Benefit Assessment

At randomization, participants have a 2/3 chance of being randomized to active IP (ASP0367). Participants randomized to placebo are unlikely to benefit from the double-blind treatment period of the study.

Additionally, enrollment in the study may be seen as an opportunity to contribute to the discovery of an effective drug for this highly unmet medical need.

2.3.3 Overall Risk-Benefit Conclusion

ASP0367 has the potential to address fatigue, exercise intolerance and muscle weakness and improve quality of life in participants with PMM. With appropriate precautions (e.g., cardiac monitoring), the potential risks mentioned above do not preclude initiation of a clinical study in PMM patients. This phase 2 study in participants with PMM will continue to evaluate the safety profile of ASP0367 and apply careful risk characterization and minimization activities.

The sponsor considers the overall benefit-risk to participants with PMM taking part in Study 0367-CL-1201 to be favorable.

3 OBJECTIVES, ENDPOINTS AND ESTIMANDS

The study objectives and endpoints are outlined in [Table 3].

Table 3 Study Objectives and Endpoints

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: ○ Nature, frequency and severity of TEAEs ○ 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

Objectives	Endpoints
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.

[redacted]

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 6-minute walk test (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.

4 STUDY DESIGN AND DOSE RATIONALE

4.1 Overall 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. Since participants enrolled in the study prior to implementation of Protocol Version 9.0 (Substantial Amendment 8), which removes the 52-week OLE period, may have a treatment duration of up to 76 weeks, a separate treatment analysis will also be conducted at later timepoints up to 76 weeks as per available data [Section 9]. An unblinded, independent Data and Safety

Monitoring Board (DSMB) will assess the overall safety and tolerability of ASP0367 throughout the course of the study as summarized in [Section 10.1.5.1.1].

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.

At week 24 while enrollment continues, data from the first approximately 30 evaluable participants will be reviewed in an interim analysis, as described in [Section 9.6], to determine if the futility or efficacy stopping criteria are met while the study is ongoing. An Astellas internal DMC for interim analysis will assess the futility and efficacy of the study treatments and make recommendations about the ongoing conduct of the study as summarized in [Section 10.1.5.1.2]. An interim analysis of pharmacokinetic and pharmacodynamic data will also be reviewed by the DMC.

4.2 Scientific Rationale for Study Design

A randomized, placebo-controlled, parallel group design was chosen to detect changes in functional capacity and assess the safety/tolerability in PMM patients. Participants will be randomized to treatment to reduce selection bias, and further, both participants and the investigator will be blinded to treatment to reduce bias in the measurement of both safety and efficacy endpoints. Placebo was chosen as a comparator because there are no approved treatments for PMM.

The double-blind treatment period of the study will assess the dose response of ASP0367 on functional improvement relative to placebo to confirm that the appropriate dose level within the biologically active dose window is being selected with the new ASP0367 tablet formulation [Section 4.3] and assess the safety and tolerability of ASP0367 relative to placebo. A 6MWT will be employed as the primary efficacy endpoint, since exercise intolerance was one of the most key symptoms motivating PMM patients to participate in a clinical trial [Zolkipli-Cunningham et al, 2018] and the 6MWT is a sub-maximal exercise test used to assess functional capacity by measuring distance walked in six minutes. With the support of available nonclinical toxicity studies up to 52 weeks, 24 weeks randomized study duration for 6MWT has been chosen in considering disease progress.

As this current study is focusing on mitochondrial myopathies in which mitochondria are primarily involved, participants who demonstrate molecular genetic abnormality known to be associated with mitochondrial dysfunction will be enrolled in the study. Further, participant-reported symptoms (i.e., muscle weakness, fatigue and exercise intolerance) or physical examination findings of myopathy that are the predominant symptoms of the participant's mitochondrial disorder are required for the enrollment.

4.2.1 Summary of Changes to Study Design

The present study was originally designed as a randomized, double-blind, placebo-controlled, oral dose, adaptive phase 2/3 study with open-label extension (OLE) to evaluate the efficacy,

safety and tolerability of ASP0367 in participants with PMM. The original study design consisted of the following portions: screening (4 weeks); phase 2 dose selection portion with 2 doses of ASP0367 vs matching placebo (2 weeks); phase 3 portion with selected, single dose treatment vs placebo (up to 52 weeks); OLE (24 weeks); and follow-up (4 weeks).

Effective with implementation of Protocol Version 9.0 (Substantial Amendment 8), the original study design will be modified to become a phase 2 study (originally referred to as the "Phase 2 Portion") with a double-blind treatment period (24 weeks) and no OLE period. An interim analysis of approximately 30 evaluable participants who have reached week 24 of the double-blind treatment period will be conducted to evaluate futility and efficacy.

4.3 Dose Rationale

In Study 0367-CL-0001, ASP0367 in a capsule formulation was used. In the current study (0367-CL-1201), tablet formulation, which showed no appreciable difference of in vitro dissolution profile compared with the capsule formulation, will be used.

ASP0367 was safe and well tolerated at a single dose of up to 120 mg and multiple doses of up to 75 mg for 14 days in healthy adults. No dose-liming toxicity was found, and maximum tolerated dose was not determined. ASP0367 showed treatment- and dose-dependent FAO-related PPARδ target gene up-regulation in blood in healthy adults [Section 2.2.3.2 Clinical]. The safety profile of ASP0367 was further supported by the data from the GLP toxicity studies (13-week [both rats and monkey], 26-week [rat] and 52-week [monkey] repeated oral dose toxicity studies, and 11-week juvenile rat toxicity study), which showed higher no observed adverse effect levels (NOAELs) and associated systemic exposures than those seen in the 4-week repeated oral dose rat toxicity study [Section 2.2.3.1 Nonclinical]. Of note, in the GLP 26-week rat toxicity study, the NOAEL was considered to be 50 mg/kg per day, the highest dose level tested (equivalent to C_{max} values of 15500 and 30400 ng/mL and AUC_{24} values of 41200 and 111000 ng·h/mL for males and females, respectively, on day 182). In addition, in the GLP 52-week monkey toxicity study, the NOAEL was considered to be 500 mg/kg per day, the highest dose level tested (equivalent to C_{max} values of 1480 and 523 ng/mL and AUC_{24} values of 8400 and 4090 ng·h/mL for males and females, respectively, on day 365). More details can be found in the Investigator's Brochure.

The planned doses of 30 and 75 mg once daily are expected to be safe and biologically active and chosen to support evaluation of a dose response. These doses were therefore included in this study. The study design was modified (Protocol Version 9.0 Substantial Amendment 8) to assess the dose response after 24 weeks of treatment and to remove the OLE period. In addition, 2 clinical studies that recently completed clinical conduct assessed the 75 mg once daily dose for up to 20 weeks in patients with Duchenne Muscular Dystrophy (0367-CL-0102) and up to 6 weeks in patients with reduced maximum oxygen uptake due to poor systemic oxygen extraction (0367-CL-1101). In the current study, overall safety and tolerability of ASP0367 will continue to be assessed by the independent DSMB for the duration of the study.

4.4 End of Study Definition

The end of the study is defined as the last visit or scheduled procedure shown in schedule of assessments [Table 1] for the last participant in the study.

5 STUDY POPULATION

Male and female participants with PMM diagnosed by mtDNA or nDNA alterations, which are known to be associated with mitochondrial dysfunction, and by reported symptoms including muscle weakness, fatigue and exercise intolerance who are meeting following inclusion and exclusion criteria will be enrolled in the study.

Molecular genetic abnormality, which is detected by either targeted gene sequencing for the commonly affected genes in both mtDNA or nDNA that cause the phenotype or by mtDNA, exome or genome sequencing in clinically relevant or affected tissue, will be confirmed in participant's medical history.

All screening assessments must be completed and reviewed to confirm the potential participant meets all eligibility criteria. Prospective approval of protocol deviations to eligibility criteria (also known as protocol waivers or exemptions) is not permitted.

5.1 Inclusion Criteria

Participant is eligible for participation in the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization for US study sites) must be obtained from the participant prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Participant agrees and is able to adhere to the study requirements for the length of the study, including performing 6MWT.
3. Participant is ≥ 18 and < 65 years [Karaa et al, 2020] of age at the time of signing an informed consent form (ICF).
4. Diagnosed with PMM in the opinion of the investigator, consisting of the following:
    a. Molecular genetic abnormality (i.e., nuclear or mitochondrial) known to be associated with mitochondrial dysfunction (such as, but not limited to, mtDNA single deletions in CPEO and KSS; mtDNA m.3243 A > G; pathogenic nuclear or mitochondrial genome variants demonstrated to cause primary mitochondrial disease), and
    b. Participant reported symptoms (i.e., muscle weakness, fatigue and exercise intolerance) or physical examination findings of myopathy that are the predominant symptoms of the participant's mitochondrial disorder.
5. Participant has been on stable dose regimen of coenzyme Q10 (CoQ10), carnitine, creatine or other mitochondrial disease-focused vitamins or supplemental therapies for the treatment of symptoms of the mitochondrial disease for at least 3 months prior to randomization and intends to stay on a stable dose for duration of study period.

6. Participant has been on stable exercise regimen within 4 weeks prior to randomization and intends to stay on a stable regimen for duration of study period (for participants who participate in a regular exercise regimen).
7. Female participant is not pregnant (see Section 10.2, Appendix 2: Contraception Requirements) and at least one of the following conditions apply:
    a. Not a woman of childbearing potential (WOCBP) (see Section 10.2, Appendix 2: Contraception Requirements)
    b. WOCBP who agree to follow the contraceptive guidance (see Section 10.2, Appendix 2: Contraception Requirements) from the time of informed consent through at least 30 days after final study treatment administration.
8. Female participant must agree not to breastfeed starting at screening and throughout the study period and for 30 days after final study treatment administration.
9. Female participant must not donate ova starting at first dose of IP and throughout the study period and for 30 days after final study treatment administration.
10. Male participant with female partner(s) of childbearing potential (including breastfeeding partner) must agree to use contraception (see Section 10.2, Appendix 2: Contraception Requirements) throughout the treatment period and for 30 days after final study treatment administration.
11. Male participant must not donate sperm during the treatment period and for 30 days after final study treatment administration.
12. Male participant with pregnant partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy throughout the study period and for 30 days after final study treatment administration.
13. Participant agrees not to participate in another interventional study while participating in the present study.

5.2 Exclusion Criteria

Participant will be excluded from participation in the study if any of the following apply:

1. Participant has additional signs and/or symptoms due to non-myopathic process (e.g., cerebellar dysfunctions, movement disorder, peripheral neuropathy, stroke or other) or a gait problem not attributed to the myopathy that would interfere with the participant's performance during 6MWT or 5 times sit to stand (5XSTS), in the opinion of the investigator.
2. Participant has received any investigational therapy within 28 days or 5 half-lives, whichever is longer, prior to screening.
3. Participant has any condition, which, in the investigator's opinion, makes the participant unsuitable for study participation.
4. Participant has cTnI > ULN at screening.
5. Participant has estimated glomerular filtration rate (eGFR) calculated by the Chronic Kidney Disease Epidemiology Collaboration equation < 60 mL/min/1.73 m^2 at screening or a history of chronic kidney disease stage 3 or greater.*
6. Participant has at screening**: total bilirubin (TBL) > ULN or transaminase(s) (aspartate aminotransferase [AST] or alanine aminotransferase [ALT]) > ULN in the absence of

elevations in CK. Participants who have a slightly elevated TBL and/or ALT and/or AST and are suitable candidates for the study may be enrolled after discussion of the case with the medical monitor and completion of further evaluation as warranted.

7. Participant has psychiatric conditions such as schizophrenia, bipolar disorder or major depressive disorder that has not been under control within 3 months prior to screening.
8. Participant has a history of suicide attempt, suicidal behavior or has any suicidal ideation within 1 year prior to screening that meets criteria at a level of 4 or 5 by using the Columbia-Suicide Severity Rating Scale (C-SSRS) or who is at significant risk to commit suicide, as assessed by the investigator at screening.
9. Participant has severe behavioral or cognitive problems that preclude participation in the study, in the opinion of the investigator.
10. Participant has undergone an in-patient hospitalization that precludes participation in the study, in the opinion of the investigator, within the 30 days prior to the randomization.
11. Participant has a planned hospitalization or a surgical procedure during the study, which may affect the study assessments in the opinion of the investigator.
12. Participant has clinically significant and unstable respiratory disease and/or cardiac disease (medical history or current clinical findings) in the opinion of the investigator, or prior interventional cardiac procedure (e.g., cardiac catheterization, angioplasty/ percutaneous coronary intervention, balloon valvuloplasty, etc.) within 3 months prior to randomization. Participants with pacemakers are allowed in the study per investigator's discretion and after discussion with the medical monitor, and as long as it is used for prevention and there is no underlying cardiac dysfunction.
13. Participant has a corrected mean QTcF > 450 msec for male participants and > 480 msec for female participants at screening or randomization. If QTcF exceeds these limits, 1 additional triplicate ECG can be repeated on the same day in order to determine the participant's eligibility.
14. ECG evidence of acute ischemia, atrial fibrillation or active conduction system abnormalities. The following conduction system abnormalities may be permitted per the investigator's discretion, only after discussing the case with the medical monitor:
    a. First degree atrioventricular (AV)-block
    b. Second degree AV-block Type 1 (Mobitz Type 1/Wenckebach type)
    c. Right bundle branch block
    d. Left fascicular block
    e. Bi-fascicular block
15. Participant requires any ventilatory support, inclusive of any respiratory device to support breathing such as home ventilators and any form of non-invasive positive pressure ventilation (including continuous positive airway pressure [CPAP], bilevel positive airway pressure [BiPAP], and average volume-assured pressure support [AVAPS]). Participants who require oxygen therapy (even by low-flow nasal cannula [LFNC]) are not candidates for this study.
16. Participant has severe vision impairment that, in the opinion of the investigator, may interfere with their ability to complete all study requirements.
17. Participant has an intractable seizure disorder that, in the opinion of the investigator, may interfere with their ability to complete all study requirements.

18. Active malignancy or any other cancer from which the participant has been disease-free for < 5 years, except for curative treated localized non-melanoma skin cancer (e.g., basal cell or squamous cell carcinoma).
19. Participant has a solid organ transplant and/or is currently receiving treatment with therapy for immunosuppression.
20. Participant has severe scoliosis or kyphoscoliosis that significantly impair respiratory capacity and pulmonary function tests or limit positioning due to pain who would be likely to require orthopedic surgical intervention within a year after study randomization.
21. Participant has a positive test for human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection at screening.
22. Participant has previously received ASP0367.
23. Participant has, in the opinion of the investigator, a history of active substance abuse within 1 year prior to randomization.
24. Participant has used any peroxisome proliferator-activated receptor (PPAR) ligands such as fibrates and thiazolidinediones within 4 weeks prior to randomization.
25. Participant has initiated the use of CoQ10, carnitine, creatine or other mitochondrial disease-focused supplements for the treatment of symptoms of the mitochondrial disease within 3 months prior to study randomization.
26. Participant has a known or suspected hypersensitivity to ASP0367 or any components of the formulation used.
27. Participant has walked longer than the distance that 85% of healthy people can walk in 6MWT at the baseline visit (week 0) [see Section 7.1.1, 6-minute Walk Test].
28. Participant has symptomatic COVID-19 infection within 3 months prior to study randomization that required treatment (Monoclonal antibodies, ventilator support, hospitalization) and/or led to long-term sequelae or lingering symptoms.
29. Participant has body mass index (BMI) below 17 kg/m^2 [Karaa et al, 2020] or above 35 kg/m^2 at screening.
30. Participant has signs or symptoms of bulbar weakness, such as dysphagia, dysphonia, hoarseness or drooling/sialorrhea, due to either neuropathy or myopathy.

*https://www.kidney.org/atoz/content/stages-chronic-kidney-disease-ckd

**Note: Laboratory parameters of eGFR, TBL, AST and ALT may be repeated once within the screening window at the investigator's discretion after consulting with the medical monitor. If increased AST and/or ALT values are observed, association with increased CK values will be evaluated by the investigator and medical monitor.

5.3 Lifestyle Considerations

For the participants who are on exercise regimen, participants will be encouraged to keep a stable exercise regimen within 4 weeks prior to randomization and during the study. For participants enrolled in the study, participants will be asked to refrain from strenuous exercise on the day preceding and on the day of the on-site visit when 6MWT and 5XSTS will be performed (weeks 0, 4, 12 and 24).

5.4 Screen Failures

A screen failure is defined as a potential participant who signed the ICF but did not meet 1 or more criteria required for participation in the study and was not randomized.

For screen failures, the demographic data, date of signing the ICF, inclusion and exclusion criteria, AEs up to the time of screen failure and reason for screen failure will be collected in the case report form (CRF).

5.4.1 Rescreening

Rescreening after screen failure is allowed one time when there is a clinically relevant justification. If the participant meets exclusion criteria that cannot resolve during the screening period, the participant must be documented as a screen failure. In order to rescreen after prior screen failure, a new ICF must be signed and the participant entered into screening with a new participant identification number. Rescreening is only allowed once for an individual participant after consulting with the medical monitor if the participant was deemed ineligible due to a transient condition or if otherwise justified.

During screening, laboratory parameters of eGFR, TBL, AST and ALT may be repeated once within the screening window at the investigator's discretion after consulting with the medical monitor if ineligible due to a transient condition, which would not otherwise prevent the participant from taking part. ECG may be repeated once at the investigator's discretion on the same day at screening or randomization. These can be repeated without the need to register the participant as a screen failure.

6 INVESTIGATIONAL PRODUCT(S)

6.1 Investigational Product(s) Administered

Table 4 Investigational Product(s)

Name	**ASP0367**	**Placebo for ASP0367**
Use	Test product	Placebo
Dosage Form	Tablets	Tablets
Physical Description	Round, light yellowish red film-coated tablet	Matching placebo for ASP0367
Unit Dose Strength	10 and 25 mg	Matching placebo for ASP0367
Inactive Excipients	Lactose monohydrate, croscarmellose sodium, hydroxypropyl cellulose, microcrystalline cellulose, magnesium stearate, hypromellose, polyethylene glycol, talc, titanium dioxide, yellow ferric oxide, red ferric oxide	Mannitol, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, hypromellose, polyethylene glycol, talc, titanium dioxide, yellow ferric oxide, red ferric oxide
Storage	20°C to 25°C (68°F to 77°F); excursions permitted between 15°C and 30°C (59°F and 86°F) (as per USP Controlled Room Temperature). Protect from light.	20°C to 25°C (68°F to 77°F); excursions permitted between 15°C and 30°C (59°F and 86°F) (as per USP Controlled Room Temperature). Protect from light.
Packaging and Labeling	HDPE bottles	HDPE bottles
Route of Administration	Oral	Oral
Administration	Once daily with or without food Participants should be instructed to take the IP in the morning at the same time each day as much as possible. Crushing of tablets is not allowed.	Once daily with or without food Participants should be instructed to take the IP in the morning at the same time each day as much as possible. Crushing of tablets is not allowed.
IMP or Non-IMP	IMP	IMP
Sourcing	Provided centrally by sponsor	Provided centrally by sponsor

HDPE: High-density polyethylene; IMP: Investigational Medicinal Product; IP: investigational product; USP: US Pharmacopeia

The anticipated duration of the study for each participant, including screening and follow-up, is approximately 32 weeks.

Participants should be instructed to take the IP in the morning at the same time each day as close as possible. Crushing of tablets is not allowed. IP will be administered orally with or without food.

At study site visits, participants will visit the study site without taking the IP and take IP at the study site as instructed.

Table 5 Treatment Groups and Duration

Arm/IP Name	ASP0367	Placebo
Use	Test product	Matching placebo for ASP0367
Dose	75 mg (3 × 25 mg tablet) 30 mg (3 × 10 mg tablet)	NA (3 × tablet)
Frequency	Once daily	Once daily
Route	Oral	Oral
Duration	24 weeks	24 weeks

IP: investigational product; NA: not applicable

Refer to the pharmacy manual for detailed information regarding preparation, handling and storage of the IP.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Packaging and Labeling

All IP used in this study will be prepared, packaged and labeled under the responsibility of qualified personnel at Astellas Pharma Inc. (API) or sponsor's designee in accordance with API or sponsor's designee standard operating procedures (SOPs), current Good Manufacturing Practice (GMP) guidelines, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local laws/regulations.

Each bottle will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as investigational drug.

Refer to the pharmacy manual for detailed information regarding packaging and labeling of the IP.

6.2.2 Handling, Storage and Accountability

The handling, storage and accountability of the IP will be managed according to the following guidelines:

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all IP received and any discrepancies are reported and resolved before use of the IP.
- Only participants enrolled in the study may receive IP and only authorized study site personnel may supply or administer IP. Only IP with appropriate expiry/retest dating may be dispensed.
- All IP must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions and access must be limited to the investigator and authorized study site personnel.
- The investigator, institution or the head of the medical institution (where applicable) is responsible for accountability, reconciliation and record maintenance (i.e., receipt, reconciliation and final disposition records).

- Further guidance and instruction on final disposition of used and unused IP is provided in the pharmacy manual.

Refer to the pharmacy manual for detailed information regarding handling, storage and accountability of the IP.

6.3 Randomization and Blinding

6.3.1 Blinding Method

This is a double-blinded study. Participants will be randomized to receive ASP0367 or placebo in a blinded manner such that neither the investigator, sponsor's study management team, clinical personnel, caregiver, nor the participant will know which IP is being administered in the double-blind treatment period of the study. The randomization number will be assigned based on information obtained from the Interactive Response Technology (IRT) system.

6.3.2 Confirmation of the Indistinguishability of the Investigational Product

The appearance of both the dosage form and packaging of ASP0367 are identical to those of the matching placebo.

6.3.3 Retention of the Assignment Schedule and Procedures for Treatment Code Breaking

The randomization list and treatment assignment blind will be maintained by the IRT system.

The independent DSMB will be provided with access to the treatment assignment for periodic review of the overall safety and tolerability data. Its operation will be documented in the DSMB Charter.

The DMC will be provided with access to the treatment assignment for review of the interim analysis data. Its operation will be documented in the DMC Charter.

6.3.4 Breaking the Treatment Code for Emergency

The treatment code for each randomized participant will be obtained from the IRT system in the event of a medical emergency requiring knowledge of the treatment assigned to the participant. The IRT system will be programmed with blind-breaking instructions that can only be requested by the investigator or subinvestigators designated to have access to perform blind-breaking. In case of a medical emergency, the investigator (or his/her designated backup) has the sole responsibility for determining if unblinding of the participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a participant's treatment assignment unless this could delay emergency treatment for the participant.

Prior to the initial IP shipment, the investigator must have confirmed ability to access code-break through the IRT system and must have a designated backup (e.g., redundant processes) to support emergency unblinding requirements.

Prior to randomization, participants should be provided with information that includes the study site emergency contact number and back-up contact number in case of a medical emergency. Any unblinding by the investigational personnel must be reported immediately to the sponsor and include an explanation of why the IP was unblinded. If unblinding is associated with a SAE, the investigator is to follow the instructions in [Section 10.3.6 Reporting Procedures for Serious Adverse Events].

Care will be taken to limit knowledge of the treatment assignment, in case this can affect the blinding of other participants or future study assessment for the participant.

6.3.5 Breaking the Treatment Code by the Sponsor

The sponsor may break the treatment code for participants who experience a suspected unexpected serious adverse reaction (SUSAR), in order to determine if the individual case or a group of cases requires expedited regulatory reporting. Individual emergency codes will be provided to the limited personnel who are responsible to break the codes for all SUSAR cases for reporting purposes.

6.3.6 Assignment and Allocation

Participants enrolled in this study will be randomized in a 1:1:1 ratio to the 30 mg ASP0367, 75 mg ASP0367 or placebo arm.

Randomization will be performed according to the schedule obtained via the IRT system and stratified by site group (2 level: Mayo clinic_Minnesota; Non-Mayo clinic_Minnesota). The study site personnel will dispense either ASP0367 or placebo according to the IRT system's assignment. Specific procedures for randomization through the IRT system are contained in the study procedures manual.

6.4 Investigational Product Compliance

Participants should be counseled on the need to meet 100% compliance with IP. Investigator or designee should ensure that participants meet this goal throughout the study. Participant compliance with IP will be assessed at each visit. Compliance will be assessed by counting returned tablets. Deviations from the prescribed dose regimen will be recorded. When IP is administered at the study site, it will be administered under the supervision of study personnel.

6.5 Dose Modification

Dose modification for individual participants is not allowed.

Dosing may be interrupted if further evaluation is required for ongoing AE(s), clinical laboratory abnormality or intercurrent illness. The investigator will assess the resolution of the event and determine, in consultation with the medical monitor, whether resuming dosing or continued participation in the clinical study is in the best interest of the participant.

6.6 Continued Access to Investigational Product After the End of the Study

Access to ASP0367 will not be available after the end of this study. The compound is still in an early phase of clinical development prior to the determination of the safety and efficacy data that would support continued development with the dose selected and the intent to pursue marketing approval.

6.7 Treatment of Overdose

Any dose of IP greater than 75 mg/day will be considered an overdose. This overdose level may be adjusted based on emerging safety and tolerability data.

Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator should:

- Contact the medical monitor immediately.
- Assess the participant and provide appropriate supportive care as needed.
- Evaluate the participant along with safety information available to determine, in consultation with the medical monitor and the sponsor, whether study treatment should be discontinued.
- Document the quantity of the excess dose as well as the duration of the overdose.

Refer to [Section 10.3.7 Reporting Procedures for Special Situations] for reporting requirements for suspected overdose or other medication error.

6.8 Concomitant Therapy

6.8.1 Previous and Concomitant Treatment

The investigator must record the use of all previous and concomitant treatment from 3 months prior to randomization to follow-up visit in the CRF, both medication and nonmedication therapy. The investigator may record the use of previous treatment before 3 months prior to randomization in the CRF as long as participants remember. Previous and concomitant treatment includes all vitamins, herbal remedies, over the counter and prescription medications, as well as exercise regimen and physical therapy.

6.8.2 Prohibited Concomitant Treatment

- PPAR ligands listed in [Section 10.5 Appendix 5 List of Excluded Concomitant Medications] are not allowed within 4 weeks prior to randomization and during the study.
- Substrates for P-gp, BCRP, OATP1B1, OATP1B3 and organic anion transporter (OAT)3 are not allowed during the study. For participants using atorvastatin, rosuvastatin and simvastatin, switching to a less-interacting statin (pravastatin, pitavastatin and lovastatin) is allowed at investigator's discretion.
- Moderate and strong inhibitors of CYP3A are not allowed during the study.
- Inhibitors for transporter BCRP are not allowed during the study.
- Inhibitors of UGT1A3, UGT1A8 and UGT1A9 are not allowed during the study.

- Strong and moderate inducers of CYP3A and inducers for UGT1A3, UGT1A8, UGT1A9 and transporters are not allowed during the study.
- Triheptanoin, a synthetic medium chain triglyceride indicated in long-chain FAO disorders, is not allowed during the study.

A list of prohibited medications is provided in [Section 10.5, Appendix 5: List of Excluded Concomitant Medications].

6.8.3 Restricted Concomitant Treatment

CoQ10, carnitine, creatine or other mitochondrial disease-focused vitamins or supplemental therapies for the treatment of symptoms of the mitochondrial disease must be stable for 3 months prior to randomization and for the duration of the study.

6.8.4 Other Restrictions

See [Section 5.3 Lifestyle Considerations].

7 STUDY PROCEDURES AND ASSESSMENTS

- Study procedures and their timing are summarized in the schedule of assessments [Table 1]. Adherence to the study design requirements, including those specified in the schedule of assessments, is essential and required for study conduct. Prospective protocol waivers or exemptions are not allowed.
- Any change, divergence or departure from the study design or procedures identified in the protocol is considered a protocol deviation. All deviations from the protocol are to be recorded.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., imaging, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the schedule of assessments.
- Laboratory/analyte results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

7.1 Efficacy Assessments

7.1.1 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 unless restricted by policies of the study site.

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 asked to refrain from strenuous exercise on the day preceding and on the day of the 6MWT. 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 as outlined in the schedule of assessments [Table 1].

The "1000 Norms Project Consortium" has established normative distance walked 6MWT data based on age, gender, weight, and height [McKay et al, 2017]. Study participants who walked longer than the distance that 85% of healthy people can walk based on "1000 Norms Project Consortium" at the baseline visit (week 0) can be considered as normal and will be excluded from the study. Each participant's individual z-score will be calculated by entering age and gender, as well as distance walked in 6MWT at baseline (week 0) as indicated at "1000 Norms Project Consortium" website (https://clinicaloutcomemeasures.org/). The z-score > - 1.03 corresponds to the distance longer than 85% of healthy people can walk.

7.1.2 5 Times Sit to Stand

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 a 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 as outlined in the schedule of assessments [Table 1].

7.1.3 Quality of Life in Neurological Disorders

The Quality of Life in Neurological Disorders (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 as outlined in the schedule of assessments [Table 1].

7.1.4 Modified Fatigue Impact Scale

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 as outlined in the schedule of assessments [Table 1].

7.1.5 Patient Global Impression of Change

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. PGIC will be performed as outlined in the schedule of assessments [Table 1].

7.1.6 The Patient Global Impression of Severity

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. PGIS will be performed as outlined in the schedule of assessments [Table 1]..

7.1.7 5-level EQ-5D Version (EQ-5D-5L)

European Quality of Life (EuroQol) 5-Dimensions, 5-Level Questionnaire (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 performed as outlined in the schedule of assessments [Table 1].

7.1.8 Primary Mitochondrial Myopathy Video Assessments

Effective with implementation of Protocol Version 8.0 (Substantial Amendment 7), PMMVA will no longer be collected. The Primary Mitochondrial Myopathy Video Assessments (PMMVA) tool provides a standardized way to document and assess patient quality of movement. A caregiver or a close observer will video record specific movement tasks at home using a secure mobile application. In addition to assigned movement tasks, the participant will complete one participant-choice activity. All participants will also have the option to submit a new ability video (if applicable). The participant-choice activity is an activity that the participant will select prior to baseline data capture that the participant is struggling with. The caregiver/observer will video record the same participant's choice activity at each subsequent time point throughout the study. If the participant gains a new ability during the study, the caregiver/observer may video record the new ability at any time during the study in the study mobile application.

7.1.9 Individualized Activity Assessments

Effective with implementation of Protocol Version 8.0 (Substantial Amendment 7), individualized activity assessments will no longer be collected. Participants will identify 3

activities during the screening period that are difficult due to their PMM and/or make them notice their PMM. They will rate the magnitude of interference of their PMM symptoms in the previous 2 weeks for each of their 3 identified activities and the change in ease/difficulty of performing that activity since baseline.

7.1.10 Qualitative Interview

Effective with the implementation of Protocol Version 8.0 (Substantial Amendment 7), qualitative interviews will no longer be conducted. Participants will complete qualitative audio interviews remotely to collect direct feedback about their experience in the study, particularly with respect to treatment and changes in disease burden.

Trained interviewers will conduct the interviews using a semi-structured interview guide in a secure telemedicine platform.

7.2 Safety Assessments

Study procedures and their timing are summarized in the schedule of assessments [Table 1]. Protocol waivers or exemptions are not allowed. Procedures conducted as part of a participant's routine clinical management (i.e., standard of care) obtained before signing the ICF may be utilized for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the timeframe, as defined in the schedule of assessments [Table 1].

7.2.1 Laboratory Assessments

- See [Section 10.6, Appendix 6: Clinical Laboratory Assessments] for the list of clinical laboratory tests to be performed and refer to schedule of assessments [Table 1] for timing and frequency.
- At follow-up phone call visits, a blood sample for abbreviated biochemistry will be collected at the participant's home by a qualified healthcare provider from the home healthcare vendor. In addition, at week 2, a blood sample for abbreviated biochemistry and hematology will be collected. These samples should be collected at the participant's home. In case the home healthcare visit is not feasible, the visit can be conducted at the study site. The detailed procedures will be described in the lab manual.
- The investigator or subinvestigator must review the laboratory report, document this review and record any clinically significant changes occurring during the study as an AE. The laboratory reports must be filed with the source documents.
- Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or subinvestigator who is a qualified physician. Abnormal laboratory findings associated with the underlying disease are not considered clinically significant unless judged by the investigator to be more severe than expected for the participant's condition.

7.2.2 Vital Signs

- Body temperature, pulse and blood pressure will be assessed as outlined in the schedule of assessments [Table 1].
- Blood pressure (systolic blood pressure and diastolic blood pressure) and pulse measurements will be measured using a semiautomatic blood pressure recording device with an appropriate cuff size (same cuff size and same arm will be used for each time point).
- When vital signs are scheduled for the same time as a blood sample, the vital signs will be taken before the blood sample.
- Blood pressure and pulse will be measured in the sitting or supine position after the participant has rested for at least 5 minutes.

7.2.3 Physical Examination

- Physical examinations will be conducted at study site visits as outlined in the schedule of assessments [Table 1]. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardio-vascular, abdomen, extremities, musculoskeletal and neuromuscular. Any clinically relevant abnormality at screening, including baseline symptoms for mitochondrial myopathy should be recorded as medical history.
- Any clinically relevant change from baseline to the end of study will be recorded as an AE in the CRF.

7.2.4 Electrocardiogram

- A standard 12-lead ECG will be conducted as outlined in the schedule of assessments [Table 1] 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 for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 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 as described in [Section 8.1 Discontinuation of Individual Participant(s) from Study Treatment]. Participants with persistent cTnI or

cTnT elevations, abnormal ECG or abnormal echocardiogram should undergo cardiology follow up.

7.2.5 Echocardiogram

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 for reference range), or when there is an elevation of cTnT above the ULN (see Appendix 10.9 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 as described in [Section 8.1 Discontinuation of Individual Participant(s) from Study Treatment]. Participants with persistent cTnI or cTnT elevations, abnormal ECG or abnormal echocardiogram should undergo cardiology follow up.

7.2.6 Body Weight

Body weight will be collected as outlined in the schedule of assessments [Table 1].

7.2.7 Columbia-Suicide Severity Rating Scale

- The C-SSRS was developed as a screening tool to identify suicide risk. The interviewer asks participants detailed questions regarding suicidal ideation, behaviors, intensity of ideation and attempts. Response options and recall periods vary in accordance with the nature of the question.
- The C-SSRS will be performed by trained site staff via interview as outlined in the schedule of assessments [Table 1]. 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 participant.
- Participant who has a history of suicide attempt, suicidal behavior or has any suicidal ideation within 1 year prior to screening that meets criteria at a level of 4 or 5 by using the C-SSRS or who is at significant risk to commit suicide, as assessed by the investigator at screening will be excluded.

7.2.8 Order of Assessments

It is recommended that the participants follow the following order of assessments within each of the given visits [Table 6] to fulfill the requirements in the protocol.

Table 6 Order of Assessments under Protocol Version 9.0 (Substantial Amendment 8 and Subsequent Versions)

Week 0	Week 4	Week 12	Week 24
▪ Vital signs ▪ 3 separate 12-lead ECG	▪ Vital signs ▪ 3 separate 12-lead ECG	▪ Vital signs ▪ 3 separate 12-lead ECG	▪ Body Weight ▪ Vital signs ▪ 3 separate 12-lead ECG
▪ Hematology, biochemistry, urinalysis ▪ Serum and urinary biomarkers ▪ Pregnancy test ▪ Blood sample collection predose for genotyping ▪ Blood sample collection predose for PGx (banking) ▪ Blood sample collection predose for pharmacodynamics (PPARδ target gene expression)	▪ Hematology, biochemistry, urinalysis ▪ Pregnancy test	▪ Hematology, biochemistry, urinalysis ▪ Serum and urinary biomarkers ▪ Pregnancy test ▪ Blood for plasma pharmacokinetics at predose (-90 min to 0 min)* ▪ Blood for pharmacodynamics (PPARδ target gene expression) at predose (-90 min to 0 min)*	▪ Hematology, biochemistry, urinalysis ▪ Serum and urinary biomarkers ▪ Pregnancy test ▪ Blood for plasma pharmacokinetics at predose ▪ Blood for pharmacodynamics (PPARδ target gene expression) at predose
▪ 6MWT ▪ Patient reported outcomes ▪ C-SSRS ▪ 5XSTS ▪ Physical examination	▪ 6MWT ▪ Patient reported outcomes ▪ C-SSRS ▪ 5XSTS ▪ Physical examination	▪ 6MWT ▪ Patient reported outcomes ▪ C-SSRS ▪ 5XSTS ▪ Physical examination	▪ 6MWT ▪ Patient reported outcomes ▪ C-SSRS ▪ 5XSTS ▪ Physical examination
▪ Verify inclusion/ exclusion criteria	▪ Dispense IP	▪ Dispense IP	
▪ Randomization	▪ IP dosing	▪ IP dosing	
▪ Dispense IP		▪ Blood for plasma pharmacokinetics at postdose (0.5-4 h)	
▪ IP dosing			
▪ Blood for plasma pharmacokinetics at postdose (0.5-4 h)			

5XSTS: 5 Times Sit to Stand; 6MWT: 6-minute walk test; C-SSRS: Columbia-Suicide Severity Rating Scale; ECG: electrocardiogram; IP: investigational product; PGx: pharmacogenomics; PPARδ: peroxisome proliferator-activated receptor delta

Note: Assessments in the same box may be performed in any order.

*Blood samples for pharmacokinetics and pharmacodynamics (PPARδ target gene expression) at week 12 will be collected within 90 minutes prior to IP dosing. When it is not feasible to complete 6MWT, Patient reported outcomes, C-SSRS, 5XSTS and physical examination in 90 minutes, collect blood samples for pharmacokinetics and pharmacodynamics after the assessments to comply with the sample collection window.

7.3 Adverse Events and Other Safety Aspects

The definitions of an AE or SAE can be found in [Sections 10.3.1 and 10.3.2], respectively.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate or the participant's legally authorized representative).

The investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study IP or that caused the participant to discontinue the IP and/or study [see Section 10.3, Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting].

The method of recording, evaluating and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in [Section 10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting].

7.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All SAEs or AEs ([S]AEs) will be collected from the signing of the ICF until the follow-up visit or 28 days after the final IP administration, whichever is later, or when the participant is determined to be a screen failure, at the time points specified in the schedule of assessments [Table 1] and reported on the CRF.

If the severity of an (S)AE changes, the event should be relisted on the CRF with the new severity and new onset date.

If the severity decreases, the (S)AE should be relisted on the CRF with the new severity and new onset date. The exception is ongoing pre-dose events that continue post-dose and improve post-dose. Such events should not be re-listed.

If the severity of an SAE reduces, the details of the AE should be provided on the SAE worksheet for the medical assessor to be able to assess the course of the event.

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in [Section 10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting]. The investigator will submit any updated SAE data to the sponsor within 24 hours of them being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study IP or study participation, the investigator must promptly notify the sponsor.

7.3.2 Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

In previous versions of the protocol, if an AE had been reported or observed by the vendor during a qualitative interview or PMMVA, it would have been reported to the investigators and designated contract research organization (CRO). The investigator and any qualified designees are responsible for assessing, documenting, recording and reporting to the IRB/IEC any events that meet the definition of an AE or SAE.

7.3.3 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AEs of special interest (as defined in [Section 7.3.6 Adverse Events of Special Interest]) will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in [Section 8.3 Lost to Follow-up]). Further information on follow-up procedures is provided in [Section 10.3, Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting].

If after the protocol-defined AE collection period [see Section 7.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information], an AE progresses to an SAE, or the investigator learns of any (S)AE including death, where he/she considers there is reasonable possibility it is related to the IP or study participation, the investigator must promptly notify the sponsor.

7.3.4 Regulatory Reporting Requirements for Serious Adverse Events

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study IP under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study IP under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC and investigators.
- Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

7.3.5 Disease-related Events and/or Disease-related Outcomes Not Qualifying as Adverse Events or Serious Adverse Events

Under this protocol, the following event(s) will not be considered as an (S)AE:

- Pre-planned and elective hospital/clinical procedures/interventions or procedures for diagnostic, therapeutic or surgical procedures for a pre-existing condition that did not worsen during the course of the study. These procedures are collected per the completion guidelines on the CRF.

7.3.6 Adverse Events of Special Interest

The following (S)AEs are considered of special interest:

- Cardiac tissue injury, indicated by cTnI above ULN (see Appendix 10.9 for reference range) or if the cTnT is above ULN (see Appendix 10.9 for reference range) or baseline if the participant's baseline value of cTnT was above ULN
- Cardiac tissue injury with any signs or symptoms reflecting cardiac involvement, inclusive of new onset shortness of breath confirmed by ECG or echocardiogram
- Skeletal muscle injury indicated by CK increase ≥ 5 times the value of the ULN or the individual's baseline value, whichever is higher judged not related to an explainable increase, including excessive movement, fall or disease state
- Suspected abuse of IP. ASP0367 has high-risk of doping potential and was assigned to category D (i.e., high-risk doping potential in sport). Hence, suspected abuse of IP will be analyzed for its muscle function improving characteristics per Astellas Doping Potential Guideline and will be closely followed up via compliance and participants, who will be expected to meet 100% compliance with IP. Compliance will be assessed by counting returned tablets. Deviations from the prescribed dose regimen will be recorded. When IP is administered at the study site, it will be administered under the supervision of study personnel [Section 6.4 Investigational Product Compliance]. Suspected abuse during study will be reported as special situations per [Section 7.3.7 Special Situations].

AEs (serious or nonserious) of special interest are to be collected in the CRF. If the AEs of special interest are classified as serious, they are to be collected via the SAE worksheet and reported within 24 hours as described in [Section 10.3.6 Reporting Procedures for Serious Adverse Events].

7.3.7 Special Situations

Certain special situations observed in association with the IP, such as incorrect administration (e.g., wrong dose of IP or background therapy) are reported as protocol deviations and/or may require special reporting, as described below. These special situations are not considered AEs but do require to be communicated to Astellas as per the timelines defined below.

If a special situation is associated with, or results in, an AE, the AE is to be assessed separately from the special situation and captured as an AE in the CRF. If the AE meets the definition of an SAE, the SAE is to be reported as described in [Section 10.3.6 Reporting Procedures for Serious Adverse Events] and the details of the associated special situation are

to be included in the clinical description on the special situation worksheet or pregnancy reporting form.

The special situations are:

- Pregnancy
- Lactation
- Medication error, overdose and use outside protocol
- Suspected misuse/abuse of IP
- Occupational exposure
- Suspected drug-drug interaction

Instructions and procedures for reporting special situations are provided in [Section 10.3.7 Reporting Procedures for Special Situations].

7.4 Pharmacokinetics

7.4.1 Analysis of ASP0367 in Plasma

Blood samples for the analysis of ASP0367 in plasma will be collected as described in the schedule of assessments [Table 1]. The actual date and time of each blood sample collection and the actual date and time of IP administration on the site visit day and day before pharmacokinetic sampling visits will be collected in the source documents. Site staff should document the type (high fat defined as ≥ 55 g fat, non-high fat defined as < 55 g fat or unknown and completion time of the meal immediately prior to on-site dosing. Meal type and completion time are not collected at visits with only predose sample collection. Plasma will be prepared according to procedures further specified in the laboratory manual. Standard high-quality screw-cap sample tubes will be used. Samples will be shipped on dry ice to the designated CRO and analyzed using a validated method.

The plasma samples remaining after the pharmacokinetic analysis may be used for exploratory metabolic profiling. The plasma samples will be sent to Astellas Pharma Inc. (API, Tsukuba, Japan) or designated CRO. Observed C_{trough} data will be summarized in the clinical study report (CSR), along with exploratory/graphical C_{trough} vs response for key endpoints, as appropriate. Modeled pharmacokinetic and exposure response analyses will be summarized in a separate report.

7.5 Pharmacodynamics

Blood samples for PPARδ target gene expression assay will be collected at time points as detailed in the schedule of assessments [Table 1]. Blood sample collection, processing, handling and storage will be described in the laboratory manual.

7.6 Pharmacogenomics

7.6.1 Sample for Genotyping

Knowledge of polymorphisms of genes PPARδ and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A) may help understand/explain observed differences in efficacy of ASP0367. A 2 mL whole blood sample for the analysis of these

genes (pharmacogenomics [PGx]) will be collected as indicated in the schedule of assessments [Table 1]. For detailed sample collection, sample labeling and sample shipment procedures, refer to the laboratory manual. All samples will be transferred to the central laboratory and then shipped to the analytical laboratory where they will be analyzed using appropriate validated methods.

7.6.2 Sample for Banked Pharmacogenomics

PGx research may be conducted in the future to analyze or determine genes of relevance to clinical response, pharmacokinetics and toxicity/safety. A 4-mL sample of whole blood for possible banked PGx analysis will be collected as indicated in the schedule of assessments [Table 1]. Samples will be shipped to a sponsor-designated banking CRO. Details on sample collection, labeling, storage and shipment procedures will be provided in a separate laboratory manual. See [Section 10.7, Appendix 7: Pharmacogenomic Analysis with Banked Sample] for further details on the banking procedures.

7.7 Biomarkers

7.7.1 Urine Sample Analysis for TITIN

Urine samples for TITIN of ASP0367 will be collected according to the schedule of assessments [Table 1].

Details on sampling, processing, storage and shipment procedures will be provided in the laboratory manual.

7.7.2 Blood Sample for Serum Target Proteins

A blood sample for serum target proteins (aldolase A [ALDOA], cytochrome c [CYC] and filamin-C [FLNC]) will be done in blood collection tubes at the time points indicated in the schedule of assessments [Table 1].

For detailed sampling, processing of samples, storage and shipment procedures refer to the laboratory manual.

7.8 Immunogenicity Assessments

Not applicable

7.9 Electronic Clinical Outcome Assessment

The following clinical outcome assessments will be captured on an electronic device.

The collected electronic source data will be hosted by the vendor. The investigator or site designee will review the questionnaire data throughout the study to ensure completion and protocol compliance.

The questionnaire data will be transferred electronically to sponsor or designee at predefined intervals during the study. The vendor will provide the investigator with a complete and clean copy of their site's data and will provide the sponsor or designee with a complete and clean copy of the study data. The ownership of this data is with the investigator and subsequently

any changes requested by the investigator or designee to these participant- or clinician-reported data will be made following the vendor's process. The requested change must be supported by documented evidence at the site.

7.9.1 Quality of Life in Neurological Disorders

Neuro-QoL will be performed at the study site during study site visits. The assessments will be answered by the participant and collected on an electronic device.

7.9.2 Modified Fatigue Impact Scale

MFIS will be performed at the study site during study site visits. The assessments will be completed by the participant on an electronic device.

7.9.3 Patient Global Impression of Change

PGIC will be performed at the study site during study site visits. The assessments will be completed by the participant on an electronic device.

7.9.4 Patient Global Impression of Severity

PGIS will be performed at the study site during study site visits. The assessments will be completed by the participant on an electronic device.

7.9.5 Columbia-Suicide Severity Rating Scale

The C-SSRS will be performed at the study site during study site visits. The assessments will be completed by trained site staff via interview on an electronic device.

7.9.6 European Quality of Life 5-Dimension, 5-Level Questionnaire

The EQ-5D-5L will be performed at the study site during site visits. The assessments will be completed by the participant on an electronic device.

7.9.7 Primary Mitochondrial Myopathy Video Assessments

Effective with implementation of Protocol Version 8.0 (Substantial Amendment 7), PMMVA will no longer be collected. In previous versions of the protocol, PMMVA was performed at participant's home setting by participants using a secure mobile application. Participants registered for the study mobile application and downloaded the mobile application on an Apple (IOS 10+) or Android (5.1+) smartphone. Participants were provided with training materials in the study mobile application and in the study kit to learn how to video record the assigned movement activities. Participants provided an electronic signature within the study mobile application to document training completion. The specific movement activities were detailed in the Training Manual.

7.9.8 Individualized Activity Assessments

Effective with implementation of Protocol Version 8.0 (Substantial Amendment 7), individualized activity assessments will no longer be collected. In previous versions of the protocol, the assessments for individualized activities were rated at participant's home using a secure mobile application.

7.9.9 Qualitative Interview

Effective with implementation of Protocol Version 8.0 (Substantial Amendment 7), qualitative interviews will no longer be conducted. In previous versions of the protocol, Qualitative audio interviews were conducted remotely by trained interviewers using a semi-structured interview guide in a secure telemedicine platform in participant's home setting.

7.10 Other Assessments

7.10.1 Exercise Status

For the participants who are on an exercise regimen, changes to the status of the exercise regimen will be collected at each visit (on site or phone call).

7.11 Total Amount of Blood

The total amount of blood for each participant will vary depending on the course of their disease, duration on treatment and local laboratory requirements. At any time during the study, if any laboratory abnormalities are found for a participant, additional blood may be drawn for safety monitoring [Table 7].

Table 7 Blood Volume

Sample Type	Number of Samples	Approximate Sample Volume (mL)	Approximate Total Volume (mL)
Hematology, biochemistry, serology and serum pregnancy test (if applicable) at screening	1	22	22
Biochemistry and hematology at weeks 0, 4, 12, 24 and 28	5	16	80
Abbreviated biochemistry and abbreviated hematology at week 2	1	11.5	11.5
Abbreviated biochemistry at phone call visits	5	10.5	52.5
Pharmacokinetics of ASP0367	4	2	8
Serum biomarkers	3	4	12
Genotyping	1	2	2
Pharmacogenomics (banking)*	1	4	4
Pharmacodynamics of PPARδ target gene expression	3	5	15
Total			207

PPARδ: peroxisome proliferator-activated receptor delta

*Pharmacogenomics (banking) will be performed for the participants who were given the consent.

8 PARTICIPANT DISCONTINUATION

Refer to [Section 10.1.9 Study and Site Start and Closure] regarding discontinuation of study sites or of the study as a whole. An interim analysis will be performed to determine if efficacy or futility stopping criteria are met while the study is ongoing. Refer to [Section 9.6] for details regarding the interim analysis and stopping criteria.

8.1 Discontinuation of Individual Participant(s) from Study Treatment

A discontinuation from treatment is defined as a participant who enrolled in the study and for whom study treatment is permanently discontinued for any reason.

The participant is free to withdraw from the study treatment and/or study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to discontinue the participant from study treatment or to terminate a participant's involvement in the study at any time if the participant's clinical condition warrants it.

The reason for discontinuation from study treatment must be documented in the participant's medical records.

A participant must discontinue study treatment for any of the following reasons:

- Participant requests to stop treatment.
- Any clinical AE, laboratory abnormality or intercurrent illness, in the opinion of the investigator, indicates continued treatment is not in the best interest of the participant.
- Participant remains non-compliant with the protocol based on investigator's or sponsor's assessment.
- Female participant becomes pregnant.

An individual participant may be required to discontinue further administration of IP if any of the following occurs, which is considered to be clinically significant and to be considered related to IP:

- SUSAR
- Participant has a mean QTcF > 500 msec measured by a triplicate ECG. If the mean QTcF exceeds 500 msec, a follow-up triplicate ECG may be performed on the same day. If the follow-up mean QTcF is still > 500 msec, the participant must discontinue IP.
- Participant who experiences a cTnI elevation (i.e., > ULN [see Appendix 10.9 for reference range]) or a cTnT elevation above the ULN (see Appendix 10.9 for reference range) or above the participant's baseline value if it was elevated should undergo ECG and echocardiogram within 24 hours. The investigator should determine whether it is appropriate to continue administration of IP to the participant while the evaluations are being completed. If the ECG and echocardiogram are within normal limits, a repeat of the abnormal value (cTnI or cTnT) should be obtained. Participants with persistent cTnI or cTnT elevations, abnormal ECG or echocardiogram should interrupt IP and undergo cardiology follow up.

- Participant who develops any signs or symptoms potentially reflecting cardiac involvement, inclusive of new onset shortness of breath, should interrupt IP and undergo ECG, echocardiogram, and have a cTnI drawn to be evaluated prior to continuing IP.
- Participant who develops an elevation in CK levels ≥ 5 times the value of the ULN or the individual's baseline value, whichever is higher and it has been judged that this elevation is not related to an explainable cause such as excessive activity or disease state.
- Participant who develops elevated levels of ALT or AST > 3 × ULN, or TBL > 2 × ULN persisting after 72 hours in the absence of elevated CK levels must discontinue IP. Generally, liver chemistry will be followed by repeated measurements until they return to baseline or stable values.
- Participant who presents evidence of suicidality during the C-SSRS assessments by meeting the criteria at a level of 4 or 5 since last visit must discontinue IP and upon immediate evaluation, the investigator will decide an appropriate referral.
- Participant who develops symptomatic viral infection should be evaluated by the Investigator and assessed for the need to stop IP until clinically improved.
- Participant who newly develops signs or symptoms of bulbar weakness, such as dysphagia, dysphonia, hoarseness or drooling/sialorrhea, should discontinue IP.
- Participant with a new requirement for oxygen therapy or for ventilatory support, inclusive of any respiratory device to support breathing, such as home ventilators and any form of non-invasive positive pressure ventilation (including CPAP, BiPAP, and AVAPS), should discontinue IP.

8.2 Discontinuation of Individual Participant(s) from Study

All participants who discontinue study treatment will remain in the study and must continue to be followed for protocol-specific follow-up procedures as outlined in the schedule of assessments [Table 1]. The only exception to this is when the participant specifically withdraws consent for any further contact with him/her or persons previously authorized by the participant to provide this information.

To encourage participants who discontinue assigned treatment to remain in the study for efficacy and safety evaluations, the ICF will highlight continued collection of data in participants that do not adhere to treatment but remain in the study and distinguish the difference between withdrawing from the treatment and withdrawing from the study.

In addition, to minimize the amount of missing data, the sponsor will keep track of the amount of missing data of 6MWTs and the reasons for withdrawal from the treatment or study. If any issue is identified, a root cause analysis will be conducted and it will address the issue appropriately.

8.3 Lost to Follow-up

Every reasonable effort is to be made to contact any participant lost to follow-up during the course of the study to complete study-related assessments, record outstanding data and retrieve IP. These contact attempts should be documented in the participant's medical record.

9 STATISTICAL CONSIDERATIONS

Given the design, a 24-week treatment analysis, which is of the primary focus, will be provided to assess efficacy and safety during the 24-week double-blind treatment period. This will occur after all participants have completed 24 weeks of treatment. Unblinding will be performed after database lock for 24-week data. Once the entire study is completed and the database is locked, additional efficacy and safety data will be summarized and a separate treatment analysis will be conducted at later timepoints up to 76 weeks as per available data. This section includes the principal feature of the analysis for the 24-week double-blind treatment period and interim analysis. Available data collected after the 24-week double-blind treatment period will be summarized, and details will be provided in a separate statistical analysis plan (SAP). An interim analysis will be performed when approximately 30 participants complete the 6MWT assessment at week 24.

9.1 Statistical Hypotheses

The hypothesis for the primary endpoint is given as follows:

H0: The change from baseline in distance walked on the 6MWT at week 24 for each dose of ASP0367 and placebo are the same.

H1: 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) under the nominal significance level of 0.10 2-sided, a two-stage group sequential design with Lan-DeMets alpha-spending function determined by means of the O'Brien-Fleming approach will be used for the interim analysis. Additionally, the hierarchical testing procedure in the following order will be applied for the primary analysis in the 24-week treatment analysis:

1. Comparison between ASP0367 75 mg and placebo
2. Comparison between ASP0367 30 mg and placebo

9.2 Sample Size Determination

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.

9.3 Populations for Analyses

The following populations are defined for the 24-week treatment analysis:

Population	Description
Full Analysis Set (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 FAS will be used for all summaries of efficacy data.
Safety Analysis Set (SAF)	All participants who receive at least 1 dose of ASP0367 or placebo for the double-blind treatment period. The SAF will be used for all summaries of the safety data.
Pharmacokinetic analysis set (PKAS)	All participants who receive at least 1 dose of IP for which concentration data are available for at least 1 time point. Inclusion of participants in the PKAS with missing data or major protocol deviations will be considered by the pharmacokineticist on a case-by-case basis. The PKAS will be used for all summaries and analyses of the pharmacokinetic data.
Extended pharmacokinetic analysis set (EPKAS)	All participants who receive at least 1 dose of IP for which at least 1 pharmacokinetic parameter is available at week 0 and/or week 2. Inclusion of participants in the EPKAS with missing data or major protocol deviations will be considered by the pharmacokineticist on a case-by-case basis. The EPKAS will be used for noncompartmental analyses of the pharmacokinetic data and concentration and pharmacokinetic parameter summaries at weeks 0 and 2.
Biomarker analysis set (BMAS)	All participants who receive at least 1 dose of ASP0367 or placebo and have data from at least 1 post baseline biomarker test or pharmacodynamic test available. The BMAS will be used for all summaries and analyses of the biomarker as well as pharmacodynamic data.

9.4 Statistical Analyses

A SAP will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. SAPs will be prepared for analyses at the end of 24 weeks of double-blind treatment for objectives and endpoints of this protocol and additional objectives and endpoints specific to the endpoints in the 0367-CL-1201 [MAYO] Protocol. Other SAPs will be prepared for analysis at the end of 76 weeks of treatment for objectives and endpoints of this protocol and additional objectives and endpoints specific to the 0367-CL-1201 [MAYO] Protocol. The SAPs for the 24-week double-blind treatment analysis and 76-week treatment analysis will be finalized before unblinding for 24-week data and before database lock for 76-week data, respectively. Changes from the planned analyses in the final SAP that impact the statistical analyses will be justified in the CSR. Additionally, the interim analysis plans will be prepared to provide the details of the interim analysis. The interim analysis plans will be finalized prior to the interim analyses.

9.4.1 General Considerations

In general, continuous data will be summarized with descriptive statistics (number of participants, mean, SD, minimum, median and maximum), frequency and percentage for categorical data. Percentage by categories will be based on the number of participants with no missing data (i.e., will add up to 100%).

Baseline will be defined as the last non-missing observation prior to first administration of IP, unless otherwise specified.

Demographics and baseline characteristics (age, sex, race and ethnicity, body weight, height and BMI) and genetic abnormality for PMM will be summarized by treatment group and overall for the Safety Analysis Set (SAF).

The number and percentage of participants who complete and discontinue 24 weeks of treatment and reasons for treatment discontinuation will be presented for all randomized participants by treatment group and overall. All disposition details and dates of first and last evaluations for each participant will be listed.

All previous and concomitant treatment and medical history will be listed. Medical history will be coded using MedDRA and will be summarized by system organ class (SOC) and preferred term (PT) by treatment group and overall for the SAF. All IP exposure data will be listed.

9.4.2 Analysis of Efficacy

Efficacy analysis will be conducted on the Full Analysis Set and statistical testing will be performed to compare each dose group of ASP0367 and placebo group at the 2-sided 0.10 significance level. Details will be provided in the SAP.

9.4.2.1 Analysis of Primary Endpoint

For primary efficacy endpoint, change from baseline in distance walked on the 6MWT at week 24, a mixed model for repeated measures (MMRM) with treatment groups (3 level: ASP0367 75 mg, ASP0367 30 mg, and placebo group), week (weeks 4, 12 and 24) and site group (2 level: Mayo clinic_Minnesota; Non-Mayo clinic_Minnesota) 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. If the number of participants from either site group is small, the site group will be removed from the model. It will be determined before the unblinding and pre-defined in the SAP.

The 6MWT evaluated below will be included in the analysis.

- For participants who consented for this study in Protocol Version 7.0 or former version and did not re-consent to Protocol Version 8.0 or later by week 24: the 6MWT evaluated until 7 days after the last dose of IP for the double-blind treatment period of the study or moving to the OLE or day 182, whichever comes first.

- For participants who consented or re-consented this study in Protocol Version 8.0 or later version by week 24: the 6MWT evaluated until 7 days after the last dose of IP for the double-blind treatment period of the study.

9.4.2.2 Sensitivity Analysis/Supplementary Analysis

Sensitivity analysis and supplementary analysis will be performed for the primary endpoint using the MMRM based on the Full Analysis Set to assess the robustness of the primary efficacy results. The following analyses are planned:

- MMRM with additional covariate(s).
- MMRM including the data after treatment discontinuation.

9.4.2.3 Analysis of Secondary Endpoints

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

For Neuro-QoL Short Form Fatigue and Lower Extremity Function (Mobility) scores, a transformed T-score will be used, which rescale raw scores into standardized scores with a mean of 50 and a standard deviation of 10. Change of T-scores from baseline to week 24 will be analyzed using the same MMRM as with the primary endpoint.

For PGIC score at week 24, generalized estimating equation model will be used with treatment group (3 level: ASP0367 75 mg, ASP0367 30 mg, and placebo group), week (weeks 4, 12 and 24) and site group (2 level: Mayo clinic_Minnesota; Non-Mayo clinic_Minnesota) as factors, as well as an interaction of treatment by week. In addition, 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, change from baseline to week 24 will be analyzed using generalized estimating equation model with treatment group (3 level: ASP0367 75 mg, ASP0367 30 mg, and placebo group), week (weeks 4, 12 and 24) and site group (2 level: Mayo clinic_Minnesota; Non-Mayo clinic_Minnesota) as factors, baseline value as a covariate, as well as an interaction of treatment by week and an interaction of baseline value by week.

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

9.4.2.4 Analysis of Exploratory Endpoints

For 6MWT, 5XSTS, Neuro-QoL Short Form Fatigue and Lower Extremity Function (Mobility) scores, change from baseline to week 4 and 12 will be summarized by treatment group and time point. The change from baseline in minute-by-minute analyses (i.e., 6^{th} vs 1^{st}) of 6MWT at weeks 4, 12 and 24 will be summarized by treatment group.

For MFIS score and PGIS scores, change from baseline to weeks 4 and 12 will be summarized by treatment group and time point. Moreover, PGIC scores at weeks 4 and 12 will also be displayed in the same way.

For the EQ-5D-5L descriptive systems, the number and percentage of participants will be summarized by scored level (1 to 5) of each dimension and treatment group, as well as time point. Change from baseline in EQ-VAS and EQ-5D-5L index values at weeks 4, 12 and 24 will be summarized by treatment group and time point.

9.4.3 Analysis of Safety

Safety analyses will be conducted on the SAF. Summary tables and listings of safety information will be provided in the SAPs.

9.4.3.1 Adverse Events

AEs will be coded using MedDRA.

A TEAE is defined as below:

- For participants who consented for this study in Protocol Version 7.0 or former version and did not re-consent to Protocol Version 8.0 or later version by week 24: an AE observed after starting administration of the IP to 28 days after the last dose of IP for the double-blind treatment period of the study or moving to the OLE or day 182, whichever comes first.
- For participants who consented or re-consented to this study in Protocol Version 8.0 or later version by week 24: an AE observed after starting administration of the IP 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 with TEAEs, SAEs, AEs leading to withdrawal of treatment and AEs related to IP will be summarized by SOC, PT and treatment group. The number and percentage of AEs by severity will also be summarized. The worst severity will be summarized if the same AE is recorded more than once for a participant.

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

AE data will be listed.

9.4.3.2 Laboratory Assessments

For quantitative clinical laboratory measurements (hematology, biochemistry and urinalysis), descriptive statistics will be used to summarize results and change from baseline by treatment group and time point.

Laboratory data will be listed.

9.4.3.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline by treatment group and time point.

Vital signs data will be listed.

9.4.3.4 Body Weight

Body weight data will be displayed in listings.

9.4.3.5 Electrocardiogram

9.4.3.5.1 Routine 12-lead Electrocardiogram

The routine 12-lead ECG results will be summarized by treatment group and time point.

All ECG interpretations will be displayed in listings.

9.4.3.5.2 Continuous 12-lead Electrocardiogram

For all analyses, replicates at each time point will be averaged for each continuous 12-lead ECG parameter.

Descriptive statistics will be used to summarize the continuous 12-lead ECG parameter results and changes from baseline by treatment group and time point.

The number and percentage of participants with absolute QTcF interval ≤ 450 msec, > 450 msec, > 480 msec, > 500 msec will be provided by treatment group at each time point and overall by visit. Similar summary information will be provided for QTcF interval increases from baseline of ≤ 30 msec, > 30 msec and > 60 msec.

Continuous 12-lead ECG data (individual replicates and the averages) will be listed.

9.4.3.6 Echocardiogram

Echocardiogram results will be displayed in listings.

9.4.3.7 Columbia-Suicide Severity Rating Scale

C-SSRS data will be summarized by treatment group and time point.

9.4.4 Analysis of Pharmacokinetics

Descriptive statistics will include n, mean, SD, minimum, median, maximum, coefficient of variation (CV), geometric mean and geometric CV, where applicable. Noncompartmental analysis will be performed on the dense pharmacokinetic samples from participants who have already provided these and will be reported in the CSR. Further details on the calculation of the pharmacokinetic parameters will be provided in the SAPs. No noncompartmental analysis will be conducted on samples from new participants. Only C_{trough} will be reported for new participants in the CSR, and pharmacokinetic profiles will be analyzed via population pharmacokinetics.

9.4.5 Analysis of Pharmacodynamics

Analysis of pharmacodynamics data for participants enrolled prior to implementation of Protocol Version 9.0 (Substantial Amendment 8) will differ from analysis of data for participants enrolled starting with Protocol Version 9.0 (Substantial Amendment 8).

9.4.5.1 Analysis of Treatment-emergent Gene Expression

Summaries of absolute values and relative changes from baseline for each gene by scheduled sample time point and treatment group will be provided. Baseline for gene expression is predose measurements on day 1. Absolute values and relative changes from baseline for gene expression will be listed. If deemed appropriate, gene expression data may be log-transformed and/or graphically summarized.

9.4.5.2 Estimation of Pharmacodynamic Parameters

Further details on the calculation of the pharmacodynamic parameters will be provided in the SAP.

9.4.6 Other Analyses

9.4.6.1 Analysis of Biomarkers

Descriptive statistics (e.g., n, mean, SD, median, minimum CV, geometric mean and geometric CV where applicable) will be used to summarize the effects of ASP0367 (result and relative change from baseline) for serum FLNC, CYC and ALDOA, and urinary TITIN. The summaries will be presented by treatment group and by scheduled sampling time point.

For 24-week treatment analysis, a summary will be provided by treatment group.

Descriptive statistics will be used to summarize results and relative change of biomarker measurements from baseline by time point.

All biomarker data (results and relative change from baseline) will be listed. In addition, biomarker data may be summarized graphically and/or descriptively as they relate to clinical measures, as applicable. Additional exploratory biomarker analyses may be performed and defined in a biomarker SAP.

9.4.6.2 Analysis of Genotyping

Polymorphisms of genes PPARδ and PPARGC1A will be tabulated. In addition, polymorphisms of genes may be summarized graphically and/or descriptively as they relate to clinical measures, as applicable. All analyses described in this section are based on availability of the data.

9.4.6.3 Exposure-response Analysis

The relationship between PK parameters (e.g., C_{trough}) of ASP0367 and efficacy, safety and pharmacodynamics will be investigated. The details of this analysis will be described in the SAP. Other exposure metrics such as $C_{ave,ss}$ may be investigated using pharmacokinetic parameters estimated in population pharmacokinetic analysis and reported separately from the CSR, as calculable pending the population PK model.

9.5 Additional Conventions

If the start and stop dates of AEs and concomitant medications are incomplete, imputed dates will be used to determine whether an AE is/is not treatment emergent or to allocate a concomitant medication to the study period it was taken.

Refer to the SAPs for details of the definition for analysis windows to be used for analyses by visit.

9.6 Interim Analysis

9.6.1 Interim Analysis of Efficacy

The analysis of futility and of efficacy will be performed when approximately 30 participants complete the 6MWT assessment at week 24 for the evaluation of whether a change may be observed in the primary endpoint. If no appreciable change is noted, the study may be stopped due to futility. If efficacy is observed, the study may continue or may be brought to a completion in order to proceed to a final full analysis of the study.

The change from baseline in distance walked on the 6MWT at week 24 will be 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 may be analyzed at the interim analysis.

The interim analysis outputs will be prepared by an external statistician and presented to the DMC for interim analysis who will make recommendations about the ongoing conduct of the study.

Details of the analysis will be defined in the interim analysis plan.

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 will be 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.

9.6.2 Interim Analysis of Pharmacokinetics and Pharmacodynamics

An interim analysis of pharmacokinetic and pharmacodynamic data may be conducted with data from approximately 30 participants. These analysis results will help in understanding the results of the interim analysis for futility and efficacy. The interim analysis will be detailed in

a separate interim pharmacokinetic and pharmacodynamic analysis plan. Personnel external to the study team will be specified in the interim analysis plan and will analyze the pharmacokinetic and pharmacodynamic data and prepare a report to be shared with the DMC and others. Only mean values and figures with variability will be shared with the study team and DMC to maintain the study team blind before database lock.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Ethical, Regulatory and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
  - Applicable ICH GCP Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator's Brochure and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable) and all other applicable local regulations

10.1.2 Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent of Participants

10.1.3.1 Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of

21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.

- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the signed ICF must be provided to the participant.

10.1.3.2 Supply of New and Important Information Influencing the Participant's Consent and Revision of the Written Information

- The investigator or his/her representative will immediately inform the participant verbally whenever new information becomes available that may be relevant to the participant's consent or may influence the participant's willingness to continue participating in the study (e.g., report of serious adverse drug reaction). The communication must be documented in the participant's medical records and whether the participant is willing to remain in the study or not must be confirmed and documented.
- The investigator must update the participant's ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the participant on all updated ICFs throughout their participation in the study. The investigator or his/her designee must reconsent participants with the updated ICF even if relevant information was provided verbally. The investigator or his/her representative who obtained the written informed consent and the participant should sign and date the ICF. A copy of the signed ICF will be given to the participant and the original will be placed in the participant's medical record. An entry must be made in the participant's records documenting the reconsent process.

10.1.4 Data Protection

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited unless the participant provides written consent or approval. Additional medical information may be given only after approval of the participant to the investigator or to other appropriate medical personnel responsible for the participant's well-being.

The sponsor shall not disclose any confidential information on participants obtained during the performance of their duties in the study without justifiable reasons.

Even though any individuals involved in the study, including the study monitors and auditors, may get to know matters related to a participant's privacy due to direct access to source documents, or from other sources, they may not disclose the content to third parties.

The sponsor affirms the participant's right to protection against invasion of privacy. Only a participant identification number will identify participant data retrieved by the sponsor. However, the sponsor requires the investigator to permit the sponsor, sponsor's

representative(s), the IRB/IEC and when necessary, representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

The sponsor agrees to comply and process personal data in accordance with all applicable privacy laws and regulations, including, without limitation, the Personal Information Protection Law in Japan and privacy laws in the US. If the services will involve the collection or processing of personal data (as defined by applicable data protection legislation) within the European Economic Area (EEA), then the sponsor shall serve as the controller of such data, as defined by the EU Data Protection Directive (DPD), and investigator and/or third party shall act only under the instructions of the sponsor in regard to personal data. If the sponsor is not based in the EEA, the sponsor must appoint a third party to act as its local data protection representative or arrange for a co-controller established in the EU for data protection purposes in order to comply with the DPD.

10.1.5 Committee(s) Structure

10.1.5.1 Data Monitoring Committees

This study will utilize an independent DSMB and a separate DMC for the interim analysis and their operation will be defined in detail in the charter documents.

10.1.5.1.1 Data and Safety Monitoring Board

The DSMB will be an independent and external multidisciplinary group consisting of at least 2 physicians and a biostatistician. One of the physicians will be named DSMB Chair and at least 1 of these physicians must be an expert in mitochondrial disorders. The third member of the DSMB will be a biostatistician with relevant experience in drug development. All DSMB members will be familiar with the general guidelines for data and safety monitoring set forth by FDA and other relevant regulatory authorities. The DSMB meeting(s) will be conducted periodically (i.e., every 3 months) following the first meeting to be held after the first 30 participants complete week 2 assessments during the double-blind treatment period in person or via teleconference with both an open and closed session according to the DSMB charter.

The DSMB will assess the overall safety and tolerability of ASP0367 throughout the course of the study. Safety data including AEs, vital signs, routine 12-lead ECGs, safety laboratory tests and cumulative AE data will be reviewed in an unblinded fashion. The DSMB may provide recommendations to continue or terminate the study if deemed in the best interest of the participants.

10.1.5.1.2 Data Monitoring Committee for Interim Analysis

The DMC for interim analysis will be an Astellas internal multidisciplinary group.

The DMC for interim analysis will assess the efficacy of the study treatments and make recommendations about the ongoing conduct of the study. The DMC for interim analysis will be responsible for examining the data reported by treatment assignment, including the 6MWT assessment at week 24, using statistical boundaries specified in the interim analysis plan. The DMC could recommend the discontinuation of the study due to efficacy or futility. The

interim analysis will be detailed in an interim analysis plan and presented to the DMC who will make recommendations about the ongoing conduct of the study.

10.1.6 Dissemination of Clinical Study Data

ICH E3 guidelines recommend, and EU Directive 2001/83/EC requires, that a final CSR that forms part of a marketing authorization application, be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator(s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. The representative for the coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the sponsor prior to database lock.

10.1.7 Data Quality Assurance

- All participant data relating to the study will be recorded on the CRF unless transmitted to the sponsor or designee electronically in an external data file (e.g., central laboratory data). The investigator is responsible for verifying that data entries on the CRF are accurate and correct by physically or electronically signing the CRF.
- Guidance on completion of CRFs will be provided in a separate CRF Completion Guideline.
- The investigator must permit study-related monitoring, audits, IRB/IEC review and regulatory agency inspections and provide direct access to source data documents.
- Quality tolerance limits (QTLs) will be predefined in the applicable plan(s) to identify systematic issues that can impact participant safety and/or reliability of study results. These predefined parameters will be monitored during the study, and important deviations from the QTLs and remedial actions taken will be summarized in the CSR.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., CROs).
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator according to ICH or applicable local regulatory requirements, whichever is longer, after study completion. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source Documents

1. Source data must be available at the study site to document the existence of the participants and to substantiate the integrity of study data collected. Source data must

include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

2. The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
3. The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are handwritten on paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved or transmitted electronically via computerized systems (and/or other kind of electronic devices) as part of regulated study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol-related assessments, AE tracking, electronic clinical outcome assessment and/or drug accountability.
4. Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments and audit trail information, if applicable). All printed records must be kept in the participant's file and be available for archiving.
5. Study monitors will perform ongoing source data review to confirm that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP and all applicable regulatory requirements.

10.1.9 Study and Site Start and Closure

The study start date is the date the first participant signs the ICF for the study.

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further study test product development

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines

- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
- Total number of participants included earlier than expected

If the study is prematurely terminated or suspended, the sponsor or designee shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

10.1.10 Arrangement for Use of Information and Publication of the Study

Information concerning the test product, patent applications, processes, unpublished scientific data, the Investigator's Brochure and other pertinent information is confidential and remains the property of the sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the sponsor will use the information obtained during the study in connection with the development of the product and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this study, the investigator understands that he/she has an obligation to provide the sponsor with all data obtained during the study.

Publication of the study results is discussed in the study agreement.

10.1.11 Quality Assurance

The sponsor is implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded and reported in compliance with the protocol, GCP and applicable regulatory requirement(s). Where applicable, the QA and QC systems and written SOPs of the CRO will be applied.

The sponsor or sponsor's designee may arrange to audit the study at any or all study sites and facilities. The audit may include on-site review of regulatory documents, CRFs and source documents. Direct access to these documents will be required by the auditors.

10.2 Appendix 2: Contraception Requirements

WOCBP who are eligible for participation in the study, including those who choose complete abstinence, must have pregnancy tests as specified in the schedule of assessments. Pregnancy test results must confirm that the participant is not pregnant.

WOMEN OF CHILDBEARING POTENTIAL DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS

A female is considered fertile (i.e., WOCBP) following menarche and until becoming postmenopausal unless permanently sterile.

Females in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal with one of the following (i.e., permanently sterile):
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
- Postmenopausal

A postmenopausal state is defined as at least 12 months after last menstrual bleeding without an alternative medical cause.

In case the last menstrual bleeding cannot be clearly determined, confirmation with more than one follicle-stimulating hormone (FSH) measurement of at least > 40 IU/L (or higher per local institutional guidelines) is required.

Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status by repeated FSH measurements before study enrollment.

Documentation of any of these categories can come from the study site personnel's review of the female participant's medical records, medical examination or medical history interview.

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILDBEARING POTENTIAL

Female participants of childbearing potential are eligible for participation in the study if they agree to use one of the highly effective methods of contraception listed below from the time of signing the ICF and until the end of relevant systemic exposure, defined as 30 days after the final IP administration.[a]

Highly effective methods of contraception (failure rate of < 1% per year when used consistently and correctly):[b]

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
  - Oral
  - Injectable
  - Implantable
- Other combined (estrogen- and progesterone-containing) methods
  - Vaginal ring
  - Injectable
  - Implantable
  - Intrauterine hormone-releasing system or intrauterine device
  - Bilateral tubal occlusion
- Vasectomized partner
  A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
- Sexual abstinence
  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the test product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant. It is not necessary to use any other method of contraception when complete abstinence is elected.

[a]Local laws and regulations may require use of alternative and/or additional contraception methods.

[b]Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILDBEARING POTENTIAL

Male participants with female partners of childbearing potential are eligible for participation in the study if they agree to the following during treatment and until the end of relevant systemic exposure defined as 30 days after final drug administration.[a]

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator
- Use a condom
- Female partners of male participants who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception

[a]Local laws and regulations may require use of alternative and/or additional contraception methods.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

10.3.1 Definition of Adverse Events

AE Definition:

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study IP, whether or not considered related to the study IP.

"Adverse event" means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study IP. This includes events related to the comparator and events related to the (study) procedures.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study IP administration even though it may have been present before the start of the study.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, biochemistry or urinalysis) or other safety assessment (e.g., vital signs, physical examination or ECGs), including those that

worsen from baseline, that is considered to be clinically significant in the medical and scientific judgment of the investigator and not related to underlying disease, is to be reported as an (S)AE.

Any clinically significant abnormal laboratory finding or other abnormal safety assessment, which is associated with the underlying disease, does not require reporting as an (S)AE, unless judged by the investigator to be more severe than expected for the participant's condition.

Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

10.3.1.2 Potential Cases of Drug-induced Liver Injury

Refer to [Section 10.4, Appendix 4: Liver Safety Monitoring and Assessment] for detailed instructions on drug induced liver injury. Abnormal values in AST and/or ALT concurrent or with abnormal elevations in TBL that meet the criteria outlined in [Section 10.4, Appendix 4: Liver Safety Monitoring and Assessment], in the absence of other causes of liver injury, are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and are always to be considered important medical events and reported per [Section 10.3.6 Reporting Procedures for Serious Adverse Events].

10.3.2 Definition of Serious Adverse Events

An SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death
- Is life-threatening

  The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization
  - In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
  - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent or significant disability/incapacity
  - The term disability means a substantial disruption of a person's ability to conduct normal life functions.

  - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza and accidental trauma (e.g., sprained ankle), which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect
- Other situations:
  - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
  - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

If an event is not an AE per definition in [Section 10.3.1 Definition of Adverse Events], then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

10.3.3 Assessment of Causality

- The investigator is obligated to assess the relationship between study IP and each occurrence of each AE/SAE.
- A “reasonable possibility” of a relationship conveys that there are facts, evidence and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy and other risk factors, as well as the temporal relationship of the event to study IP administration will be considered and investigated.
- The investigator will also consult the Investigator’s Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each (S)AE, the investigator must document in the medical notes that he/she has reviewed the (S)AE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the sponsor.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Following a review of the relevant data, the causal relationship between the IP and each (S)AE will be assessed by answering "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the IP?"

When making an assessment of causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a "reasonable possibility" that an (S)AE may have been caused by the IP (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Has the participant been administered IP?
- Plausibility (i.e., could the event have been caused by the suspect IP? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and study data, etc.)
- Dechallenge/dose reduction/rechallenge:
  - Dechallenge: Did the (S)AE resolve or improve after only stopping the dose of the suspect drug without any treatment?
  - Dose reduction: Did the (S)AE resolve or improve after reducing the dose of the suspect drug?
  - Rechallenge: Did the (S)AE reoccur if the suspected drug was reintroduced after having been stopped?
- Laboratory or other test results: a specific lab investigation supports the assessment of the relationship between the (S)AE and the IP (e.g., based on values pre-, during and post-treatment)
- Available alternative explanations independent of IP exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation
- Temporal relationship between exposure to the IP and (S)AE onset and/or resolution. Did the (S)AE occur in a reasonable temporal relationship to the administration of the IP?
- Finally, judging which are more likely based on all the above contents, factors of reasonable possibility or confounding factors, comprehensive judgment of plausible will be provided.

There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. While it is very important that the investigator always assesses causality for every event before the initial transmission of the SAE data to the sponsor, the initial report should be submitted without delay (i.e., within 24 hours of awareness). With limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of "no" is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise his/her assessment of causality in light of new

information regarding the SAE and shall send an SAE follow-up report and update the CRF with the new information and updated causality assessment.

10.3.4 Assessment of Severity

AEs, including abnormal clinical laboratory values, will be graded using the National Cancer Institute-Common Terminology Criteria for Adverse Event (NCI-CTCAE) guidelines (version 5.0). The items that are not stipulated in the NCI-CTCAE version 5.0 will be assessed according to the criteria below and entered into the CRF:

Table 8 Grading Scale Defining the Severity of an Adverse Event

Grade	Assessment Standard
1 – Mild	Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
2 – Moderate	Minimal local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL†
3 – Severe	Medically significant but not immediately life threatening, hospitalization or prolonged hospitalization indicated; disabling; limiting self-care ADL‡
4 – Life-threatening	Life threatening consequences, urgent intervention indicated
5 – Death	Death related to AE

ADL: activities of daily living; AE: adverse event

†Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

‡Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

10.3.5 Recording and Follow-Up of AEs and/or SAEs

AE and SAE Recording

- When an (S)AE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports and diagnostics reports) related to the event.
- The investigator will then record all relevant (S)AE information in the CRF.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to the sponsor in lieu of completion of the CRF.
- There may be instances when copies of medical records for certain cases are requested by the sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the sponsor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the (S)AE.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the (S)AE as fully as possible. This may

include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any post mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.6 Reporting Procedures for Serious Adverse Events

The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the sponsor by fax or email immediately (within 24 hours of awareness).

The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data, as well as the investigator causality assessment including the explanation for the causality assessment.

If the SAE is associated with emergency unblinding by the investigator as outlined in [Section 6.3.4 Breaking the Treatment Code for Emergency], this is to be recorded on the SAE worksheet. On the SAE worksheet, the investigator is to include when unblinding took place in association with the SAE.

For contact details, see [Contact Details of Sponsor's Key Personnel]. Fax or email the SAE/special situations/product defect worksheet to:

Astellas Pharma Global Development Inc.
Pharmacovigilance
North America fax number: +1-888-396-3750
North America alternate fax number: +1-847-317-1241
International Fax: +44-800-471-5263
Email: safety-us@astellas.com

If there are any questions, or if clarification is needed regarding the SAE, please contact the sponsor's medical monitor/study physician or their designee [Contact Details of Sponsor's Key Personnel].

Follow-up information for the event should be sent promptly (as soon as available, but no longer than within 7 days of the initial notification).

Full details of the SAE should be recorded on the medical records, SAE/special situation worksheet and on the CRF.

The following minimum information is **required**:

- International study number/study number
- Participant number, sex and age

- Date of report
- Description of the SAE (event and seriousness criteria)
- Causal relationship to the IP (including reason)
- Drug provided (if any)

The sponsor or sponsor's designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality and expectedness of the events (e.g., SUSAR reporting) according to current local/regional regulatory requirements. The sponsor or sponsor's designee will submit expedited safety reports to competent authorities and concerned ethics committee per current local regulations, and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports as required by their IRB/IEC within timelines set by regional regulations (e.g., EMA, FDA) where required. Documentation of the submission to and receipt by the IRB/IEC of expedited safety reports should be retained by the study site. In the US, FDA expedited IND reporting guidelines will be followed.

The sponsor will notify all investigators responsible for ongoing clinical studies with the test product of all SUSARs, which require submission per local requirements.

The investigators should provide written documentation of IRB/IEC notification for each report to the sponsor.

The investigator may contact the sponsor's medical monitor/study physician for any other problem related to the rights, safety or well-being of the participant.

10.3.7 Reporting Procedures for Special Situations

10.3.7.1 Contraceptive Guidance and Collection of Pregnancy Information

If a female participant becomes pregnant during the study dosing period or within 30 days from the discontinuation of dosing, the investigator is to report the information to the sponsor according to the timelines in [Section 10.3.6 Reporting Procedures for Serious Adverse Events] using the SAE worksheet as a special situation and in the CRF.

The investigator will attempt to collect pregnancy information on any female partner of a male participant who becomes pregnant during the study dosing period or within 30 days from the discontinuation of dosing and report the information to the sponsor according to the timelines in [Section 10.3.6 Reporting Procedures for Serious Adverse Events] using the special situation worksheet or pregnancy form.

The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data, etc., should be included in this information.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female participant as an AE in the CRF or SAE per [Section 10.3.6 Reporting Procedures for Serious Adverse Events]. Participant pregnancy outcomes listed below are to be reported as SAEs:

- Spontaneous abortion/miscarriage, abortion and missed abortion
- Death of a newborn or infant within 1 month after birth is to be reported as an SAE regardless of its relationship with the IP.
- If an infant dies more than 1 month after the birth, it is to be reported if a relationship between the death and intrauterine exposure to the IP is judged as *"*possible*"* by the investigator.
- Congenital anomaly (including anomaly in miscarried fetus)
- Benign hydatidiform mole
- Blighted ovum

Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination or other means as appropriate. (S)AEs experienced by the newborn/infant should be reported via the pregnancy reporting form. Generally, follow up will be no longer than 6 to 8 weeks following the estimated delivery date.

10.3.7.2 Medication Error, Overdose and "Off-label Use"

If a medication error (defined as an unintended failure in the treatment process that leads to, or has the potential to lead to, harm to the participant), overdose or "off-label use" (i.e., use outside of the target disease defined in the protocol) is suspected, refer to [Section 6.7 Treatment of Overdose]. Any associated (S)AEs are to be reported in the CRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.6 Reporting Procedures for Serious Adverse Events] together with the details of the medication error, overdose and/or "off-label use."

10.3.7.3 Suspected Misuse/Abuse of Investigational Product

Definition of misuse: Situations where the IP is/are intentionally and inappropriately used not in accordance with the intended use as defined in the protocol.

Definition of abuse: Persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects.

If misuse or abuse of the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the CRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.6 Reporting Procedures for Serious Adverse Events] together with details of the misuse or abuse of the IP.

10.3.7.4 Occupational Exposure

If occupational exposure (e.g., inadvertent exposure to the IP of study site personnel while preparing it for administration to the participant) to the IP occurs, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs occurring to the individual associated with or resulting from the special situation are to be reported on the special situations worksheet.

10.3.7.5 Suspected Drug-drug Interaction

If a drug-drug interaction associated with the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the CRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.6 Reporting Procedures for Serious Adverse Events] together with details of the suspected drug-drug interaction.

10.3.8 Supply of New Information Affecting the Conduct of the Study

When new information becomes available that is necessary for conducting the study properly, the sponsor will inform all investigators involved in the study, as well as the appropriate regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

The investigator will also inform the participants who will be required to sign an updated ICF in order to continue in the study.

10.3.9 Urgent Safety Measures

An urgent safety measure (USM) is an intervention that is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the sponsor, relevant competent authorities, IRB/IEC, where applicable, in order to protect participants from any immediate hazard to their health and/or safety. Either the investigator or the sponsor can initiate a USM. The cause of a USM can be safety-, product- or procedure-related.

10.3.10 Reporting Urgent Safety Measures

In the event of a potential USM, the investigator must contact the study physician (within 24 hours of awareness). Full details of the potential USM are to be recorded in the participant's medical records. The sponsor may request additional information related to the event to support their evaluation.

If the event is confirmed to be a USM, the sponsor will take appropriate action to ensure the safety and welfare of the participants. These actions may include but are not limited to a change in study procedures or study treatment, halting further enrollment in the study, or stopping the study in its entirety. The sponsor or sponsor's designee will notify the relevant competent authorities and concerned ethics committee within the timelines required per current local regulations, and will inform the investigators, as required. When required, investigators must notify their IRB/IEC within timelines set by regional regulations.

10.4 Appendix 4: Liver Safety Monitoring and Assessment

The purpose of this appendix is to provide guidance for the monitoring of drug-induced liver injury during the course of the study. It should be noted that this section does not specify the end-of-study analyses of liver enzymes. The end-of-study liver enzymes analyses will be described in the SAP. Any participant enrolled in the study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × ULN or TBL > 2 × ULN should undergo detailed testing for liver enzymes (including at least gamma-glutamyltransferase [GGT], ALT, AST, alkaline phosphatase [ALP], TBL and glutamate dehydrogenase). Participants whose baseline ALT or AST values were > 3 × ULN (with elevated creatine kinase [CK]) and who have a clinically significant rise as assessed by the investigator should also undergo detailed testing for liver enzymes. Testing should be repeated within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator and study team. Participants should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

In addition, free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) should be measured to monitor the thyroid function in case of hepatobiliary dysfunction. If FT3, FT4 or TSH show any abnormalities and/or clinically significant symptoms, the investigator should determine if further follow up of liver chemistry tests and/or thyroid function tests are required.

For cases with concurrent CK elevation, participants should undergo detailed testing for liver enzymes (including at least GGT, ALT, AST, ALP, TBL and glutamate dehydrogenase). Once the investigator can confirm absence of hepatobiliary injury based on the detailed liver enzyme testing, the investigator will determine if further follow up of liver chemistry tests are required. If concurrent hepatobiliary injury cannot be ruled out, the guidance should be followed as described in Follow-up Procedures in this appendix.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN is as shown below.

Table 9 Moderate and Severe Liver Abnormalities

	ALT or AST		TBL
Moderate	> 3 × ULN	or	> 2 × ULN
Severe	> 3 × ULN	and†	> 2 × ULN

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBL: total bilirubin; ULN: upper limit of normal

†Samples taken simultaneously or within maximum 24 hours.

In addition, the participant should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks

- ALT or AST > 3 × ULN and† international normalized ratio (INR) > 1.5 (If INR testing is applicable/evaluated)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

†Samples taken simultaneously or within a maximum of 24 hours.

The investigator may determine that abnormal liver chemistry results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and clinical laboratory tests. The study site personnel are to complete the liver abnormality case report form (LA-CRF). Participants with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated 2 to 3 times weekly, and then weekly or less if abnormalities stabilize or the IP has been discontinued and the participant is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a SAE. The sponsor should be contacted and informed of all participants for whom severe hepatic liver function abnormalities possibly attributable to IP are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases are to be recorded as “AEs” within the CRF. Illnesses and conditions such as hypotensive events and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic participants and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications are to be entered in the CRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the participant’s history, other testing may be appropriate including:
  - Acute viral hepatitis (A, B, C, D, E or other infectious agents)
  - Ultrasound or other imaging to assess biliary tract disease
  - Other clinical laboratory tests, including INR and direct bilirubin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Study Treatment Discontinuation

In the absence of an explanation for increased liver chemistry tests, such as viral hepatitis, preexisting or acute liver disease, or exposure to other agents associated with liver injury, the participant may be discontinued from study treatment. The investigator may determine that it is not in the participant's best interest to continue study treatment. Discontinuation of study treatment should be considered if the participant has severe hepatic abnormalities.

In addition, if close monitoring for a participant with moderate or severe hepatic laboratory tests is not possible, study treatment should be discontinued.

Hy's Law definition: Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10% to 50% mortality (or transplant).

The 2 "requirements" for Hy's Law are:

1. Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in AT elevations > 3 × ULN ("2 × ULN elevations are too common in treated and untreated participants to be discriminating").
2. Cases of increased TBL (at least 2 × ULN) with concurrent AT elevations at least 3 × ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert's syndrome [Temple, 2006].

FDA Guidance for Industry titled, "Drug-induced Liver Injury: Premarketing Clinical Evaluation" issued by the FDA on July 2009:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations > ULN of ALT or AST than the (nonhepatotoxic) control drug or placebo.
2. Among participants showing such AT elevations, often with ATs much greater than 3 × ULN, one or more also show elevation of serum TBL to > 2 × ULN, without initial findings of cholestasis (elevated serum ALP).
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.

10.5 Appendix 5: List of Excluded Concomitant Medications

Type of Prohibited Medication	Name of Medication
PPAR ligands	clinofibrate, clofibrate, fenofibrate, gemfibrozil, fibrates, thiazolidinediones
P-gp substrates	dabigatran, digoxin, fexofenadine
BCRP substrates	rosuvastatin, sulfasalazine
OATP1B1 and OATP1B3 substrates	asunaprevir, atorvastatin, bosentan, danoprevir, docetaxel, fexofenadine, nateglinide, paclitaxel, repaglinide, rosuvastatin, simvastatin
OAT3 substrates	cefaclor, ceftizoxime, famotidine, furosemide, methotrexate, oseltamivir carboxylate, penicillin G
Strong and Moderate Inhibitors of CYP3A	boceprevir, cobicistat, danoprevir/ritonavir, elvitegravir/ritonavir, grapefruit juice, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, paritaprevir/ritonavir/(ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir/ritonavir, telaprevir, tipranavir/ritonavir, telithromycin, troleandomycin, voriconazole, clarithromycin, idelalisib, nefazodone, nelfinavir, aprepitant, ciprofloxacin, conivaptan, crizotinib, cyclosporine, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, verapamil
BCRP inhibitors	curcumin, cyclosporine A, eltrombopag
Strong and moderate inducers of CYP3A and inducers for UGT1A3, UGT1A8, UGT1A9 and transporters	apalutamide, carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's Wort, bosentan, efavirenz, etravirine, phenobarbital, primidone, ritonavir, telmisartan
Inhibitors of UGT1A3, UGT1A8 and UGT1A9	probenecid, mefenamic acid, tipranavir, tipranavir/ritonavir, atazanavir, atazanavir/ritonavir
Triheptanoin, a synthetic medium chain triglyceride	Triheptanoin

BCRP: breast cancer resistance protein; CYP: cytochrome P450; OAT: organic anion transporter; OATP: organic anion transporting polypeptide; P-gp: P-glycoprotein; PPAR: peroxisome proliferator-activated receptor; UGT: uridine diphosphate-glucuronosyltransferase

10.6 Appendix 6: Clinical Laboratory Assessments

Laboratory tests will be performed according to the schedule of assessments.

Table 10 Clinical Laboratory Tests

Panel/Assessments	Parameters to be Analyzed
Hematology	Hematocrit (Hct) Hemoglobin (Hgb) Mean corpuscular hemoglobin concentrations (MCHC) Mean corpuscular volume Platelet count Red blood cell count (RBC) White blood cell count (WBC) White blood cell differential
Biochemistry	Alanine aminotransferase (ALT) Albumin (Alb) Alkaline phosphatase (ALP) Amylase Aspartate aminotransferase (AST) Blood urea nitrogen (BUN) Calcium (Ca) Cardiac troponin I (cTnI) Cardiac troponin T (cTnT) Chloride (Cl) C-reacting protein (CRP) Creatinine Creatine kinase (CK) Creatine kinase isoenzymes: • Heart muscle (CK-MB)¶ • Skeletal and heart muscle (CK-MM)¶ Cystatin C Gamma glutamyl transferase (GGT) Glucose (Gluc) Glutamate dehydrogenase (GLDH) HbA1c High-density lipoproteins (HDL) Inorganic phosphorus (P) Lactate dehydrogenase (LDH) Lipase Low-density lipoproteins (LDL) Magnesium (Mg) Plasma lactate Potassium (K) Sodium (Na) Total bilirubin (TBL) Total cholesterol (TC) Total protein (TP) Triglycerides (TG) Uric acid (UA)
Table continued on next page	

Panel/Assessments	Parameters to be Analyzed
Abbreviated hematology	Hemoglobin (Hgb) Platelet count Red blood cell count (RBC) White blood cell count (WBC) White blood cell differential
Abbreviated biochemistry	Alanine transaminase (ALT) Alkaline phosphatase (ALP) Amylase Aspartate transaminase (AST) Blood urea nitrogen (BUN) Calcium (Ca) Cardiac troponin I (cTnI) Cardiac troponin T (cTnT) Chloride (Cl) Creatinine Creatine kinase (CK) Creatine kinase isoenzymes: • Heart muscle (CK-MB)¶ • Skeletal and heart muscle (CK-MM)¶ Cystatin C Gamma glutamyl transferase (GGT) HbA_{1c} Lipase Potassium (K) Sodium (Na) Total bilirubin (TBL)
Serology	Hepatitis A virus antibodies (immunoglobulin M) Hepatitis B surface antigen Hepatitis C virus antibodies Human immunodeficiency virus 1+2 antibodies
Urinalysis	Blood Creatinine (Cr) Glucose pH Protein Urine specific gravity
Pregnancy test (serum)	Human chorionic gonadotropin
Pregnancy test (urine)	Human chorionic gonadotropin
Urine drug test	Amphetamines Barbiturates Benzodiazepines Cannabinoids Cocaine MDMA Methadone Methamphetamines Opiates Oxycodone
Urine alcohol test	Alcohol

Note: FT3, FT4 and TSH should be measured to monitor thyroid function in case of hepatobiliary dysfunction.

¶Creatine kinase isoenzymes (CK-MB and CK-MM) are measured in reflex test based on CK result.

10.7 Appendix 7: Pharmacogenomic Analysis with Banked Sample

INTRODUCTION

PGx research aims to provide information regarding how naturally occurring differences in a participant's gene and/or expression of genes based on genetic variation may impact what treatment options are best suited for the participant. Through investigation of PGx by technologies such as genotyping, gene sequencing, statistical genetics and Genome-Wide Association studies, the relationship between gene profiles and a drug's kinetics, efficacy, toxicity or disease may be better understood. As many diseases may be influenced by one or more genetic variations, PGx research may identify which genes are involved in determining the way a participant may or may not respond to a drug.

OBJECTIVES

The PGx research that may be conducted in the future with acquired blood samples is exploratory. The objective of this research will be to analyze or determine genes of relevance to clinical response, pharmacokinetics and/or toxicity/safety and/or disease.

By analyzing genetic variations, it may be possible to predict an individual participant's response to treatment in terms of efficacy and/or toxicity and/or disease.

PARTICIPANT PARTICIPATION

Participants who have consented to participate in this study may participate in the PGx substudy. Participants must provide written consent prior to providing any blood samples that may be used at a later time for PGx analysis.

SAMPLE COLLECTION AND STORAGE

Participants who consent to participate in this substudy will provide 4 mL sample of whole blood per Astellas' instructions. Each sample will be identified by the unique participant number. Samples will be shipped to a designated banking CRO as directed by Astellas.

PGx ANALYSIS

Details on the potential PGx analysis cannot be established yet. Astellas may initiate the PGx analysis if evidence suggests that genetic variants may be influencing the drug's pharmacokinetics, efficacy and/or safety and/or disease.

DISPOSAL OF PGx SAMPLES/DATA

All PGx samples collected will be stored for a period of up to 15 years following study database lock. If there is no requirement for analysis, the whole blood sample will be destroyed after the planned storage period. The participant has the right to withdraw consent at any time. When a participant's withdraw notification is received, the PGx sample will be destroyed. The results of any PGx analysis conducted on a sample prior to its withdrawal will be retained at Astellas indefinitely unless otherwise specified by local regulation.

INFORMATION DISCLOSURE TO THE PARTICIPANTS

Exploratory PGx analysis may be conducted following the conclusion of the study, if applicable. The results of the PGx analysis will not be provided to any investigators or participants, nor can the results be requested at a later date. Any information that is obtained from the PGx analysis will be the property of Astellas.

10.8 Appendix 8 Clinical Study Continuity

INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).

BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated schedule of assessments [Table 1] unless the site principal investigator discusses the need with the Astellas medical monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study-required assessments and procedures if study activities are unable to be performed as described in [Section 7] due to a crisis.

INFORMED CONSENT

Participants who need to follow any or all of the alternate measures outlined in this Appendix will be required to provide informed consent, which explicitly informs them of the nature of and rationale for these changes and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the principal investigator or designee will obtain oral agreement from the participant followed by written documentation as soon as is feasible. A separate addendum to the study ICF will be provided to document the participant's consent to the changes.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if 1 or more of the following conditions are met due to the crisis:

- Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible.
- Site facilities have been closed for clinical study conduct.
- Site has been restricted to treating patients with conditions outside of the scope of the study.
- Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel.
- Participant(s) have temporarily relocated from the current study site to an alternate study site to avoid placing a burden on the participant with respect to travel.

- Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel.
- Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety.

Adherence to the original protocol as reflected in the schedule of assessments [Table 1] is expected, where plausible, in the case of a crisis. The alternate measures as noted in [Table 11] below are only permissible in the event of a crisis, and after discussing the need with the Astellas medical monitor to implement the alternate measures. This is to allow for continuity of receiving study intervention and maintaining critical safety and efficacy assessments for participants in the study at a time of crisis.

If 1 or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding timepoint(s).

Table 11 Alternative Schedule of Assessments in Response to a Crisis

Critical Assessments	Alternate Approach(es)	Timepoints
Physical examination	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	Refer to protocol schedule of assessments.
Body weight	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
Vital signs	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
Hematology, biochemistry	Home visits with follow-up phone call are allowed. At home visits, the assessments can be replaced with abbreviated biochemistry and abbreviated hematology.	
Urinalysis	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
Abbreviated biochemistry, abbreviated hematology	Home visits with follow-up phone call are allowed.	
Pregnancy test (urine)	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
12-lead ECG	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
C-SSRS	Interview via phone by trained site staff is acceptable.	

Table continued on next page

Critical Assessments	Alternate Approach(es)	Timepoints
6MWT	V2 (week 0) and V11 (week 24) are required but missed other timepoints are acceptable when the duration of the site visit should be minimized.	Refer to protocol schedule of assessments.
5XSTS	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
Blood for plasma pharmacokinetics	Expected to be performed onsite but missed assessments can be acceptable where unfeasible.	
Serum and urinary biomarkers	Missed assessments are acceptable when the duration of the site visit should be minimized.	
Blood for pharmacodynamics (PPARδ target gene expression)	Expected to be performed onsite but missed assessments can be acceptable where unfeasible.	
Neuro-QoL Short Form Fatigue, Neuro-QoL Short Form Lower Extremity Function (Mobility), MFIS, PGIC, and PGIS	Recommended to be performed onsite but missed assessments are acceptable when the duration of the site visit should be minimized.	
Dispense/Collect IP	Direct-to-patient shipment is allowed.	
Previous and concomitant treatment	Home visits with follow-up phone call are allowed.	
Exercise status	Home visits with follow-up phone call are allowed.	
Adverse event assessment	Home visits with follow-up phone call are allowed.	

STUDY INTERVENTION SUPPLY

If any of the conditions outlined above in the Participants Procedures Assessment are met, the following mitigating strategy will be employed, as needed, to ensure continuity of study intervention supply to the participants:

- Direct-to-Participant (DTP) shipments of study intervention from the site to the participant's home.

DATA COLLECTION REQUIREMENTS

Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.

- Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window or other modifications) due to the crisis.

10.9 Appendix 9 List of Normal Ranges for Selected Laboratory Assessments

Analyte	Sex	Age	Reference Range
Troponin T (cTnT)	Both	≥ 18 years	≤ 14.0 pg/mL
Troponin I (cTnI)	Both	≥ 0 years	≤ 0.06 ng/mL

Source: Laboratory Specifications Document v11 (06 Nov 2023)

10.10 List of Abbreviations and Definition of Key Study Terms

List of Abbreviations

Abbreviations	Description of abbreviations
5XSTS	5 Times Sit to Stand
6MWT	6-minute walk test
AE	adverse event
ALDOA	aldolase A
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AME	absorption, metabolism and excretion
API	Astellas Pharma Inc.
AST	aspartate aminotransferase
AT	aminotransferases
AUC_{24}	area under the concentration-time curve from the time of dosing to 24 hours
AUC_{inf}	area under the concentration-time curve from zero to infinity
AUC_{last}	area under the concentration-time curve from the time of dosing to the last measurable concentration
AV	atrioventricular
AVAPS	average volume-assured pressure support
BCRP	breast cancer resistance protein
BiPAP	bilevel positive airway pressure
BMAS	biomarker analysis set
BMI	body mass index
CFR	Code of Federal Regulations
CK	creatine kinase
CK-MB	creatine kinase isoenzymes found in heart muscle
CK-MM	creatine kinase isoenzymes found in skeletal and heart muscle
C_{max}	maximum concentration
CoQ10	coenzyme Q10
CPAP	continuous positive airway pressure
CPEO	chronic progressive external ophthalmoplegia
CRF	case report form
CRO	contract research organization
CSR	clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
C_{trough}	concentration immediately prior to dosing at multiple dosing
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CV	coefficient of variation
CYC	cytochrome c
CYP	cytochrome P450
DB	double-blind

Abbreviations	Description of abbreviations
DIO	diet-induced obese
DMC	Data Monitoring Committee
DMD	Duchenne Muscular Dystrophy
DPD	Data Protection Directive
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram
EEA	European Economic Area
EU	European Union
eGFR	estimated glomerular filtration rate
EPKAS	extended pharmacokinetic analysis set
EQ-5D	European Quality of Life 5-dimension questionnaire
EQ-5D-5L	European Quality of Life 5-dimension, 5-level questionnaire
EuroQol	European Quality of Life
FAO	fatty acid oxidation
FAS	Full Analysis Set
FDA	Food and Drug Administration
FLNC	filamin-C
FSH	follicle-stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HEK293	human embryonic kidney 293
hERG	human ether-à-go-go-related gene
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
INR	international normalized ratio
IP	investigational product
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISN	international study number
KSS	Kearns-Sayre syndrome
LA-CRF	liver abnormality case report form
LOCF	last observation carried forward
MAD	multiple ascending dose
MATE	multidrug and toxin extrusion
mdx	X-linked mutation of the murine dystrophin gene

Abbreviations	Description of abbreviations
MELAS	mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes
MERRF	myoclonic epilepsy with ragged-red fibers
MFIS	Modified Fatigue Impact Scale
MMRM	mixed model for repeated measures
mRNA	messenger RNA
mtDNA	mitochondrial DNA
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
nDNA	nuclear DNA
Neuro-QoL	Quality of Life in Neurological Disorders
NOAEL	no observed adverse effect level
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
OLE	open-label extension
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
P-gp	P-glycoprotein
PGx	pharmacogenomics
PKAS	pharmacokinetic analysis set
PMM	Primary Mitochondrial Myopathy
PMMVA	Primary Mitochondrial Myopathy Video Assessment
PPAR	peroxisome proliferator-activated receptor
PPARδ	peroxisome proliferator-activated receptor delta
PPARGC1A	peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PT	preferred term
QA	quality assurance
QC	quality control
QTcF	QT interval using Fridericia's correction
QTL	quality tolerance limits
SAD	single ascending dose
(S)AE	serious adverse event or adverse event
SAE	serious adverse event
SAF	Safety Analysis Set
SAP	statistical analysis plan
SOC	system organ class
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reactions
TBL	total bilirubin
TEAE	treatment-emergent adverse event
TSH	thyroid stimulating hormone
t_{max}	time of maximum concentration
UGT	uridine diphosphate-glucuronosyltransferase

Abbreviations	Description of abbreviations
ULN	upper limit of normal
USM	urgent safety measure
WOCBP	woman/women of childbearing potential

Definition of Key Study 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.
Screening period	Period of time before entering the study period, usually from the time when a participant signs the consent form until just before the test product or comparative drug (sometimes without randomization) is given to a participant.
Study period	Period of time from the first study site initiation date to the last study site completing the study.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

11 REFERENCES

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12 COORDINATING INVESTIGATOR'S SIGNATURE

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/Protocol 0367-CL-1201

Version 9.0, Incorporating Substantial Amendment 8

24 Jan 2024

I have read all pages of this protocol for which Astellas is the sponsor. I agree that it contains all the information required to conduct this study.
Coordinating Investigator: Signature: _____ Date (DD-MMM-YYYY) Printed Name: _____ Address: _____ _____

13 SPONSOR SIGNATURES

Required sponsor signatures as required by ICH GCP 4.5.1 are located in the first attachment

Attachment 1	Electronic Sponsor Signatures

Global Protocol Format V9.0