CLINICAL STUDY PROTOCOL

Title: A Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of 24 Weeks Treatment With REN001 in Patients With Primary Mitochondrial Myopathy (PMM)

NCTID: NCT04535609

Document Date: 24 June 2022

CLINICAL STUDY PROTOCOL

A DOUBLE-BLIND, PLACEBO-CONTROLLED, STUDY TO EVALUATE THE EFFICACY AND SAFETY OF 24 WEEKS TREATMENT WITH REN001 IN PATIENTS WITH PRIMARY MITOCHONDRIAL MYOPATHY (PMM)

Sponsor:	Reneo Pharma Ltd. Innovation House, Discovery Park Ramsgate Road Sandwich Kent CT13 9FF United Kingdom
Clinical Research Organization:	Orphan-Reach Ltd. The Old School, Newport Road, Woughton Park, Milton Keynes MK6 3AP United Kingdom
Chief Investigator:	[redacted] Massachusetts General Hospital 175 Cambridge St. Boston MA 02114
Sponsor Protocol No.:	REN001-201
EudraCT No.:	2020-002855-40
IND No.:	148211
Study Drug Name:	REN001
Development Phase:	Phase 2b
Date of Protocol:	24 June 2022
Protocol version:	Final V4.0 Global Amendment 3.0

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki, and with other applicable regulatory requirements.

SIGNATURE PAGE

Declaration of Sponsor Chief Medical Officer

Title: A double-blind, placebo-controlled, study to evaluate the efficacy and safety of 24 weeks treatment with REN001 in patients with primary mitochondrial myopathy (PMM)

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2013 and the guidelines on Good Clinical Practice.

DocuSigned by:
[redacted]

_____ As time stamp____
Date

[redacted]

Institution: Reneo Pharma Ltd.

PROTOCOL SYNOPSIS

Title:	**A DOUBLE-BLIND, PLACEBO-CONTROLLED, STUDY TO EVALUATE THE EFFICACY AND SAFETY OF 24 WEEKS TREATMENT WITH REN001 IN PATIENTS WITH PRIMARY MITOCHONDRIAL MYOPATHY (PMM)**
Sponsor Study No:	REN001-201
Compound:	REN001
Phase:	Phase 2b
Sponsor:	Reneo Pharma Ltd.
Chief Investigator:	[redacted]
Design:	This is a randomized, double-blind, placebo-controlled, parallel group, multi-center, study designed to investigate the efficacy and safety of REN001 administered once daily over a 24-week period to patients with PMM. Subjects will be randomized (allocation 1:1) to the following two treatment groups: • REN001 100 mg/day • Placebo An independent Patient Screening Oversight Committee (PSOC) will review screening criteria to ensure subjects enrolled into the study have the correct PMM diagnosis prior to randomization. The structure, function and operation of the PSOC will be detailed in the REN001-201 PSOC Charter. An independently chaired Safety Review Committee (SRC) will review safety data at specified intervals for the duration of the study. The structure, function and operation of the SRC will be detailed in the REN001-201 SRC Charter. An exploratory sub-study (REN001-201-DXA) to assess change in the bone mineral density using Dual Energy X-ray Absorptiometry (DXA) scans after 24 weeks of treatment with REN001 is open to subjects at sites where regulatory and ethical approval is in place.
Objectives:	<u>Primary:</u> To evaluate the efficacy of REN001 in subjects with PMM treated for 24 weeks, assessed by the effect on exercise endurance. <u>Secondary:</u> To evaluate the efficacy of REN001 in subjects with PMM treated for 24 weeks, assessed by the effect on fatigue. <u>Safety:</u>

	To evaluate the safety and tolerability of REN001 in subjects with PMM during 24 weeks of treatment. Pharmacokinetic (PK): To investigate the pharmacokinetics of REN001 in subjects with PMM. Exploratory: To evaluate the efficacy of REN001 in subjects with PMM treated for 24 weeks, assessed by the effect on functional capacity. To evaluate the effect of REN001 on quality of life (QoL) of subjects with PMM after treatment for 24 weeks.
Endpoints	Primary: Change from Baseline at Week 24 in distance walked during the 12-Minute Walk Test (12MWT). Secondary: Change from Baseline at Week 24 in the Modified Fatigue Impact Scale (MFIS) Physical sub-scale score. Patient Global Impression of Change (PGIC) score (muscle symptoms) at the end of treatment. Safety: Number and severity of adverse events (AE). Absolute values, changes from Baseline, and incidence of potentially clinically significant changes in: • Laboratory safety tests • Electrocardiograms (ECG) • Supine vital signs • Eye assessments Pharmacokinetic (PK): REN001 plasma concentrations. Exploratory: Change from Baseline at Week 12 and Week 24 in: • Number of sit to stands in the 30 second sit to stand (30STS) test • Step count from pedometer • Patient Global Impression of Severity (PGIS) score – muscle symptoms • Patient Global Impression of Severity (PGIS) score – fatigue symptoms • Patient Reported Outcomes Measurement Informal System (PROMIS) Short Form – Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue 13a

- 36-Item Short Form Health Survey (SF-36) domain scores
- Modified Fatigue Impact Scale (MFIS) Total, Cognitive and Psychosocial sub-scale scores
- Brief Pain Inventory (BPI) pain severity and pain interference scores
- Work Productivity and Activity Impairment Questionnaire: Specific Health Problem (WPAI:SHP) scores

PGIC score (fatigue symptoms) at the end of treatment.

Number of Subjects:

Approximately 200 subjects will be randomized into the study.

Treatment:

Investigational Medicinal Product (IMP) and Mode of Administration:
IMP and matched placebo will be presented as Swedish orange, hard gelatin, size 0 capsules. Subjects will be required to take 2 capsules once each day with food. The dosage strength of REN001 capsules is 50 mg.

Doses and Randomization:

- REN001 100 mg orally once daily
- Placebo orally once daily

The randomization will be stratified by both mutational genotype (m.3243A>G or other mitochondrial DNA defects) and the screening 12-minute walk distance (12MWD) (≤ 500 meters or > 500 meters).

Duration of Treatment:
24 weeks

Eligibility Criteria:

Inclusion:
Subjects must meet all of the following inclusion criteria to be eligible for inclusion in the study:

1. Subjects aged 18 years or older with PMM as defined by the International Workshop: Outcome measures and clinical trial readiness in primary mitochondrial myopathies in children and adults (Mancuso et al 2017) (see Appendix 1).
2. A confirmed PMM diagnosis due to a known pathogenic gene mutation or deletion of the mitochondrial genome. The Sponsor may authorize local genetic testing at Screening, if required, but results must be available prior to randomization of the subject (see Sections 6.1 and 7.1.24 for details).
3. Documented PMM primarily characterized by exercise intolerance or active muscle pain.

4. Subjects must be ambulatory and able to perform the 12MWT independently (walking aids are allowed).
5. Distance walked of ≤1000 meters at Screening in the 12MWT (must be obtained at least 4 weeks before randomization).
6. Have no changes to any therapeutic exercise regimen within 30 days prior to Day 1 and be willing to remain on the same therapeutic exercise regimen for the duration of the study.
7. Be willing and able to swallow gelatin capsules.
8. Females should be either of non-child-bearing potential or must agree to use highly effective methods of contraception from Screening through to 30 days after last dose in the study. Where females are using hormonal contraception therapy, an additional effective non-hormonal method of contraception is advised. Males with partners who are women of child-bearing potential (WOCBP) must also use contraception (See Section 5.10 for details).
9. Concomitant medications (including supplements) intended for the treatment of PMM or other co-morbidities must be stable for at least 1 month prior to randomization and throughout participation in the study.
10. For subjects under 25 years old only: confirmation of bone growth plate closure by wrist radiograph.
11. Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study.

Exclusion:
Subjects presenting with any of the following will not be included in the study:

1. Participation in a prior REN001 (previously known as HPP-593) study.
2. Currently taking or anticipated to need a peroxisome proliferator-activated receptor (PPAR) agonist during the study.
3. Bone deformities or motor abnormalities other than those related to mitochondrial myopathy that may interfere with the outcome measures.
4. Treatment with an investigational drug within 3 months or 5 drug half-lives, whichever is longer, prior to Day 1.
5. Anticipated to need a prescription and/or non-prescription drug that might interfere with the study endpoints (See Table 1).

6. Currently taking drugs with a narrow therapeutic index and Breast Cancer Resistance Protein (BCRP) mediated absorption, distribution, metabolism and excretion (ADME) e.g., aliskiren, ambrisentan, colchicine, digoxin, everolimus, imatinib, lapatinib, maraviroc, nilotinib, posaconazole, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan, methotrexate, mitoxantrone, irinotecan, rosuvastatin, and sulfasalazine (See Table 1).
7. Clinically significant kidney disease or impairment, with an estimated Glomerular Filtration Rate (eGFR) less than 60 ml/min/1.73m^2 using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation at Screening.
8. Clinically significant liver disease or impairment, with aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 x Upper limit of normal (ULN), or Total bilirubin >1.6 x ULN or >ULN with other signs and symptoms of hepatotoxicity at Screening. *(Subjects with an isolated elevated bilirubin (e.g. < 2 x ULN) may be included after discussion with the Medical Monitor if the cause is due to a benign hereditary disorder of metabolism such as Gilbert's syndrome.)*
9. Uncontrolled diabetes and/or a Screening glycosylated hemoglobin (HbA1c) of ≥11%.
10. Uncontrolled epilepsy.
11. Evidence of significant concomitant clinical disease that may need a change in management during the study or could interfere with the conduct or safety of this study. *(Stable well-controlled chronic conditions such hypercholesterolemia, gastroesophageal reflux, or depression under control with medication (other than tricyclic antidepressants), are acceptable provided the symptoms and medications would not be predicted to compromise safety or interfere with the tests and interpretations of this study.)*
12. A history of cancer. A history of *in situ* basal cell carcinoma in the skin is allowed.
13. Have been hospitalized within the 3 months prior to Screening for any major medical condition (as deemed by the Investigator).
14. Clinically significant cardiac disease and/or clinically significant ECG abnormalities including a screening QTcF of ≥ 450 msec, 2nd degree heart block, symptomatic tachyarrhythmia or unstable arrythmia that in the opinion of the Investigator should exclude the subject from

completing the study exercise tests (i.e. 12MWT and 30STS tests). (*Subjects with right bundle branch block, left fascicular block and long PR interval which are common in PMM may be enrolled if the Investigator considers that the condition would not compromise safety or interfere with the tests and interpretations of this study.*)

15. Any condition possibly reducing drug absorption (e.g., gastrectomy or increased motility).
16. Evidence of hospitalization for rhabdomyolysis within the year prior to enrolment.
17. Positive hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (HBcAb) at Screening, or positive for hepatitis C or human immunodeficiency virus (HIV) at Screening.
18. Pregnant or nursing females.
19. History of sensitivity to PPAR agonists.
20. Donation or intent to donate blood, or blood components during the study or within one month after completion of the study.
21. A history of drug dependency. Use of opiates/cannabis for medical reasons is acceptable with prescription evidence or at the Investigators discretion.
22. A history of alcohol dependency.
23. Significant impairment due to central or peripheral nervous system involvement that would interfere with the exercise tests.
24. Significant weakness not caused by the underlying primary muscle disease such as post stroke or neurogenic weakness.
25. Have had an organ transplant.
26. Are not eligible or have a contraindication for cataract surgery.
27. A history of osteoporosis as evidenced by non-traumatic (stress) fractures or a prior T-score of -2.5 or worse which has not been adequately addressed.
28. Inability to comprehend or unwilling to follow the study requirements including restrictions on treatments, attendance at the study center, completion of questionnaires and participation in laboratory testing as called for by the protocol.
29. Any other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational

product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, and in discussion with the Medical Monitor, would make the subject inappropriate for entry into this study.

Study Visits:

There will be a total of 8 visits for each subject.

Each subject who provides informed consent will complete screening activities to confirm eligibility to enter the study. The Screening Visit (Visit 1) must be completed no more than 8 weeks prior to the start of dosing and will take place at the Study Center. There must be a minimum of 4 weeks between the Screening 12MWT and the Baseline 12MWT. If laboratory tests are outside of the normal range at initial screening, tests may be repeated once (this can be done as a home nurse visit). Where possible subjects should be pre-screened to assess the requirement for genotyping. The Screening Visit may be accomplished over more than 1 day. Subjects who are receiving prohibited medications must suspend the medications if this can safely and appropriately be done and have sufficient washout during the screening period (See Table 1) prior to randomization. Re-screening of subjects is allowed only once and requires prior approval by the Sponsor, for instance, if the wash-out of prior medications or genotyping is longer than 6-8 weeks. Re-screened subjects will not be required to have either the eye exam or DXA scan (REN001-201 DXA sub-study subjects) repeated within 6 months of the original assessments. Where a Study Center requires it, subjects may be asked to have a negative COVID-19 test prior to undertaking scheduled visits at the Study Center.

The PSOC will assess if subjects have the correct diagnosis of PMM for the study prior to randomization.

Subjects who successfully complete screening, and are approved by the PSOC, will undergo a Baseline Visit (Visit 2) which may be completed over two days if required. Once the Baseline assessments have been completed subjects will be randomized 1:1 to receive oral REN001, 100 mg or placebo once daily with food for 24 weeks.

In centers where the Regulatory Authority and the Ethics Committee/Institutional Review Board and Institution allows Visit 3 (Week 2), Visit 4 (Week 4), Visit 6 (Week 18) and Visit 8 (Follow Up) may be conducted either in the Study Center or at the subject's home using a home nursing service.

Visit 5 (Week 12) and Visit 7 (Week 24) will be completed in the Study Center and which may be completed over two days if required. A Follow Up Visit (Visit 8) will be performed, which can be carried out either in the Study Center or at the

subject's home. Visit 8 will be completed 21-28 days after the last dose of study medication. This visit will not be required if a subject chooses to enter the REN001 open label extension study (REN001-202) at their Week 24 visit; applicable only for countries where the open label extension study is being conducted.

A qualified nurse will carry out all the required study assessments for Home Visits. Additional Home Visits instead of Study Center visits, or delays to Visits beyond the stated visit windows, may be allowed in some circumstances e.g. global pandemic, with the Sponsor's prior approval.

A specialist concierge service will be available to subjects to arrange hotel accommodation and transport to and from Study Center visits and reimburse any subject study expenses.

The planned maximum study duration for each subject in the study will be 36 weeks (assuming 8 weeks screening, 24 weeks treatment and 4 weeks follow up).

Efficacy, safety, pharmacokinetic and exploratory assessments are detailed in the study Schedule of Activities (Table 2).

Statistical Methods:

On the basis of preliminary results of an open-label pilot study (REN001-101) involving 17 subjects with PMM who received REN001 100 mg/day for 12 weeks, a mean improvement of 94 meters was observed in the 12MWT.

A sample size of 186 subjects (93 per treatment group) provides 90% power, with a two-sided significance level of 5%, of obtaining a statistically significant difference between two treatment groups in distance walked during the 12MWT. This assumes a treatment effect (difference to placebo in mean change from baseline) size of 60 meters. The standard deviation for the changes from baseline observed in Study REN001-101 was approximately 100 meters. A higher standard deviation, 125 meters, has been assumed for this study due to the longer assessment period (24 weeks instead of 12 weeks) and the increased number of centers participating in the study. Allowing for a reduction in power due to subject withdrawals a total of approximately 200 subjects will be randomized.

To control the Type 1 error rate a fixed sequence testing procedure will be adopted. The order of testing will be:

1. Change from Baseline at Week 24 in distance walked during the 12MWT
2. Change from Baseline at Week 24 in the MFIS Physical subscale score

3. PGIC score (muscle symptoms) at the end of treatment

The primary population for efficacy will be the full analysis set (FAS), defined as all subjects randomized, who receive at least one dose of study treatment. Subjects in this population will be analyzed by assigned treatment.

The change from baseline in distance walked during the 12MWT will be analyzed using a mixed effects model for repeated measures (MMRM). The model will include fixed terms for treatment, visit and the treatment by visit interaction. The model will also include the stratification mutation factor and continuous baseline distance walked during the 12MWT as covariates. An unstructured covariance structure will be used for the repeated visits within a subject. The treatment comparison (REN001 100 mg vs placebo) for the change from baseline at Week 24 will be estimated. The estimate, standard error, 95% confidence intervals and associated p-values will be presented.
Treatment comparisons will also be made at Week 12.

The primary endpoint will also be analyzed for a per protocol population; defined as those subjects in the FAS population who have not violated inclusion or exclusion criteria and/or deviated from the protocol, in a way that could influence their efficacy assessment.

The MFIS Physical subscale score changes from baseline will be analyzed using a MMRM. The model will include fixed terms for treatment, visit and the treatment by visit interaction. The model will also include the stratification mutation factor, continuous baseline MFIS Physical subscale score and continuous baseline distance walked during the 12MWT as covariates. An unstructured covariance structure will be used. The treatment comparison for the change from baseline at each visit will be estimated. The estimates, standard errors, 95% confidence intervals and associated p-values will be presented for each visit. The primary comparison is at Week 24.

The PGIC (muscle symptom) data will be converted to a numerical scale from 3 (very much improved) to -3 (very much worse); hence a value of 0 would signify no change. This score will then be analyzed using analysis of covariance (ANCOVA), using a fixed term for treatment and the mutation stratification factor and continuous baseline distance walked during the 12MWT as covariates. The treatment comparison (REN001 vs placebo) will be estimated, along with its standard error, 95% confidence interval and associated p-value.

Safety analyses will be performed on the Safety Population, defined as all subjects who are randomized and receive at least one dose of study drug. Subjects will be analyzed by the treatment received.

Treatment-emergent adverse events (TEAEs) will be summarized by treatment group and by System Organ Class (SOC) and Preferred Term, in accordance with the MedDRA coding dictionary. The number of subjects reporting each AE preferred term will be tabulated for all TEAEs and separately for those considered as at least possibly related to study treatment by the Investigator. Number of subjects reporting serious adverse events (SAEs) and adverse events of special interest (AESI) will also be tabulated.

Observed values and changes from baseline in clinical laboratory tests, vital signs, ECG data and eye examination data will be summarized using descriptive statistics by treatment group and visit.

Plasma concentrations of REN001 will be summarized over time using descriptive statistics for Day 1, Week 12 and Week 24. These data will also be included in a population PK analysis.

LIST OF STUDY PERSONNEL

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ADME	Absorption, distribution, metabolism and excretion
ADR	Adverse Drug Reaction
AE	Adverse event
AESI	Adverse event of Special Interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BCRP	Breast Cancer Resistance Protein
BID	*Bis in die* – twice daily
BMI	Body mass index
BP	Blood Pressure
BPI	Brief Pain Inventory
BSAP	Bone specific alkaline phosphatase
CA	Competent Authorities
CFR	Code of Federal Regulations
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CHMP	Committee for Human Medicinal Products
CoQ10	Coenzyme Q10
CK	Creatine phosphokinase
CTX	Carboxy-terminal collagen crosslinks
DNA	Deoxyribonucleic acid
DSUR	Drug Safety Update Report
DXA	Dual-energy X-ray absorptiometery
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
eGFR	Estimated Glomerular Filtration Rate
EIA	Enzyme immunoassay
EMA	European Medicines Agency
ET	Early Termination
FACIT	Functional Assessment of Chronic Illness Therapy
FAO	Fatty Acid Oxidation
FAS	Full Analysis Set
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
FU	Follow Up
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GMP	Good Manufacturing Practice
HbA1c	Glycosylated hemoglobin
HBcAb	Hepatitis B core Antibody
HBsAg	Hepatitis B surface Antigen
hCG	Human Chorionic Gonadotropin
HDL	High Density Lipoprotein
HDPE	High Density Polyethylene
HIV	Human Immunodeficiency Virus
Hr	Hour

ICH	International Council on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IU/L	International Units per Liter
IWRS	Integrated Web-based Response System
kg	Kilogram
LDL	Low Density Lipoprotein
LFT	Liver Function Tests
LoQ	Level of Quantification
MAR	Missing at random
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MFIS	Modified Fatigue Impact Scale
mg	milligrams
mL	milliliter
mmHg	millimeters of mercury
MMRM	mixed effect model for repeated measures
msec	millisecond
mtDNA	mitochondrial Deoxyribonucleic Acid
nDNA	nuclear Deoxyribonucleic Acid
NASH	Non-alcoholic steatohepatitis
NMDAS	Newcastle Mitochondrial Disease Adult Scale
NTX	N-terminal telopeptide
OXPHOS	Oxidative phosphorylation
P-Cr	Phospho-creatine
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
P-gp	P-glycoprotein
PK	Pharmacokinetic
PMM	Primary mitochondrial myopathy
PP	Per protocol
PRO	Patient Reported Outcome
PROMIS	Patient-Reported Outcomes Measurement Information System
PPAR	peroxisome proliferator-activated receptor
PSOC	Patient Screening Oversight Committee
PT	Prothrombin time
PTE	Pre-treatment events
PTH	Parathyroid hormone
PV	Pharmacovigilance
QD	*Quaque die* - once daily
QoL	Quality of Life
QT	The interval between the start of the Q wave and the end of the T wave
QTcF	Heart rate corrected QT interval using Fridericia's formula
RR	The interval between the successive R waves
SAE	Serious adverse event
SAP	Statistical Analysis Plan

SF-36	36 Item Health Survey
SOC	System Organ Class
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
WPAI:SHP	Work Productivity and Activity Impairment Questionnaire: Specific Health Problem
WOCBP	Women of childbearing potential
6MWT	6-minute walk test
12MWD	12-minute walk distance
12MWT	12-minute walk test
30STS	30 Second Sit to Stand

1 INTRODUCTION

1.1 Indication

Primary mitochondrial myopathies are genetic disorders associated with pathogenic variants in the mitochondrial or nuclear deoxyribonucleic acid (DNA) that primarily lead to a) oxidative phosphorylation (OXPHOS) dysfunction or other disturbances of mitochondrial structure and function and b) predominantly, but not exclusively, affect skeletal muscle, with motor function deficits affecting endurance not better explained by other systemic or neurological conditions (Parikh et al 2014; Mancuso et al 2017). In patients with mitochondrial DNA (mtDNA) mutations, inheritance and clinical presentation are further complicated by the presence of multiple mtDNA genomes in an individual cell leading to a mixture of mutated and wild-type genomes (heteroplasmy) in the same cell or tissue.

Onset of PMM can occur at any age, although typically the more severe phenotypes present earlier in life, and milder phenotypes present later in life. One of the challenges of mitochondrial diseases is the marked clinical variation seen in patients, which can delay diagnosis. Because mitochondria are the main source of energy production in mammalian cells, clinical features typically involve the tissues with the highest energy requirements. Furthermore, the presence of mtDNA in all human tissues means that dysfunction occurs in multiple organ systems. The most commonly affected organ systems are the nervous, muscular, cardiac and endocrine systems. Individuals with PMM often present with a constellation of clinical features that may be compatible with a discrete clinical syndrome. However, categorizing patients into syndromes is of limited value, as the majority of patients do not fit into discrete categories (Gorman et al, 2016). PMMs are usually progressive conditions which produce significant disability and, in some instances, premature death.

The molecular genetic investigation of suspected mitochondrial disease can be complex, and has to be guided by previous clinical, histochemical and biochemical findings. Definitive diagnostic confirmation now relies on genetic testing and the identification of a pathogenic mutation in a recognized mitochondrial gene.

The management of patients with mitochondrial diseases is focused on strategies to reduce morbidity and mortality and early treatment of organ-specific complications. Several agents (mostly nutritional supplements) have been investigated in double-blind, placebo-controlled studies. These include carnitine, creatine, Coenzyme Q10 (CoQ10), cysteine, dichloroacetate, dimethylglycine, and the combination of creatine and lipoic acid. No agent or combination regimen has demonstrated efficacy in clinical disease endpoints (Pfeffer & Chinnery, 2013). PMMs represent an area of significant unmet medical need; there is currently no available disease -modifying therapy for patients with PMM.

1.2 Background

Full details of the REN001 chemistry, non-clinical and clinical data can be found in the current Investigator's Brochure.

1.3 Study Rationale

There is a strong rationale for the use of REN001, a selective PPARδ agonist, in patients with PMM. The PPARδ receptor is a nuclear receptor primarily found in skeletal muscle. Patients with PMM caused by genetic mutations in the mitochondrial genome have impaired oxidative phosphorylation. Selective PPAR agonists may ameliorate the cellular energy deficit in patients with these mutations by:

- Increasing fatty acid oxidation (FAO) and OXPHOS activity resulting in enhanced mitochondrial adenosine triphosphate (ATP) generating capacity;

- Increasing the proportion of wild-type mitochondria. Although mitochondrial disease induced mitochondrial proliferation may favor mutated mitochondria, pharmacological upregulation of mitochondrial biosynthesis increases the number of both mutated and wild-type mitochondria, which allows wild-type mitochondria to compensate for mutant mitochondria; and,
- Increasing mitochondrial biogenesis and thereby increasing residual OXPHOS activity, which would enhance the cellular ATP synthesis capacity.

1.4 Risk-Benefit Assessment

Benefits

In the open-label Phase 1b study (REN001-101) in subjects with PMM, oral REN001 dosed at 100 mg per day was safe and well tolerated for 12 weeks (Part A) and up to an additional 24 weeks in the subjects who continued into an extension part of the study (Part B). Following 12 weeks of dosing, the majority of patients with PMM experienced improvement in the outcome measures of distance walked in the 12MWT and symptoms of fatigue and pain evaluated with questionnaires.

Risks

Elevated creatine phosphokinase

Subjects with PMM may be found to have asymptomatic benign raised baseline creatine phosphokinase (CK) levels as part of the PMM disease, and even among healthy individuals CK levels transiently rise after exercise or heavy manual labor. In patients with PMM serum CK levels may increase to as much as 30 times the ULN within 24 hours of strenuous physical activity, then slowly decline over the next 7 days. The degree of CK elevation depends on the type and duration of exercise, with greater elevation in those who are untrained (Moghadam-Kia, Oddis, & Aggarwal, 2016). It is possible that improvements in the cellular energy deficit with REN001 treatment may result in increased muscle use and transient benign muscle enzyme elevations.

Serum CK data from subjects with PMM in the Phase 1b study (REN001-101) demonstrated a pattern of transient, elevated CKs following exercise and the collection of muscle biopsies; none of these elevations were associated with myoglobinuria. The elevations seen were self-limiting and resolved with no intervention despite continued treatment with REN001 and continuation of the subject's normal activities of daily living including exercise. In all the clinical trials that have included REN001 to date, elevations in CK tended to be modest and reversible and were usually determined by investigators as unlikely to be associated with REN001 treatment. In the current study CK levels will be assessed throughout the study with particular emphasis at Baseline and Week 2, based on the expected (asymptomatic) increases in CK previously observed in subjects with PMM following exercise testing.

Cataract formation

A single-species finding of cataract was observed in a high dose 6-month rat chronic toxicology study. Importantly, no cataracts were observed in any other toxicology studies, including in a 3-month rat study and a 12-month primate study. It is anticipated that the changes observed in the 6-month rat study are rat strain specific. Nevertheless, following this observation, taking a conservative approach, ophthalmology examinations were included in all REN001 trials. Slit lamp eye examinations will be conducted together with assessments of best corrected visual acuity at Screening and Weeks 12 and 24 in this study.

Potential Drug-Drug Interactions

REN001 is not a direct or time-dependent inhibitor of CYP3A4. However, REN001 has been shown to be a weak inducer of CYP3A4 *in vitro*. A potential for drug interactions between REN001 and drugs that are metabolized through the CYP3A4 is considered low but cannot be ruled out. Therefore, drugs that are metabolized primarily by CYP3A4 and have a narrow therapeutic index should be administered with caution in subjects participating in REN001 studies.

REN001 was identified as a P-glycoprotein (P-gp) substrate *in vitro*. Strong P-gp inhibitors may have an impact on the absorption and metabolism of a P-gp substrate, however preliminary human metabolism for REN001 indicates numerous metabolic pathways are involved in the clearance of REN001. Therefore, it is unlikely that co-administration of a strong P-gp inhibitor with REN001 will result in higher systemic exposures of REN001. Co-administration of REN001 with a strong P-gp inducer may reduce REN001 plasma concentrations.

Fertility and Contraception

In accordance with the latest European regulatory discussions and guidelines (Clinical Trials Facilitation Group, 2014 and 2020), WOCBP are eligible for the study provided they are using a highly effective form of contraception from Screening, while taking the study drug and for 30 days after stopping study drug (at least one menstrual cycle after drug exposure). Serum pregnancy testing will be completed at Screening and urine pregnancy tests will be carried out monthly thereafter and at Follow Up.

Fertile men (i.e., unless permanently sterile by bilateral orchidectomy) must use a condom during intercourse with a WOCBP from Baseline through to at least 14 weeks after stopping study drug (at least one sperm cycle after drug exposure) and should not father a child in this period. A condom is also required to be used by vasectomized men to prevent delivery of the drug via seminal fluid.

As REN001 is a weak inducer of CYP3A4 *in vitro*, caution is advised when co-administering REN001 and oral contraceptive agents. Therefore, as a precautionary measure, women receiving highly effective hormonal contraception therapy will be advised to use an additional effective non-hormonal method of contraception during treatment with REN001 and for 30 days after the final dose. Women using a highly effective non-hormonal contraception method (i.e., intrauterine device) will not be required to use additional methods of contraception.

Carcinogenicity

Long-term carcinogenicity studies have been initiated with REN001 in rats and mice. Animal carcinogenicity data in rodents suggest that some, but not all, PPAR agonists have carcinogenicity potential. The mechanism by which these compounds produce tumors in rodents is not well understood and the possibility that the rodent carcinogenicity findings may be relevant to humans cannot be ruled out at this time. Subjects with a history of cancer, except in situ basal cell carcinoma in the skin, will not be allowed to participate in the study.

Bone

a) Nonclinical finding of premature bone plate closure in rats.

This finding has not been replicated in non-human primates. As a precaution, subjects under 25 years of age in studies with REN001 over 12 weeks duration will have a wrist x-ray prior to enrolment to confirm skeletal maturity.

b) Bone turnover

Currently, it is not known if REN001 (a selective PPARδ agonist) will have any impact on bone mineral density. Nonetheless, given the study requirements for walk tests and taking a highly conservative approach, the Sponsor has excluded subjects with a history of fragility/stress fractures or an osteoporosis concomitant condition which has not been addressed, and will monitor markers of bone turnover during the study. Any reports of bone fracture will be captured as adverse events of special interest.

Safety Review Committee

The ongoing safety of study subjects will be reviewed by the Medical Monitor and study site physicians as data are received. In addition, safety data will be reviewed by an independently chaired Safety Review Committee (SRC). See Section 3.11.

Conclusion

In conclusion, the safety profile of REN001 supports further development in clinical studies subject to appropriate subject selection and safety monitoring.

1.5 Dose Selection Rationale

To date, a total of 162 subjects have been enrolled and 106 subjects received REN001 in four clinical trials (HPP593-101 (single dose, healthy volunteers); HPP593-102 (14 day, repeat dose study in obese moderately dyslipidemic subjects; HPP593-103, (28 day, healthy volunteer pharmacodynamic study in induced muscle atrophy); REN001-101, (up to 40 weeks, repeat dose in PMM subjects)). In the single dose study, doses ranged from 25 mg to 250 mg, doses in the 14-day study ranged from 50 mg to 200 mg QD or 100 mg BID, in the 28-day study the dose was 100 mg BID and in the 48-week study the dose was 100 mg QD. Overall, REN001 was considered safe and well tolerated in all four clinical studies. No treatment related SAEs were reported. No clinically significant abnormalities were observed in any safety parameters including vital signs, ECGs, and physical exams.

In study REN001-101, in subjects with PMM, the majority of subjects dosed for at least 12 weeks showed improvements in distance walked at Week 12 compared to Baseline, and also reported a reduction in muscle related symptoms and fatigue. Pharmacological activity of REN001 at 100 mg per day was also shown in Phase 1 studies in other indications (see current Investigator's Brochure for details).

The safety and the pharmacodynamic effects observed in these studies support the 100 mg QD dose level in this Phase 2b study.

2 STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the efficacy of REN001 in subjects with PMM treated for 24 weeks, assessed by the effect on exercise endurance.

2.2 Secondary Objectives

To evaluate the efficacy of REN001 in subjects with PMM treated for 24 weeks, assessed by the effect on fatigue.

2.3 Safety Objectives

To evaluate the safety and tolerability of REN001 in subjects with PMM during 24 weeks of treatment.

2.4 Pharmacokinetic Objectives

To investigate the pharmacokinetics of REN001 in subjects with PMM.

2.5 Exploratory Objectives

To evaluate the efficacy of REN001 in subjects with PMM treated for 24 weeks, assessed by the effect on functional capacity.

To evaluate the effect of REN001 on QoL of subjects with PMM after treatment for 24 weeks.

3 OVERALL DESIGN AND PLAN OF THE STUDY

3.1 Overview

There will be a total of 8 visits for each subject.

Each subject who provides informed consent will complete screening activities to confirm eligibility to enter the study. The Screening Visit (Visit 1) must be completed no more than 8 weeks prior to the start of dosing and will take place at the Study Center. There must be a minimum of 4 weeks between the Screening 12MWT and the Baseline 12MWT. If laboratory tests are outside of the normal range at initial screening, tests may be repeated once (this can be done as a home nurse visit). Where possible subjects should be pre-screened to assess the requirement for genotyping. The Screening Visit may be accomplished over more than 1 day. Subjects who are receiving prohibited medications must suspend the medications, if this can safely and appropriately be done and have sufficient washout during the screening period (See Table 1) prior to randomization. Re-screening of subjects is allowed only once and requires prior approval by the Sponsor, for instance, if the wash-out of prior medications or genotyping is longer than 6-8 weeks. Re-screened subjects will not be required to have either the eye exam or DXA scan (REN001-201 DXA sub-study subjects) repeated within 6 months of the original assessments. Where a Study Center requires it, subjects may be asked to have a negative COVID-19 test prior to undertaking scheduled visits at the Study Center.

The PSOC will assess if subjects have the correct diagnosis of PMM for the study prior to randomization.

Subjects who successfully complete screening and are approved by the PSOC will undergo a Baseline Visit (Visit 2) which may be completed over two days if required. Once the Baseline assessments have been completed subjects will be randomized 1:1 to receive oral REN001, 100 mg or placebo once daily for 24 weeks.

In centers where the Regulatory Authority and the Ethics Committee/IRB and Institution allows Visit 3 (Week 2), Visit 4 (Week 4), Visit 6 (Week 18) and Visit 8 (Follow Up) may be conducted either in the Study Center or at the subject's home using a home nursing service.

Visit 5 (Week 12) and Visit 7 (Week 24) will be completed in the Study Center and which may be completed over two days if required.

A Follow Up Visit (Visit 8) will be performed, which can be carried out either in the Study Center or at the subject's home. Visit 8 will be completed 21-28 days after the last dose of study medication. This visit will not be required if a subject chooses to enter the REN001 open label extension study (REN001-202) at their Week 24 visit; applicable only in countries where the open label extension study is being conducted.

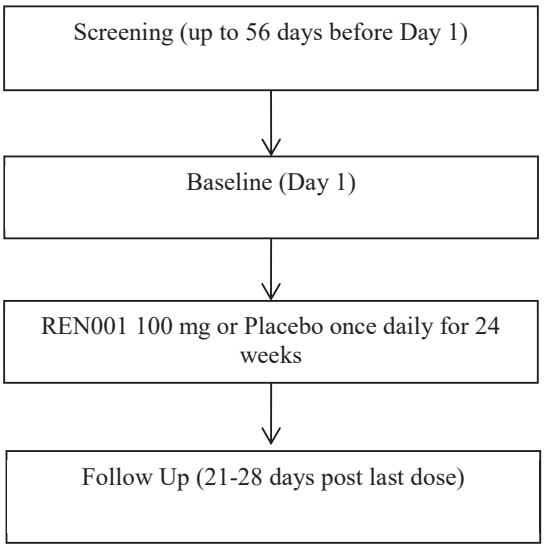
A qualified nurse will carry out all the required study assessments for home visits. Additional home visits instead of Study Center visits, or delays to visits beyond the stated visit windows, may be allowed in some circumstances e.g. global pandemic with the Sponsor's prior approval.

A specialist concierge service will be available to subjects to arrange hotel accommodation and transport to and from Study Center visits and reimburse any subject study expenses.

The planned maximum study duration for each subject in the study will be 36 weeks (assuming 8 weeks screening, 24 weeks treatment and 4 weeks follow up). See Figure 1.

Efficacy, safety, pharmacokinetic, pharmacodynamic and exploratory assessments are detailed in the study Schedule of Activities (Table 2).

Figure 1: Study Design



3.2 Endpoints

3.2.1 Primary Endpoint

Change from Baseline at Week 24 in distance walked during the 12MWT.

3.2.2 Secondary Endpoints

Change from Baseline at Week 24 in MFIS Physical sub-scale score.

PGIC score (muscle symptoms) at the end of treatment.

3.2.3 Safety Endpoints

Number and severity of adverse events.

Absolute values, changes from Baseline and incidence of potentially clinically significant changes in:

- Laboratory safety tests
- ECGs
- Supine vital signs
- Eye assessments

3.2.4 Pharmacokinetic Endpoints

REN001 plasma concentrations.

3.2.5 Exploratory Endpoints

Change from Baseline at Week 12 and 24 in:

- Number of sit to stands in 30STS test
- Step count from pedometer
- PGIS score - muscle symptoms
- PGIS score - fatigue symptoms
- PROMIS Short Form – FACIT Fatigue 13a
- SF-36 domain scores
- MFIS Total, Cognitive and Psychosocial sub-scale scores
- BPI pain severity and pain interference scores
- WPAI:SHP scores

PGIC (fatigue symptoms) score at the end of treatment.

3.3 Justification of the Study Design

There are no specific regulatory guidelines for the design of studies in patients with PMM and no approved safe and effective treatments for the disease. This study is primarily designed to assess the safety and efficacy of REN001 in patients with PMM caused by mtDNA gene mutations. PMM presents with a wide range of phenotypic heterogeneity. To address heterogeneity the study will aim to recruit 1) subjects with mt DNA defects (including the most common point mutation m.3243A>G) and 2) subjects with a primary phenotypic presentation of myopathy. In addition, the PSOC will review the screening criteria as described above.

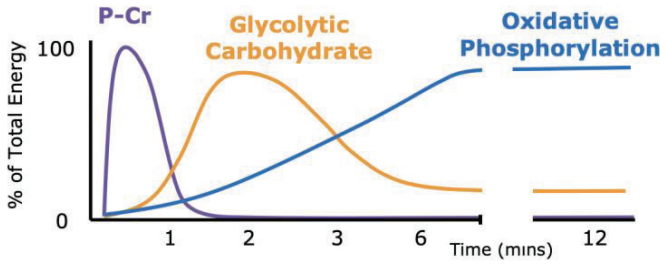
Subjects will be randomized 1:1 to receive REN001 or a matching placebo for 24 weeks. Placebo-controlled studies have been used in this indication previously. A placebo arm as

comparator has been included to establish the magnitude of change in endpoints that may occur spontaneously during the treatment period. The use of a placebo is justified as no approved therapy is available and international ethical guidelines permit the use of placebo controls in randomized trials when there are compelling methodological reasons and withholding treatment does not pose a risk of serious harm to participants (Millum & Grady, 2013).

The 24 week duration of the study offers the opportunity to evaluate the long-term effects of REN001 on physical endurance and symptoms associated with PMM. It also provides a longer exposure duration to evaluate the safety and tolerability of the drug in the intended treatment population.

The primary outcome measure of this study is the distance walked in the 12MWT. Both the 6-minute walk test (6MWT) and 12MWT have been used in clinical trials and in clinical practice. In order to demonstrate the effect of REN001 on increasing FAO and OXPHOS, a walk test of greater than 6 minutes is required. At the onset of exercise, the body relies on readily available phospho-creatine (P-Cr) stored in the cytosol (see Figure 2). P-Cr is quickly depleted. For the next 2-4 minutes of exercise, the body primarily uses glycolytic metabolism of glucose derived from skeletal glycogen. The third source of energy is the oxidative phosphorylation of fatty acids and carbohydrates that occurs in the mitochondria and is impaired in patients with PMM. This process occurs after approximately 4 minutes of exercise onset and constitutes the primary source of energy for sustained exercise.

Figure 2: Sources of Energy During Exercise



Adapted from Human Physiology: An Integrated Approach, Sixth Edition (2013) Dee Unglaub Silverthorn

Although, the 6MWT is a good measure to assess the submaximal level of functional capacity, the 6MWT duration is not sufficient to fully capture the potential benefits on physical endurance caused by improvements in mitochondrial function. In contrast, the self-paced 12MWT assesses the submaximal level of functional capacity during the period of exercise supported by mitochondrial function. A prior clinical trial conducted by the Sponsor (REN001-101), showed that subjects with PMM progressively slowed their walking speed over the course of the 12MWT, with the greatest decline occurring from minute 6 to minute 9, coinciding with the increased requirement for mitochondrial oxidative phosphorylation [data on file]. Most patients do not achieve maximal exercise capacity during the test; rather, they choose their own intensity of exercise and, if needed, are allowed to stop and rest during the test. This approximates to the activities of daily living, which are typically performed at submaximal levels of exertion for periods of more than 6 minutes.

One limitation of all walk tests is an associated 'training' effect whereby subjects may improve the distance walked on subsequent tests due to familiarity with the test (Kierkegaard & Tollback, 2007). This effect can be seen regardless of the interval duration (weeks to months) between tests (Wu et al, 2003; Prahm et al, 2014; Knak et al, 2017; Karaa et al, 2018). Various methods have been used to overcome this effect, such as 1) conducting two walk tests on the same day and using the longest distance (Kierkegaard & Tollback, 2007); 2) correcting the distance walked using average heart rate during the test (Knak et al, 2017); or 3) conducting a walk test at screening to act as a familiarization walk test (Karaa et al, 2018). In this study the training effect will be minimized using a screening, familiarization 12MWT. To minimize variability attributed to the training effect the Screening and Baseline 12MWTs in this study have been set at a minimum of at least 4 weeks apart, subsequent 12MWTs in the study will be 12 weeks apart at Weeks 12 and 24.

3.4 Termination or Suspension of Study

The study will be completed as planned unless one or more of the following criteria occur that require temporary suspension or early termination of the study:

- New information or other evaluation regarding the safety of REN001 that indicates a change in the known risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.
- Occurrence of local or global events (e.g., COVID-19 Pandemic) that would impact the safety monitoring of subjects in the study.

A Safety Review Committee will conduct periodic reviews of all safety data as described in Section 3.11.

3.5 Termination or Suspension of Investigational Sites

A study site may be terminated prematurely or suspended if the site (including the Investigator) is found to be in significant violation of GCP, the protocol, or the contractual agreement, or is unable to ensure adequate performance of the study, or for reasons as otherwise permitted by the contractual agreement.

3.6 Conditions for Individual Subject Discontinuation of Study Drug or Withdrawal from the Study

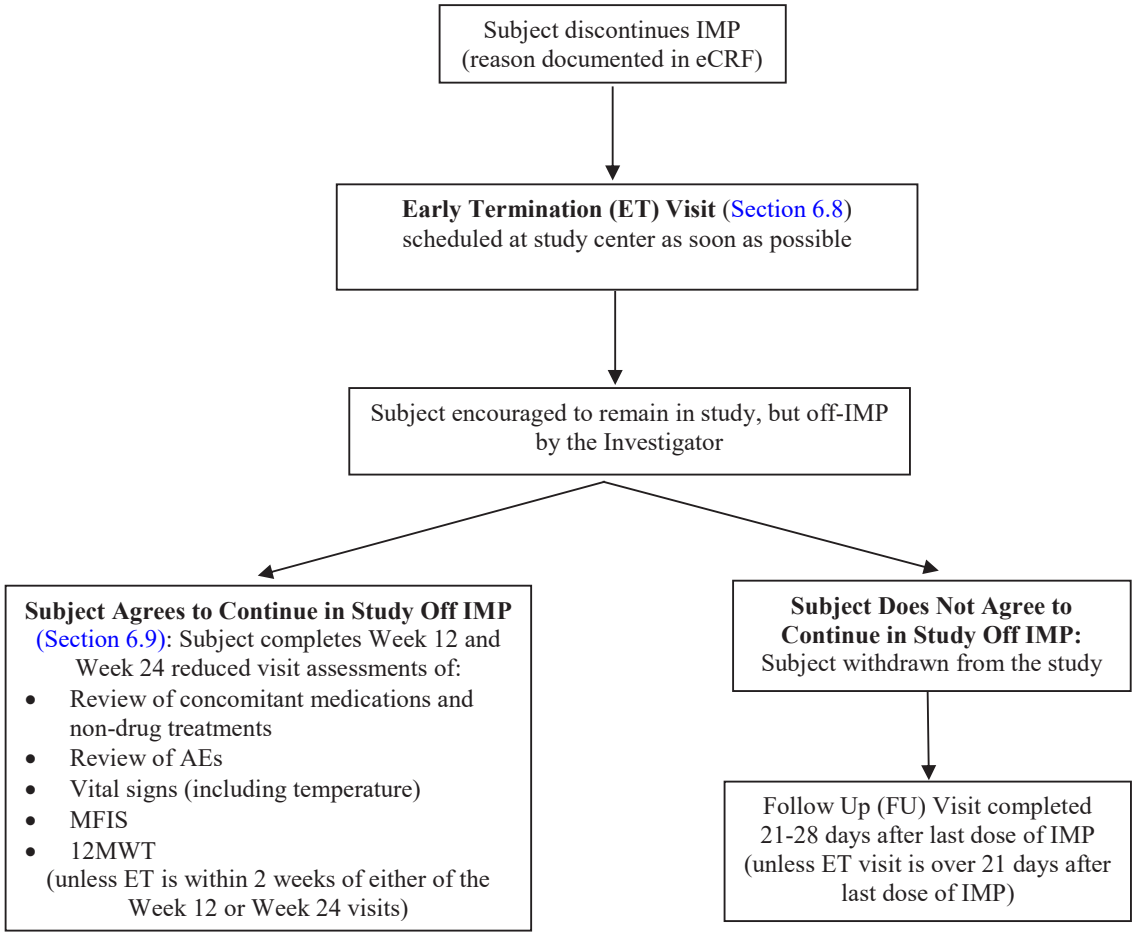
The primary reason for permanent discontinuation of a subject from the study drug should be recorded in the electronic Case Report Form (eCRF). Individual subjects may be discontinued from the study drug in the following circumstances:

1. Concurrent enrolment in other clinical studies involving investigational products or enrolment in other types of clinical research judged not to be scientifically or medically compatible with this study.
2. Use of a prohibited concomitant medication.
3. Major protocol deviation e.g., the discovery after administration of the first dose of study medication that the subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unacceptable risk to the subject's health or undermines the ability to achieve the objectives of the study.

4. Lost to follow-up. The subject did not return to the study center and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented.
5. Evidence of pregnancy during the treatment period of the study.
6. Clinically significant changes from baseline in ECG recordings considered treatment-related including an increase from baseline in heart rate corrected QT interval using Fridericia's formula (QTcF) to a value of ≥500 millisecond (msec). Single ECGs should be repeated at 5-minute intervals, 3 times, to confirm any increased QTcF interval values. In the event of an increased QTcF interval of ≥500 msec, the subject must be monitored until the value returns to below 500 msec.
7. Clinically significant muscle injury including test abnormalities (e.g., CK) as defined in Section 3.7.1.
8. Clinically significant liver toxicity including test abnormalities (e.g., ALT/AST) as defined in Section 3.7.2.
9. Clinically significant kidney toxicity including test abnormalities (e.g., creatinine levels) as defined in Section 3.7.3.
10. A TEAE that could result in a transient or permanent significant impairment. Subjects should be followed with appropriate medical management until there is a return to normal or baseline values or a clinical diagnosis of an emergent illness is confirmed.
11. Voluntary withdrawal. The subject wishes to withdraw from the study. Subjects may withdraw from the study at any time without penalty and for any reason without prejudice to their future medical care. In all cases, the reason(s) for withdrawal, including the primary reason, must be recorded in the eCRF. Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded (i.e., withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category).

If a subject discontinues study drug the following process (see Figure 3) should be followed by the Investigator in order to satisfy the regulatory desire to continue to follow subjects where possible (ICH E9-R1) after discontinuation of a study intervention. Hence, if the subject discontinues taking study drug they will be encouraged, where appropriate, to continue with reduced assessments at the Week 12 and Week 24 visits.

Figure 3: Process For Subject Discontinuation Of IMP Or Withdrawal From The Study



3.7 Strategy for Potential Withdrawal due to Laboratory Test Abnormalities

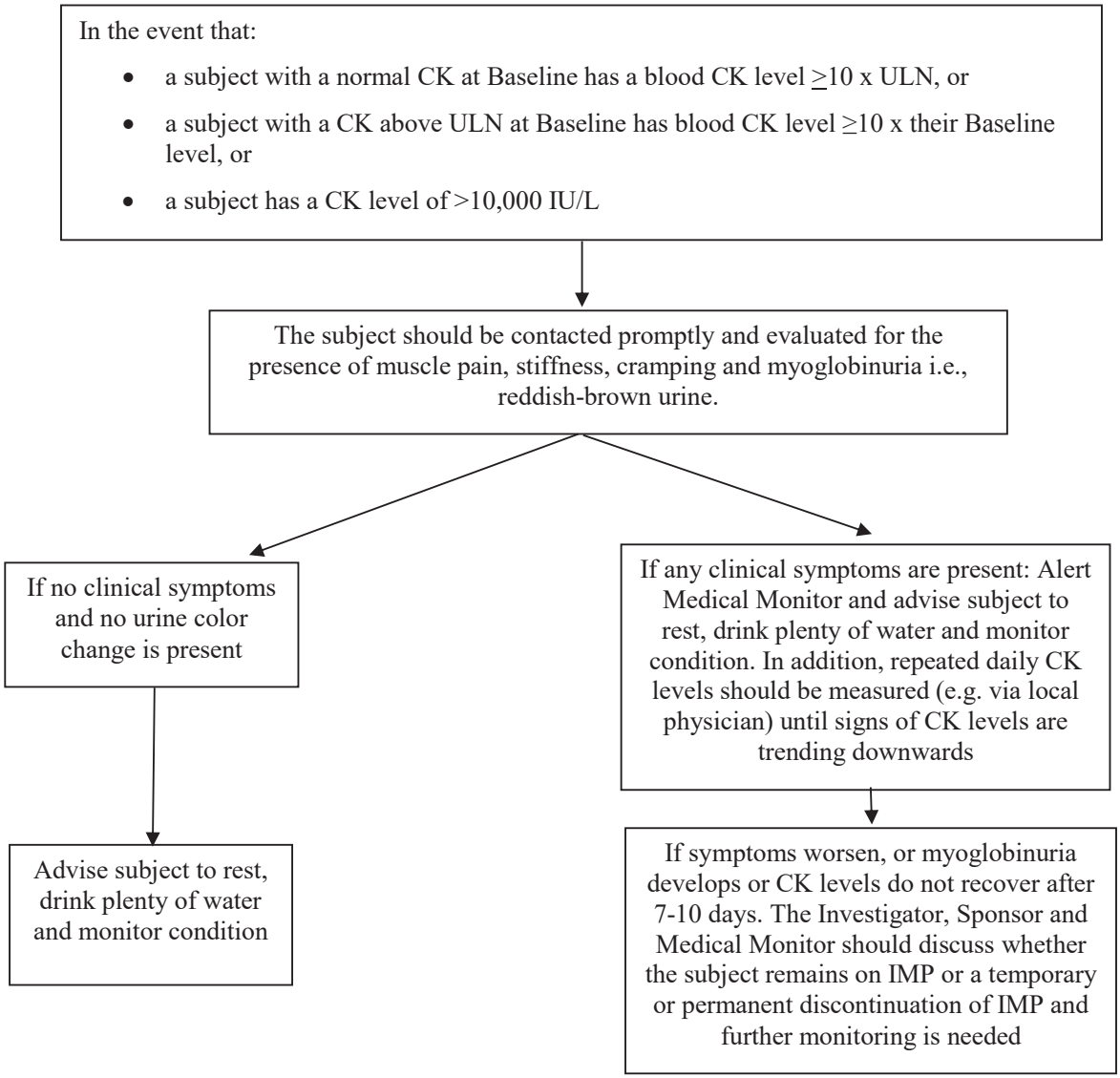
3.7.1 Safety Monitoring for Serum Creatine Phosphokinase

Patients with PMM often present with mild to moderate baseline elevations in CK and can experience transient episodes of significantly elevated CK induced by vigorous exercise, and other environmental conditions. These elevations are not associated with symptoms or signs and the patients are typically well. CK elevations are generally of the skeletal muscle fraction. However, a small proportion of the total CK may be from the myocardial fraction reflecting a small amount of this fraction found in skeletal muscle rather than the presence of myocardial injury. Importantly, these elevations are entirely to be expected in this patient population with underlying muscle disease and are not synonymous with rhabdomyolysis. Similarly, elevations in serum aminotransferases and uric acid can accompany CK elevations and may not be indicative of liver disease.

True rhabdomyolysis may also occur in these patients and must be managed appropriately. Rhabdomyolysis is characterized by severe acute muscle injury resulting in muscle pain, weakness, and/or swelling with release of myofiber contents into the bloodstream. Symptoms develop over hours to days following an inciting factor and may be associated with dark pigmentation of the urine (Nance & Mammen, 2015). Acute kidney injury is a potential

complication of rhabdomyolysis. The risk of acute kidney injury is low in patients with CK levels less than 15,000 international units per liter (IU/L). The CK algorithm below (Figure 4) has been designed to identify those subjects potentially at risk of true rhabdomyolysis and ensure appropriate early action is taken to monitor and treat the subjects accordingly.

Figure 4: CK Algorithm



3.7.2 Safety Monitoring For Liver toxicity: Management and Stopping Rules

In this study, subjects with significant liver disease or impairment of AST or ALT >2.5 x ULN, or total bilirubin >1.6 x ULN or >ULN with other signs and symptoms of hepatotoxicity at Screening will be excluded. It would be inappropriate to exclude all subjects with milder elevations in LFTs out of the normal range because patients with PMM can present with elevations in CK levels that are often accompanied by elevated LFTs (ALT and AST), which are not indicative of liver disease. Accordingly, these elevations appear to be transient and resolve spontaneously (Tarnopolsky 2016). Although serum ALT and AST

values have shown good diagnostic accuracy in patients with chronic liver disease, AST and ALT levels can be increased immediately after muscular exertion as well (Lippi 2008).

In the event that a subject presents with raised LFTs during the study the following management guidelines and stopping rules should be followed:

- For subjects who enroll with a baseline below the ULN and present with an increase in serum ALT or AST > 3xULN, repeat testing of ALT, AST, ALP and total bilirubin will be obtained as soon as possible, ideally within 48-72 hours, to confirm the abnormality and whether the abnormality is increasing or decreasing.
- For subjects who enroll with a baseline above the ULN and present with an increase in serum ALT or AST > 3xULN and at least 2x their Baseline value, repeat testing of ALT, AST, ALP and total bilirubin will be obtained as soon as possible, ideally within 48-72 hours, to confirm the abnormality and whether the abnormality is increasing or decreasing.
- If symptoms persist or repeat testing shows ALT > 3xULN for subjects with normal baseline measures or 2x their Baseline value for subjects with elevated values at Baseline, initiate close observation with repeat testing 2-3 times weekly, or as clinically mandated, to determine whether the abnormalities are improving or worsening (see below for further details).
- If close monitoring is not possible, as a precautionary measure study drug should be withheld and continued participation in the study should be discussed with the Medical Monitor (See also Section 6.9).

Guidelines for Close Observation:

- Repeated ALT, AST, ALP, and total bilirubin tests two or three times weekly.
- Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic.
- Obtain a more detailed history of symptoms and prior or concurrent diseases.
- Recheck history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and any special diets.
- Rule out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; Non-alcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., International normalized ratio (INR) or Prothrombin time (PT), direct bilirubin).
- Consider gastroenterology or hepatology consultations.

Discontinuation:

Discontinuation or interruption of study drug should be considered, in consultation with the Medical Monitor and Reneo CMO or designee where possible, if:

- ALT or AST > 8xULN.
- ALT or AST > 5xULN for more than 2 weeks.
- ALT or AST > 3xULN and total bilirubin > 2xULN or INR >1.5.
- ALT or AST > 3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

3.7.3 Safety Monitoring For Kidney toxicity: Management and Stopping Rules

If subjects develop a creatinine elevation > 2xULN and more than 2x Baseline value:

- Monitor creatinine 2 times per week, or as clinically indicated, until recovery.
- For subjects with > 3xULN creatinine elevation the study drug should be withheld and the subject should continue to be followed closely.
- In the event of either of the above continued participation in the study should be discussed with the Medical Monitor.

Discontinuation:

Discontinuation of study drug should be considered, in consultation with the Medical Monitor and Reneo CMO or designee where possible, if:

- A > 3xULN creatinine elevation is sustained or deteriorating.
- The subject is unwell and has hyperkalemia.

3.8 Replacement of Subjects

Subjects who withdraw due to reasons other than AEs or SAEs may be replaced to ensure that sufficient subjects are available to satisfy the statistical assumptions of the study.

3.9 Temporary Discontinuations

Subjects may be temporarily discontinued from study drug while AEs and/or laboratory test abnormalities are being investigated and/or at the request of the Medical Monitor. The Investigator should discuss with the Medical Monitor and Sponsor any subject for whom temporary or permanent discontinuation of study drug is being considered.

If a subject misses several consecutive doses due to personal circumstances or due to an AE not related to study drug, they may be allowed to restart dosing after discussion between the Investigator, Medical Monitor and Sponsor (See Section 5.9).

3.10 Review of Screening Eligibility Data

An independent Patient Screening Oversight Committee with experts in the diagnosis of PMM will be set up to review screening data to ensure subjects enrolled into the study have the correct diagnosis of PMM. Details regarding the structure, function and operation of the PSOC will be detailed in the REN001-201 PSOC Charter.

3.11 Review of Safety Data

Review of subject safety data (including, but not limited to, AEs, ECGs, laboratory safety tests and vital signs) will be performed by the independently chaired Safety Review

Committee (SRC) in accordance with the REN001-201 SRC Charter. Safety reviews will be conducted throughout the study in order to identify any potential safety signals during the trial that may have an impact on the safety of the participants. The SRC may make recommendations concerning continuation, termination or study modifications based on these reviews.

4 STUDY POPULATION

To fulfil the study objectives, it is essential that appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects considered appropriate for participation in the study. Subject eligibility should be reviewed and documented by the Investigator or an appropriate designee member of the site study team. In addition, subjects screening data will be reviewed independently by the PSOC for confirmation of eligibility prior to randomization.

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for inclusion in the study:

1. Subjects aged 18 years or older with PMM as defined by the International Workshop: Outcome measures and clinical trial readiness in primary mitochondrial myopathies in children and adults (Mancuso et al 2017) (see Appendix 1).
2. A confirmed PMM diagnosis due to a known pathogenic gene mutation or deletion of the mitochondrial genome. The Sponsor may authorize local genetic testing at Screening, if required, but results must be available prior to randomization of the subject (see Sections 6.1 and 7.1.24 for details).
3. Documented PMM primarily characterized by exercise intolerance or active muscle pain.
4. Subjects must be ambulatory and able to perform the 12MWT independently (walking aids are allowed).
5. Distance walked of ≤1000 meters at Screening in the 12MWT (must be obtained at least 4 weeks before randomization).
6. Have no changes to any therapeutic exercise regimen within 30 days prior to Day 1 and be willing to remain on the same therapeutic exercise regimen for the duration of the study.
7. Be willing and able to swallow gelatin capsules.
8. Females should be either of non-child-bearing potential or must agree to use highly effective methods of contraception from Screening through to 30 days after last dose in the study. Where females are using hormonal contraception therapy, an additional effective non-hormonal method of contraception is advised. Males with partners who are WOCBP must also use contraception (See Section 5.10 for details).
9. Concomitant medications (including supplements) intended for treatment of PMM or other co-morbidities must be stable for at least 1 month prior to randomization and throughout participation in the study.
10. For subjects under 25 years old only: confirmation of bone growth plate closure by wrist radiograph.
11. Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study.

4.2 Exclusion Criteria

Subjects presenting with any of the following will not be included in the study:

1. Participation in a prior REN001 (previously known as HPP-593) study.
2. Currently taking or anticipated to need a PPAR agonist during the study.

3. Bone deformities or motor abnormalities other than related to the mitochondrial myopathy that may interfere with the outcome measures.
4. Treatment with an investigational drug within 3 months or 5 drug half-lives, whichever is longer, prior to Day 1.
5. Anticipated to need a prescription and/or non-prescription drug that might interfere with the study endpoints (See Table 1).
6. Currently taking drugs with a narrow therapeutic index and BCRP mediated ADME e.g., aliskiren, ambrisentan, colchicine, digoxin, everolimus, imatinib, lapatinib, maraviroc, nilotinib, posaconazole, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan, methotrexate, mitoxantrone, irinotecan, rosuvastatin, and sulfasalazine (See Table 1).
7. Clinically significant kidney disease or impairment, with an eGFR less than 60ml/min/1.73m^2 using the CKD-EPI creatinine equation at Screening.
8. Clinically significant liver disease or impairment, with AST or ALT >2.5 x ULN, or Total bilirubin > 1.6 x ULN or >ULN with other signs and symptoms of hepatotoxicity at Screening. (*Subjects with an isolated elevated bilirubin (e.g., < 2 x ULN) may be included after discussion with the Medical Monitor if the cause is due to a benign hereditary disorder of metabolism such as Gilbert's syndrome.)*
9. Uncontrolled diabetes and/or a Screening HbA1c of ≥11%.
10. Uncontrolled epilepsy.
11. Evidence of significant concomitant clinical disease that may need a change in management during the study or could interfere with the conduct or safety of this study. *(Stable well-controlled chronic conditions such hypercholesterolemia, gastroesophageal reflux, or depression under control with medication (other than tricyclic antidepressants), are acceptable provided the symptoms and medications would not be predicted to compromise safety or interfere with the tests and interpretations of this study.)*
12. A history of cancer. A history of *in situ* basal cell carcinoma in the skin is allowed.
13. Have been hospitalized within the 3 months prior to Screening for any major medical condition (as deemed by the Investigator).
14. Clinically significant cardiac disease and/or clinically significant ECG abnormalities including a screening QTcF of ≥ 450 msec, 2nd degree heart block, symptomatic tachyarrhythmia or unstable arrythmia that in the opinion of the Investigator should exclude the subject from completing the study exercise tests (i.e., 12MWT and 30STS tests). (*Subjects with right bundle branch block, left fascicular block and long PR interval which are common in PMM may be enrolled if the Investigator considers that the condition would not compromise safety or interfere with the tests and interpretations of this study.*)
15. Any condition possibly reducing drug absorption (e.g., gastrectomy or increased motility).
16. Evidence of hospitalization for rhabdomyolysis within the year prior to enrolment.
17. Positive HBsAg and HBcAb at Screening, or positive for hepatitis C or HIV at Screening.
18. Pregnant or nursing females.
19. History of sensitivity to PPAR agonists.

20. Donation or intent to donate blood, or blood components during the study or within one month after completion of the study.
21. A history of drug dependency. Use of opiates/cannabis for medical reasons is acceptable with prescription evidence or at the Investigators discretion.
22. A history of alcohol dependency.
23. Significant impairment due to central or peripheral nervous system involvement that would interfere with the exercise tests.
24. Significant weakness not caused by the underlying primary muscle disease such as post stroke or neurogenic weakness.
25. Have had an organ transplant.
26. Are not eligible or have a contraindication for cataract surgery.
27. A history of osteoporosis as evidenced by non-traumatic (stress) fractures or a prior T-score of -2.5 or worse which has not been adequately addressed.
28. Inability to comprehend or unwilling to follow the study requirements including restrictions on treatments, attendance at the study center, completion of questionnaires and participation in laboratory testing as called for by the protocol.
29. Any other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, and in discussion with the Medical Monitor, would make the subject inappropriate for entry into this study.

4.3 Planned Sample Size and Number of Study Centers

It is envisaged that approximately 265 subjects will be screened to achieve 200 randomized subjects. Recruitment will be from across a minimum of 35 centers worldwide.

5 STUDY DRUG

5.1 Study Drug Identity

Study drug and placebo are presented as Swedish orange, hard gelatin, size 0 capsules, manufactured by Xcelience LLC, a Lonza company.

The dosage strength of REN001 capsules is 50 mg. Matched placebo capsules will also be supplied, containing approximately 30 mg microcrystalline cellulose.

Capsules are packaged in 200 mL high-density polyethylene (HDPE) bottles, with a heat induction seal and child-resistant lids, containing 100 capsules of either 50mg REN001, or placebo. Supplies will be identified by the blinded batch number and expiry date.

As each subject will take 2 capsules per day the bottle will contain sufficient capsules for 50 days of dosing.

Sufficient bottles will be supplied to ensure subjects have at least enough capsules to maintain dosing between Study Center visits.

5.2 Allocation to Treatment

Upon Screening, each subject will receive a 6 digit study number allocated by the Integrated Web-based Response System (IWRS), which will be used throughout the study. The IWRS will use a computer-generated randomization schedule to assign subjects to a treatment sequence on a 1:1 basis. The randomization will be stratified by both mutational genotype (m.3243A>G or other mitochondrial DNA defects) and the screening 12-minute walk distance (12MWD) (≤ 500 meters or > 500 meters).

Screened subjects who drop out of the study before receiving study drug or require repeat safety lab tests will retain their study number. A subject may be rescreened once only (with prior approval by the Sponsor); for instance, if a washout of prior medications of longer than 6-8 weeks is required. Rescreened subjects will be given a new study number and their previous study number will also be recorded.

5.3 Maintaining the Blind

This study is a double-blind study i.e., the study will be Sponsor, subject- and investigator-blinded. All personnel involved in the conduct of the study will remain blinded to treatment until the conclusion of the study. To minimize the potential for bias, treatment randomization information will be kept confidential by the IWRS vendor and will not be released to the Sponsor, Investigator, site personnel, or the study monitors until the conclusion of the study.

REN001 and placebo will be provided as visually matched capsules. All study drug will be supplied in identical bottles thereby maintaining the double blind conditions for the subject and Investigator.

5.4 Breaking the Blind

Details of personnel who will be unblinded to subject data during the study will be recorded in a document maintained by the Sponsor.

At the initiation of the study, the study site will be instructed on the method for breaking the blind. The study blind should not be broken except in a medical emergency, where knowledge of the study drug received would affect the treatment of the emergency or obtaining this information is a regulatory requirement. As a courtesy, and only if time permits, the Investigator may notify the Sponsor prior to unblinding. All Investigator unblinding events will be automatically reported by the IWRS to the Sponsor.

If the blind is broken, the date, time and reason must be recorded in the subject's eCRF. Information concerning the treatment allocated should only be available to those who need to know for the appropriate action to be taken with the subject.

5.5 Administration

Subjects will receive either 100 mg REN001 as 2 x 50 mg or placebo as 2 x placebo capsules for oral administration once daily for 24 weeks.

Medication should be taken with food, for consistency of exposure, at a convenient time to the subject (ideally in the morning). The exception to this will be when the subject is required to attend the Study Center for their Baseline, Week 12 and Week 24 Visits, when they should take the study medication at the Study Center, when instructed to do so, by the site staff. On these visits, sites will ensure the subjects take the study medication with food.

Subjects will be supplied with a dose recording card and will be requested to record the date and time of dosing for study visit days (other than Baseline, Week 12 and Week 24).

5.6 Release, Labelling and Storage

Bottles will be labelled and released in accordance with the Clinical Trials Directive 2001/20/EC (European Commission, 2001), GMP Directive 2003/94/EC for Investigational Medicinal Products (European Union, 2003) and US Code of Federal Regulations 21CFR312.6 and 21CFR211.165.

All study drug supplies must be stored in accordance with the manufacturer's instructions. Until dispensed to the subjects, the study medication (REN001 and placebo capsules) should be stored at refrigerated temperature (2-8^{o}C), in a securely locked area, accessible to authorized personnel only. A daily temperature log of the study drug storage area must be recorded and checked every day. Temperature excursions must be reported to the Sponsor or delegate as soon as possible.

Once the study medication has been dispensed to subjects, it should be stored refrigerated (2-8^{o}C) at the subject's home. Study medication does not need to be kept refrigerated during transport to and from the subject's home and the Study Center. Subjects should only open and use one bottle of study medication at a time. Study medication should be kept away from children.

5.7 Excessive Pharmacological Effects

No specific antidotes for REN001 are available and standard supportive measures should be used in the case of excessive pharmacological effects.

5.8 Drug Accountability

The Investigator is responsible for maintaining accurate accountability records for study drug throughout the study.

Each dispensing of study drug will be documented in the IWRS and the study supplies management system.

The subject must bring all study medication (including empty bottles) to the Study Center at each visit, for compliance, reconciliation and quarantine of any expired study medication ahead of destruction.

Any study medication remaining at the end of study will be returned to the Sponsor or their representative or destroyed locally on behalf of the Sponsor (with written permission of the Sponsor) and the destruction fully documented.

5.9 Compliance

At all study visits subject compliance with the study drug regimen will be monitored by capsule counts. Subjects failing to take 2 or more doses in a 1- or 2-week visit period or 4 or more doses in a 4-week visit period will be reminded of the importance of complying with the dosing requirements. If a subject consistently fails to take their study medication, the Investigator should alert the Sponsor, Medical Monitor and site monitor and a decision will be made as to whether the subject should be withdrawn for non-compliance.

5.10 Contraceptive Requirements

In countries where the regulatory authority allows this, WOCBP at risk for pregnancy and fertile men will be allowed to enter the study provided adequate contraceptive requirements are followed as outlined below. Contraceptive requirements for the study have been defined using the recommendations of the Clinical Trials Facilitation Group, Rome September 2014 meeting and subsequent update in September 2020 (Clinical Trials Facilitation Group, 2014 and 2020) and also the International Council on Harmonization (ICH) M3(R2) guidance (European Medicines Agency, 2009).

Females

Females of child-bearing potential must agree to use highly effective methods of contraception from Screening to 30 days after the last study dose. Highly effective methods are:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation administered by oral or intravaginal route.
- oral, injectable or implantable progestogen-only hormonal contraception associated with inhibition of ovulation.
- intrauterine device.
- intrauterine hormone-releasing system.
- bilateral tubal occlusion.
- bilaterally orchiectomized or vasectomized partner, provided that partner is the sole sexual partner of the subject, and in the case of a vasectomized partner has received medical assessment of the surgical success.
- true sexual abstinence, where this is in line with the usual lifestyle of the subject.

Periodic (calendar, ovulation, symptothermal or post-ovulation) abstinence and withdrawal are not acceptable methods of contraception.

However, caution with co-administration of REN001 and hormonal contraceptives is advised due to the potential for drug-drug interaction, see Section 1.4. In addition to highly effective hormonal contraception therapy, WOCBP at risk of pregnancy are advised to also use an additional effective non-hormonal method of contraception.

Females are considered to be of non-child-bearing potential if they fulfill at least one of the following criteria:

- Postmenopausal, defined as 45 years of age or older and amenorrhoeic for at least 1 year PLUS have a serum follicle stimulating hormone (FSH) level within the laboratory's reference range for postmenopausal women, at Screening Visit.
- Have undergone a documented hysterectomy and/or bilateral oophorectomy.

Males who are considered fertile and with partners who are WOCBP

Males are considered fertile after puberty unless permanently sterile by bilateral orchidectomy or vasectomy (following medical assessment of surgical success). Fertile males with partners who are WOCBP must agree to use contraception (condom) from Baseline until 14 weeks after the last dose of study medication. A condom is also required to be used by vasectomized men to prevent delivery of the drug via seminal fluid. In addition, they must be advised not to donate sperm during this period.

5.11 Concomitant Medications

Any medication the subject takes during the study, prescription and non-prescription, other than the study drug is considered a concomitant medication. All concomitant medications must be recorded in the eCRF. The following information must be recorded in the eCRF for each concomitant medication: generic name, route of administration, dose, start date, stop date (and whether it is a prior medication) and indication.

At Screening, subjects will be asked what medications they are currently taking. At each subsequent study visit, subjects will be asked what medications they have taken since the last study visit.

Any medications taken prior to the start of dosing will be documented as prior medications. Medications taken after the first dose of study medication will be documented as concomitant medications. Medications that start before the first dose of study medication and continue into the study are considered both prior and concomitant medications.

Subjects may not use the medications and treatments listed in Table 1 within the indicated time interval prior to Day 1 and these medications must be withheld for the duration of the study and where possible until completion of the Follow Up visit.

Table 1: Prohibited Medications

Medication	Time interval prior to Day 1
Statins (including atorvastatin, lovastatin and rosuvastatin) *	1 week
Anticoagulants *	1 week or 5 half-lives (whichever is longer)
Bezafibrate and other PPAR agonists	4 weeks
Cyclosporine and other immunosuppressive drugs	4 weeks
Oral or systemic steroids *	4 weeks
Investigational drugs	3 months or 5 half-lives (whichever is longer)
Drugs with a narrow therapeutic index and BCRP mediated ADME e.g., aliskiren, ambrisentan, colchicine, digoxin, everolimus, imatinib, lapatinib, maraviroc, nilotinib, posaconazole, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan, methotrexate, mitoxantrone, irinotecan, and sulfasalazine.	No washout period applicable, subjects should be excluded from the study (see Section 4.2)

* Other statins,anticoagulants and steroids may be allowed, if it is considered medically appropriate, following discussion with the Medical Monitor who will discuss with Sponsor.

6 STUDY CONDUCT

This is a multi-center study which will be conducted at a minimum of 35 study sites.

A schedule of Activities is presented in Table 2. Details of study assessments are provided in Section 7.

Visits should occur within the scheduled timeslot for the visit: see Table 2, Schedule of Activities. Where possible, visits should occur on the target visit day in relation to the Baseline visit. All dates should be recorded as DD-MMM-YYYY (e.g., 01 JAN 2018), and times should be recorded using the 24-hour clock (e.g., 23:20 not 11:20 pm).

Assessments should be completed in the order below:

- PROs must be completed prior to exercise testing
- The 12MWT must be done before the 30STS
- 30STS must be done last (at least 1 hour after 12MWT)

6.1 Screening: Visit 1

All subjects will be given both a written and verbal description of the study. Subjects should be given adequate time to think about whether they want to participate and to ask questions. The Investigator (or an appropriate delegate at the site) will obtain written consent from each subject prior to commencing any study related activities or assessments. Following consent, the Screening Visit (Visit 1) must be completed no more than 8 weeks prior to the start of dosing and will take place at the Study Center. There must be a minimum of 4 weeks between the Screening 12MWT and the Baseline 12MWT. If laboratory tests are outside of the normal range at initial screening, tests may be repeated once (this can be done by a home nursing visit). Where possible subjects should be pre-screened to assess the requirement for genotyping. The Screening Visit may be accomplished over more than 1 day. Subjects who are receiving prohibited medications must suspend the medications, if this can safely and appropriately be done and have sufficient washout during the screening period (see Table 1). Re-screening of subjects (once only and with prior Sponsor approval) is allowed, for instance if wash-out of prior medications or genotyping is longer than 6-8 weeks. Re-screened subjects will not be required to have either the eye exam or DXA scan (REN001-201 DXA sub-study subjects) repeated within 6 months of the original assessments. Where a Study Center requires it, subjects may be asked to have a negative COVID-19 test prior to undertaking scheduled visits at the Study Center.

At Screening, all subjects will be allocated a unique 6-digit study number, which will be used for the duration of the study.

Site staff will need to provide subjects with meals and snacks at appropriate times during the visit to ensure subjects have enough energy prior to completing the exercise tests.

The following procedures will be performed:

- Subject demography (including ethnicity for calculation of eGFR)
- Complete medical history (including prescription and non-prescription drugs/treatments, non-drug treatments, topical products, vitamins and dietary supplements taken in the last 4 weeks, alcohol, drugs of abuse and tobacco use)
- Full physical examination (including height and weight)
- 12-lead ECG
- Vital signs (including temperature)

- Obtain blood samples for:
  - Hematology and HbA1c
  - Biochemistry, HIV/Hepatitis B/C serology, serum FSH test (postmenopausal females only) and serum pregnancy test (WOCBP only)
  - Genotyping (only with prior approval of Sponsor)
- Obtain urine samples for:
  - Urinalysis
  - Drugs of abuse
- MFIS
- PGIS – muscle symptoms
- PGIS – fatigue symptoms
- PROMIS Short Form – FACIT Fatigue 13a
- 12MWT
- Eye examination (may be completed at any time between the initial Screening and Baseline visits)
- For subjects <25 years old (only) a wrist radiograph will be required to confirm bone growth plate closure.
- Input of the Pro-forma Screening data into eCRF for PSOC approval
- Subjects will be provided with a pedometer and instructions on how to use the device and complete the eDiary prior to leaving the Study Center (including the requirement for collecting the Screening, pre-treatment, pedometer data for 14 days from the Screening visit)
- Subjects will be provided with a urine collection cup and instructions for collecting a second morning void at the Baseline visit if they are unable to do this at the Study Center for any reason.

Provided the subject fulfils all the inclusion and exclusion criteria, and the PSOC has confirmed their agreement, the subject may enter the study and proceed with the Baseline Visit.

6.2 Baseline (Day 1): Visit 2

Site staff will need to provide subjects with meals and snacks at appropriate times during the visit to ensure subjects have enough energy prior to completing the exercise tests.

Subjects will attend the Study Center for the Baseline Visit when the following assessments will be carried out **prior** to dosing:

Prior to dosing:

- Obtain second morning void urine sample for:
  - Bone marker - N-terminal telopeptide (NTX)
  - Urinalysis
  - Drugs of abuse
  - Urine for pregnancy testing (WOCBP only)
- Obtain blood samples for:
  - Hematology
  - Biochemistry

  - Additional bone and calcium metabolism markers
  - Additional serum for possible use as baseline reference, to be stored frozen at the central laboratory through to completion of the study
  - Pre-dose plasma sample for pharmacokinetic analysis of REN001
- Review of concomitant medications including contraception and non-drug treatments
- Review of pre-treatment events (see Section 7.1.1.1)
- Physician completion of subjects PMM phenotypic description
- Review of Screening pedometer eDiary data
- Full physical examination (including weight)
- 12-lead ECG
- Vital signs (including temperature)
- MFIS
- BPI
- SF-36
- PGIS – muscle symptoms
- PGIS – fatigue symptoms
- PROMIS Short Form – FACIT Fatigue 13a
- WPAI:SHP
- 12MWT
- 30STS

Assessments should be conducted to ensure that the PRO questionnaires are completed prior to exercise testing and the 12MWT must be done at least 1 hour before the 30STS.

Provided the subject meets all the study entry criteria they will be randomized to study treatment. The IWRS will allocate study drug to be dispensed to the subject. The first dose should be taken with food under the supervision of site staff and the time of dosing recorded.

Subjects will remain in the Study Center after study drug administration for the following assessments to be carried out:

- Obtain plasma samples for pharmacokinetic analysis of REN001 (1, 2, 3 and 4* hours postdose)
- Review of adverse events
- Subjects will be provided with a urine collection cup and instructions for collecting a second morning void at the Week 12 visit if they are unable to do this at the Study Center for any reason

* To ease subject burden, the 4-hour post dose blood may be omitted if the visit is extending beyond what is reasonable, at the Investigator's discretion.

Subjects may then leave the Study Center at the discretion of the supervising clinical staff after being provided with sufficient study medication to last until their next scheduled Study Center visit. Subjects will be reminded of the study restrictions and instructed on study medication dosing requirements.

If feasible, and at the Investigator's discretion, the Baseline Visit may be conducted over two days.

6.3 Weeks 2 (Day 14): Visit 3

Subjects will either attend the Study Center or receive a home nursing visit (in countries where the regulatory authority allows) on Day 14. On this day, the subject will take their daily dose at home as usual and record the time of dose on the subject dosing card. The following assessments will be carried out:

- Review concomitant medications, non-drug treatments and study medication compliance
- Review of adverse events
- Vital signs (including temperature)
- Obtain blood samples for:
  - Hematology
  - Biochemistry
  - Plasma sample for pharmacokinetic analysis of REN001
- Obtain urine samples for:
  - Urinalysis
- Capsule counts of remaining capsules including any opened IMP bottles

Subjects will be reminded of the study restrictions and instructed on study medication dosing requirements.

6.4 Weeks 4 and 18 (Days 28 and 126): Visits 4 and 6

Subjects will either attend the Study Center or receive a home nursing visit (in countries where the regulatory authority allows) on Days 28 and 126. On these days, the subject will take their daily dose at home as usual and record the time of dose in the subject dosing card. The following assessments will be carried out:

- Review concomitant medications, non-drug treatments and study medication compliance
- Review of adverse events
- Vital signs (including temperature)
- Obtain blood samples for:
  - Hematology
  - Biochemistry
  - Plasma sample for pharmacokinetic analysis of REN001
- Obtain urine samples for:
  - Urinalysis
  - Urine for pregnancy testing (WOCBP Week 4 only)
- Review of last 7 days pedometer data
- MFIS
- BPI
- SF-36
- PGIS – muscle symptoms
- PGIS – fatigue symptoms
- PROMIS Short Form – FACIT Fatigue 13a

- WPAI:SHP
- Capsule counts of remaining capsules including any opened IMP bottles

Subjects will be reminded of the study restrictions and instructed on study medication dosing requirements.

6.5 Weeks 8, 16 and 20 (Days 56, 112 and 140)

Subjects who are WOCBP at risk of pregnancy will be supplied with urine pregnancy tests to use at home at Weeks 8, 16 and 20. Study Center staff will need to contact the subjects by telephone to obtain the result of the pregnancy test and enter the result into the eCRF. Subjects may be given a reminder telephone call to conduct the test if necessary.

6.6 Weeks 12 and 24 (Days 84 and 168): Visits 5 and 7

Site staff will need to provide subjects with meals and snacks at appropriate times during the visit to ensure subjects have enough energy prior to completing the exercise tests.

Subjects will attend the Study Center on Days 84 and 168. On these days, the subject will take their daily dose in the Study Center. The following assessments will be carried out:

- Obtain second morning void urine sample for:
  - Bone marker - NTX
  - Urinalysis
  - Drugs of abuse
  - Urine for pregnancy testing (WOCBP only)
- Obtain blood samples for:
  - Hematology (including HbA1c at Week 24 only)
  - Biochemistry
  - Additional bone and calcium metabolism markers
  - Pre-dose plasma sample for pharmacokinetic analysis of REN001
- Administer the study drug with food under supervision of clinical staff and the time of dosing recorded.
- Review concomitant medications, non-drug treatments and study medication compliance
- Review of adverse events
- Physician completion of subjects PMM phenotypic description (Week 24 only)
- Review of pedometer eDiary data
- Brief physical examination (including weight)
- 12-lead ECG
- Vital signs (including temperature)
- Obtain plasma samples for pharmacokinetic analysis of REN001 (1, 2, 3 and 4* hours postdose)
- Review of last 7 days pedometer data
- MFIS
- BPI
- SF-36
- PGIS – muscle symptoms

- PGIS – fatigue symptoms
- PGIC – muscle symptoms (Week 24 only)
- PGIC – fatigue symptoms (Week 24 only)
- PROMIS Short Form – FACIT Fatigue 13a
- WPAI:SHP
- 12MWT
- 30STS
- Capsule counts of remaining capsules including any opened IMP bottles
- Subjects will be provided with a urine collection cup and instructions for collecting a second morning void at the Week 24 visit if they are unable to do this at the Study Center for any reason (Week 12 only)
- Eye examinations (may be completed ± 14 days of the visit if required)

Assessments should be conducted to ensure that the PRO questionnaires are completed prior to exercise testing and the 12MWT must be done at least 1 hour before the 30STS.

*To ease subject burden, the 4-hour post dose blood may be omitted if the visit is extending beyond what is reasonable, at the Investigator's discretion.

If feasible, and at the Investigator's discretion, these Visits may be conducted over two days.

At the end of Visit 7 (Week 24) subjects must return all their unused study medication and may then leave the Study Center at the discretion of the supervising clinical staff after being provided with details of their Follow Up visit.

6.7 Follow Up Visit

Subjects will either attend the Study Center or receive a home nursing visit (in countries where the regulatory authority allows), 21-28 days following the last dose of study medication for a Follow Up Visit. This visit will not be required if a subject chooses to enter the REN001 open label extension study at their Week 24 visit.
At this visit, the following activities will be completed:

- Review concomitant medications and non-drug treatments
- Review of adverse events
- Vital signs (including temperature)
- Obtain a urine sample for pregnancy testing (WOCBP only)

If clinically significant safety laboratory findings were apparent at the subjects last visit these should be followed up until resolved with blood samples for hematology and biochemistry and a urine sample for urinalysis as appropriate.

6.8 Early Termination Visit

Subjects who discontinue taking study drug early should, if possible, have an Early Termination visit as soon as possible after the subject stops taking study drug. Where possible the Early Termination visit assessments should be completed in the study center as listed below. Site staff will need to provide subjects with meals and snacks at appropriate times during the visit to ensure subjects have enough energy prior to completing the exercise tests.

- Review concomitant medications and non-drug treatments*
- Review of adverse events*

- Brief physical examination (including weight)
- Physician completion of subjects PMM phenotypic description
- 12-lead ECG
- Vital signs (including temperature) *
- Obtain blood samples for:
  - Hematology (incl HbA1c)*
  - Biochemistry*
  - Additional bone and calcium metabolism markers
  - Plasma sample for pharmacokinetic analysis of REN001*
- Obtain second morning void urine sample for:
  - Bone marker – NTX*
  - Urinalysis*
  - Drugs of abuse*
  - Urine for pregnancy testing (WOCBP only) *
- MFIS*
- BPI*
- SF-36*
- PGIS – muscle symptoms*
- PGIS – fatigue symptoms*
- PROMIS Short Form – FACIT Fatigue 13a*
- PGIC muscle symptoms (with respect to how they were on their last dosing day) *
- PGIC fatigue symptoms (with respect to how they were on their last dosing day) *
- WPAI:SHP*
- 12MWT
- 30STS
- Capsule counts of remaining capsules including any opened IMP bottles*
- Eye examination (may be completed ± 14 days of the visit if required)

If the subject is unable to attend the Study Center for the full visit, then every effort should be made to complete the assessments marked with an asterisk (*) at the discretion of the Investigator this may be completed over 2 days if feasible.

If the subject is withdrawing from the study: after their Early Termination Visit they should be instructed to return for the Follow Up Visit 21-28 days after their last dose of study medication. If the Early Termination Visit is 21 or more days after the last dose, then the Follow Up Visit is not needed.

6.9 Subject Continuation In Study Off IMP

If a subject discontinues taking study drug, they will be strongly encouraged to continue in the study for their Week 12 and Week 24 visits (see Figure 3). If they agree to remain in the study off-IMP as a minimum they will be requested to complete the following assessments:

- Review concomitant medications and non-drug treatments
- Review of adverse events

- Vital signs (including temperature)
- MFIS
- 12MWT

Table 2: Schedule of Activities

Time and Events Table	Screening	Baseline[1, 2] (Day 1)	Week 2[3] (Day 14)	Week 4[3] (Day 28)	Week 8 (Day 56)	Week 12[2, 4] (Day 84)	Week 16 (Day 112)	Week 18[3] (Day 126)	Week 20 (Day 140)	Week 24[2, 4] (Day 168).	Early Termn[2, 5]	Follow Up[3] (21-28 days post last dose)
Visit number	**1**	**2**	**3**	**4**		**5**		**6**		**7**		**8**
Window (days)			±3	±3		±7		±7		±7		
Informed Consent	X											
Completion of Proforma and data entered into eCRF[6]	X											
Demographics	X											
Medical/medication/drug/ alcohol/tobacco history[7]	X											
Physician completion of PMM phenotypic description		X								X	X	
Physical exam[8]	X	X				X				X	X	
12-lead ECG	X	X				X				X	X	
Supine Vital signs (BP, PR and temperature)	X	X	X	X		X		X		X	X	X
Serum FSH[9]	X											
HbA1c	X									X	X	
Hepatitis B/C/HIV	X											
Safety labs (inc. urinalysis)	X	X[10]	X	X		X		X		X[11]	X	X[11]
Blood sample for bone and calcium markers		X				X				X	X	
Pregnancy Test (WOCBP only)[12]	X	X		X	X	X	X		X	X	X	X
Blood sample for genotyping[13]	X											
Population PK blood sample[14]		X	X	X		X		X		X	X	
Urine drugs of abuse	X	X				X				X	X	
Urine NTX		X				X				X	X	

Time and Events Table	Screening	Baseline[1, 2] (Day 1)	Week 2[3] (Day 14)	Week 4[3] (Day 28)	Week 8 (Day 56)	Week 12[2, 4] (Day 84)	Week 16 (Day 112)	Week 18[3] (Day 126)	Week 20 (Day 140)	Week 24[2, 4] (Day 168).	Early Termn[2, 5]	Follow Up[3] (21-28 days post last dose)
Sites to provide appropriate snacks and lunch	X	X				X				X	X	
Pedometer eDiary data collection (inc. review)	X	X[15]		X		X		X		X		
MFIS / PGIS (muscle symptoms), PGIS (fatigue symptoms) and PROMIS – Short form FACIT fatigue 13a	X	X		X		X		X		X	X	
SF-36 / BPI and WPAI:SHP		X		X		X		X		X	X	
PGIC (muscle symptoms) and PGIC (fatigue symptoms)										X	X	
12 Minute Walk Test	X[16]	X				X				X	X	
30STS		X				X				X	X	
IMP Capsule Counts			X	X		X		X		X	X	
Eye examination	X[17]					X				X	X	
Wrist radiograph	X[18]											
Concomitant medication review	X----------X											
Dosing		X[19]----------X										
AE collection and reporting	X----------X											

1. Screening visit must take place a maximum of 8 weeks before the Baseline visit.
2. If appropriate and feasible Baseline, Week 12, Week 24/Early Termination visits may be conducted over 2 days at the Investigators discretion.
3. In countries where the regulatory body allows, visit may be in the Study Center or a home nursing visit.
4. Only review of concomitant medications, AE's MFIS and 12MWT will be conducted if the subject has discontinued from study drug treatment but has not withdrawn from the study. If the subjects had an ET visit within 2 weeks of either of these a visit is not required.
5. As a minimum, subjects should have review of concomitant medication and AE's, vital signs (including temperature), complete questionnaires, safety laboratory blood (including HbA1c, bone and calcium markers and PK analysis) and urinalysis (including pregnancy tests for WOCBP, drugs of abuse and NTX) and IMP capsule counts.
6. Patient Screen Oversight Committee will review Proforma data and give a decision or request more information within 7 working days.
7. Subjects will be excluded from the study at the Investigators discretion for alcohol and/or drug dependency. Use of opiates/cannabis for medical reasons is acceptable with prescription evidence or at the Investigators discretion.
8. Full physical exam, height and weight at Screening; full physical exam and weight at Baseline; brief, symptom-directed physical exam and weight only at Weeks 12 and 24.

9. Serum FSH testing for postmenopausal females 45years and older.
10. An additional 10 mL blood sample will be taken pre-dose at baseline and the serum will be stored frozen at the central laboratory as a reference sample in the event that re-analysis of protocol stated tests are required.
11. If any clinically significant abnormalities are noted at the Week 24 visit, these should be followed up until resolved.
12. WOCBP will be supplied with urine home pregnancy test kits at Study Center visits to test at Weeks 8, 16 and 20. The Study Center must contact the subject to confirm the pregnancy test result in a timely manner.
13. Blood sample for local genotype testing will only be conducted with prior Sponsor approval and definitive results must be available prior to the Baseline visit.
14. At the Baseline, Week 12 and Week 24 visits, blood samples will be taken pre-dose and then at 1, 2, 3, and 4 hours post-dose. On the other visits, a single sample can be taken at any time post-dose provided the dosing date and time are recorded.
15. Review of Screening (pre-treatment) pedometer data at Baseline.
16. Screening 12MWT must be at least 4 weeks before the Baseline 12MWT.
17. The Screening eye examination can be performed at any time between the initial Screening and Baseline Visit for scheduling reasons.
18. Subjects <25 years old only.
19. Dosing on Baseline, Week 12 and Week 24 visits should be under the supervision of the site staff.

7 PARAMETERS AND METHODS OF ASSESSMENT

7.1 Safety Parameters

7.1.1 Adverse Events

7.1.1.1 Definitions

Pre-treatment Event (PTE)

A PTE is defined as any untoward medical occurrence in a clinical investigation subject who has signed informed consent to participate in a study but prior to administration of any study medication; it does not necessarily have to have a causal relationship with study participation.

Adverse Event (AE)

An AE is any untoward medical event that occurs in a subject who has received study medication and does not necessarily have a causal relationship with study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the product.

A treatment-emergent AE will be defined as an AE that begins or that worsens in severity after the first dose of study drug has been administered.

An adverse drug reaction (ADR) is any untoward and unintended response in a subject to an IMP which is related to any dose administered to that subject.

An unexpected adverse reaction is an adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in the Investigator's Brochure.

All PTEs/AEs, including intercurrent illnesses, occurring during the study will be documented in the eCRF.

Each event should be recorded to represent a single diagnosis. Accompanying signs (including abnormal laboratory values or ECG findings) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, sign(s) or symptom(s) should be recorded appropriately as a PTE(s) or as an AE(s).

7.1.1.2 Laboratory values and ECG findings

Changes in laboratory values or ECG parameters are only considered to be PTEs or AEs if they are judged to be clinically significant (i.e., if some action or intervention is required or if the Investigator judges the change to be beyond the range of normal physiologic fluctuation). A laboratory re-test and/or continued monitoring of an abnormal value are not considered an intervention. In addition, repeated or additional noninvasive testing for verification, evaluation or monitoring of an abnormality is not considered an intervention.

If abnormal laboratory values or ECG findings are the result of pathology for which there is an overall diagnosis (e.g., increased creatinine in renal failure), the diagnosis only should be reported appropriately as a PTE or as an AE.

7.1.1.3 Pre-existing conditions

Pre-existing conditions (present at the time of signing of informed consent) are considered concurrent medical conditions and should NOT be recorded as PTEs. Baseline evaluations (e.g., laboratory tests, ECG, X-rays etc.) should NOT be

recorded as PTEs. However, if the subject experiences a worsening or complication of such a concurrent condition, the worsening or complication should be recorded appropriately as a PTE/AE. Investigators should ensure that the event term recorded captures the change in the condition (e.g., "worsening of…").

If a subject has a pre-existing episodic condition (e.g., asthma, epilepsy) any occurrence of an episode should only be captured as a PTE/AE if the episodes become more frequent, serious or severe in nature, that is, Investigators should ensure that the AE term recorded captures the change in the condition from Baseline (e.g., "worsening of…").

If a subject has a degenerative concurrent condition (e.g., cataracts, arthritis), worsening of the condition should only be captured as a PTE/AE if occurring to a greater extent to that which would be expected. Again, Investigators should ensure that the AE term recorded captures the change in the condition from Baseline (e.g., "worsening of…").

7.1.1.4 Worsening of PTEs or AEs

If the subject experiences a worsening or complication of a PTE after starting administration of the study medication, the worsening or complication should be recorded appropriately as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g., "worsening of…").

If the subject experiences a worsening or complication of an AE after any change in study medication, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g., "worsening of…").

7.1.1.5 Adverse Events of Special Interest

Adverse events of special interest are those of scientific and medical concern specific to the Sponsor's product for which ongoing monitoring and rapid communication by the Investigator to the Sponsor may be warranted. These events require further investigation in order to characterize and understand them. Based on this assessment, the Sponsor plans to include events of special interest as follows:

- Changes in laboratory parameters of muscle injury associated with clinically significant adverse events
- Changes observed after Baseline in formation or exacerbation of cataracts or, visual acuity. A spherical equivalent change of $\geq$0.75 diopters will be considered to be a clinically significant change regardless of changes in lens clarity.
- Occurrence of fractures after Baseline.

While AESIs may not meet the definition of an SAE, they should be reported in the same manner as SAEs (See Section 7.1.1.9). This allows them to be tracked in more detail and followed up for additional information if required. When completing the SAE form there is an extra box to check to confirm that the event is an AESI. AESIs will not be reported to the Regulatory Authorities unless they are also SUSARs.

7.1.1.6 Assessment of Adverse Event

Each AE will be assessed by the Investigator with regard to the following categories.

7.1.1.6.1 Seriousness

A serious AE (SAE) (or serious ADR or unexpected serious adverse reaction) is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening. (This means that the subject is at risk of death at the time of the event; it does not mean that the event hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event(s) that may not be immediately life-threatening or result in death or hospitalization but that may jeopardize the subject or require intervention to prevent one of the above outcomes. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

Note: Cases of potential drug-induced liver injury as assessed by laboratory test values ("Hy's Law Cases") are also reportable. If a subject develops abnormal values in AST or ALT or both, concurrent with abnormal elevations in total bilirubin and no other known cause of liver injury, that event would be classified as a Hy's Law Case. In this clinical study, the term SAE will be understood to also include Hy's Law Cases.

Medical and scientific judgment should be exercised in deciding whether a case is serious and whether expedited reporting is appropriate.

7.1.1.6.2 Severity

The intensity of each PTE/AE must be assessed by the Investigator using one of the following categories, and recorded in the eCRF:

- Mild: An AE that does not interfere with usual activities
- Moderate: An AE that interferes with usual activities
- Severe: An AE that prevents usual activities

7.1.1.6.3 Causality

The Investigator will assess the causality / relationship between the study drug and the AE and record that assessment in the eCRF. Causality will be shown as Related, Possibly Related, or Not related.

7.1.1.7 Relationship to Study Procedures

Relationship (causality) to study procedures should be determined for all PTEs and AEs.

The relationship should be assessed as Yes if the Investigator considers that there is reasonable possibility that an event is due to a study procedure. Otherwise, the relationship should be assessed as No.

7.1.1.8 Collection and Reporting of AEs

7.1.1.8.1 PTE and AE Collection Period

Collection of PTEs will commence from the time the subject signs the informed consent to participate in the study and continue until the subject is first administered study medication (Day 1) or until screen failure.

Collection of AEs will commence from the time that the subject is first administered study medication (Day 1). Routine collection of AEs will continue until the Follow Up visit 21 to 28 days after the last study dose.

7.1.1.8.2 PTE and AE Reporting

At each study visit, the Investigator will assess whether any subjective AEs have occurred. A neutral question, such as "How have you been feeling since your last visit?" may be asked.

Subjects may report AEs occurring at any other time during the study. Subjects experiencing a serious PTE must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or there is a satisfactory explanation for the change.

Non-serious PTEs, related or unrelated to the study procedure, do not need to be followed-up for the purposes of the protocol.

All subjects experiencing AEs, whether considered associated with the use of the study medication or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or until there is a satisfactory explanation for the changes observed. All PTEs and AEs will be documented in the PTE/AE page of the eCRF, whether or not the Investigator concludes that the event is related to the study medication. The following information will be documented for each event:

- Event term.
- Start and stop date {and time}.
- Severity.
- Investigator's opinion of the causal relationship between the event and administration of study medication (not completed for PTEs).
- If no, provide alternative etiology or explanation.
- Investigator's opinion of the causal relationship to study procedure(s), including the details of the suspected procedure.
- Action concerning study medication (not applicable for PTEs).
- Outcome of event.
- Seriousness.

7.1.1.9 Reporting Serious Adverse Events

All SAEs that occur during the study from the signing of the informed consent and up to 30 days after receiving the last dose of study drug, whether considered to be associated with the study drug or not, must be reported within 24 hours by email or fax to the study pharmacovigilance (PV) team. The minimum information required for an initial report is:

- Sender of report (name, address of Investigator)

- Subject identification (subject study number, initials, NOT subject name)
- Protocol number
- Description of SAE and reason why the event is categorized as serious
- Causality assessment, if possible

However, as far as possible all points on the SAE form should be covered in the initial report and the completed SAE form faxed or emailed to the pharmacovigilance team (details below). The original SAE form should be sent to the address below and the Medical Monitor and the Sponsor Chief Medical Officer informed by email. In addition, the event must be documented in the eCRF.

SAE info
Bionical Emas
Suite 209, Spirella Building
Bridge Road
Letchworth Garden City
Hertfordshire
SG6 4ET
United Kingdom
Email: Drug.safety@bionical-emas.com Fax: +44 (0) 1462 600 456
US Fax: +1 206 260 7410

After receipt of the initial report, the PV team will review the information and, if necessary, contact the Investigator, to obtain further information for assessment of the event. Bionical Emas the PV vendor will be responsible for all information processing and reporting according to local legal requirements. Where necessary, Investigators will inform the authorities in their own countries.

7.1.1.10 Reporting Pregnancies

Although pregnancy should not be considered as an SAE *per se*, any pregnancies occurring in a study subject between Screening and the end of the contraception requirements (14 weeks after last dose for males, 30 days after last dose for WOCBP) must be reported and captured as if they were SAEs.

If any subject is found to be pregnant during the study, she should be withdrawn and study drug should be immediately discontinued. In addition, any pregnancies in the partner of a male subject during the study or for 14 weeks after the last dose should also be recorded following authorization from the subject's partner.

All reported pregnancies will be followed up to final outcome, using the paper Pregnancy Monitoring Report Form. The outcome, including any premature termination, must be reported to the Sponsor. An evaluation after the birth of the child will also be conducted.

Reports should be sent within 24 hours to the contacts detailed above, using the Pregnancy Monitoring Report Form. If the female subject and/or female partner of a male subject agrees to the primary care physician being informed, the Investigator should notify the primary care physician that the subject/female partner of the subject

was participating in a clinical study at the time she became pregnant and provide details of treatments the subject received.

7.1.1.11 Follow-up of Adverse Events

All AEs experienced by a subject, irrespective of the suspected causality, will be monitored until the event has resolved, any abnormal laboratory values have returned to baseline or stabilized at a level acceptable to the Investigator, Medical Monitor and/or Sponsor Chief Medical Officer or designee, or until there is a satisfactory explanation for the changes observed, or until the subject is lost to follow-up.

7.1.1.12 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities

The Sponsor will be responsible for reporting all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, Investigators and IRBs or IECs, as applicable, in accordance with national or regional regulations in the countries where the study is conducted. Relative to the first awareness (Day Zero) of the event by/or further provision to the Sponsor or Sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other serious events, unless otherwise required by national regulations. The Sponsor will also prepare an expedited report for Events Requiring Other Actions, where these might materially alter the current risk-benefit assessment of an IMP or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the trial. The investigational site also will forward a copy of all expedited reports to his or her IRB or IEC in accordance with national regulations. In addition, once a year throughout the clinical trial or upon request, a Development Safety Update Report (DSUR) will be submitted to the concerned Competent Authorities (CAs) and IEC/IRBs taking into account all new available safety information received during the reporting period.

7.1.2 Clinical Laboratory Safety Tests

Laboratory assessments will be performed by a central laboratory. Details of the urine and blood sampling procedures and any subject restrictions (e.g. withholding supplements) and subsequent storage and shipment of samples can be found in the REN001-201 Laboratory Manual. Sites will be provided with lab kits from the central laboratory. Details of parameters to be tested are listed in Table 3.

Table 3: Safety Laboratory Parameters

Biochemistry	**Hematology**
Albumin Alanine aminotransferase (ALT) Aldolase Alkaline phosphatase Aspartate aminotransferase (AST) Bilirubin (Total, direct and indirect) ***Bone specific alkaline phosphatase (BSAP)**** Calcium Cholesterol (Total, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL)) C-reactive protein Creatinine Creatine phosphokinase (CK) eGFR using CKD-EPI (at Screening only)** FSH (screening, postmenopausal females only) Gamma glutamyl transferase (GGT) Glucose (non-fasted) Human chorionic gonadotropin (hCG) (at screening, WOCBP only) Lipase Magnesium ***Parathyroid hormone (PTH)**** Phosphate Potassium Protein (Total) Sodium Triglycerides Troponin I Urea Uric acid ***Vitamin D**** **** Collected at Baseline, Week 12, Week 24 or ET visit only*** ***** subjects ethnicity data will be required to calculate eGRF accurately***	Basophils Eosinophils Hemoglobin HbA1c (Screening and Week 24/ET only) Hematocrit (Packed cell volume) Large unstained cells Lymphocytes Mean corpuscular volume (MCV) Monocytes Neutrophils Platelet count Red cell count Reticulocytes White cell count
Serological Markers (Screening only)	
HIV HBsAg HBcAb Anti-hepatitis C virus serology (by multi-antigen enzyme immunoassay (EIA))	
Urinalysis	
Blood, Glucose, Ketone, Protein, pH, Specific Gravity, Nitrite, Leukocytes, Bilirubin and Urobilinogen. Any significant abnormalities in the urinalysis should be investigated via microscopy and the findings reported in the eCRF. hCG: To be measured at the Study Center at Weeks 4, 12, 24 and Follow Up. To be measured by subject at home (using tests supplied) at Weeks 8, 16 and 20. Drugs of abuse: cocaine, cannabinoids, opiates, barbiturates, benzodiazepines, methadone, and amphetamines (at Screening, Baseline, Week 12, Week 24 or ET visit only) Urine N-terminal telopeptide (NTX) will collected at second morning void (at Baseline, Week 12, Week 24 or ET visit only)	

An extra blood sample (10 mL) will be taken pre-dose at the Baseline visit and the serum will be stored frozen at the central laboratory through to completion of the study for possible use as a baseline reference should additional safety laboratory tests

or markers of disease activity be indicated. Samples will be stored frozen until the end of the study, when they will be destroyed, on approval from the Sponsor.

Laboratory test results can be repeated if necessary. Any clinically significant abnormal laboratory test result (e.g., findings that lead to more extensive investigation and/or indicate a risk to the health of the subject) should be reported as an adverse event. (The Investigator should assess the possible relationship to study drug as is the case for all other adverse events.)

7.1.3 Blood Volume

The approximate volume of blood to be taken from each subject during the study is detailed in Table 4. Additional blood may be required for repeats of safety laboratory tests.

Table 4: Approximate Blood Volume Required for Study

Test	Number of Samples	Volume (mL)	Total (mL)
Hematology (including HbA1c)	7	2	14
Biochemistry (including aldolase, troponin I, FSH and hCG as applicable)	7	5	35
Serology (including Hep B/C/HIV)	1	8.5	8.5
PK samples	18	4	72
Baseline serum sample	1	10	10
Bone and calcium markers (including BSAP, PTH, and vitamin D)	3	4	12
Genotyping (if required)	1	5	5
Total volume (mL)			156.5

7.1.4 Physical Examination

All physical examinations will be carried out by a suitably qualified doctor or equivalent. At Screening and Baseline the subject will have a full physical examination, to include an assessment of head, neck, heart, lungs, abdomen, skin (including hair and nails), peripheral circulation, joints, general appearance, and a neurological examination. Any abnormalities should be recorded in the study eCRF.

At Weeks 12 and Weeks 24 subjects will have a brief symptom-directed physical examination, which should include general appearance, heart, lungs, skin, and a neurological examination. Clinically significant changes from the baseline examination should be assessed. Other systems examined should be determined by clinical findings and any adverse events reported.

7.1.5 Height and Weight and Body Mass Index

Subjects should have weight and height measured while wearing indoor clothing and with shoes off. The Body Mass Index (BMI) will be calculated in the eCRF (expressed to 1 decimal place) using metric units with the formula provided below:

- BMI = weight (kg)/height (cm) ^{2}

- Height will be collected in centimeters (without decimal places) at Screening only, and weight will be collected in kilograms (to 1 decimal place).

7.1.6 Vital Signs Measurements

Blood pressure (systolic and diastolic mmHg) and pulse (beats per minute) will be measured. Subjects should be supine for at least 5 minutes before taking the measurement. All measurements will be taken with the subject in a supine position. Blood pressure will be measured using the subject's dominant arm, where possible this should be done throughout the study, and recorded to the nearest mmHg.

Pulse should be measured in the brachial/radial artery for at least 30 seconds.

7.1.7 Body Temperature

Body temperature will be recorded using a digital thermometer. Any results <35ºC should be repeated to ensure correct placement of the thermometer.

7.1.8 Electrocardiogram Recording

Single ECG measurements using a 12-lead ECG machine will be taken after the subject has rested for at least 10 minutes in a supine position. To ensure safety of the subjects, a qualified individual at the site will evaluate all ECGs.

If the QTcF is ≥450 msec at Screening or QTcF is ≥500 msec for any post-baseline ECG, the ECG intervals should be inspected carefully to ensure that the RR interval has been recorded correctly, and a single ECG will be repeated at 5-minute intervals 3 times. If these values are consistently high (≥450 or 500 msec, respectively), or if the Investigator or Medical Monitor has any concern, as a precautionary measure, the subject will be screen failed or discontinue study treatment as appropriate (see Section 3.6).

If a machine read QTcF value is prolonged, as defined above, repeat measurements may not be necessary, provided a qualified physician's interpretation determines that the QTcF values are acceptable for the individual. In some cases, it may be necessary to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that leads are placed in the same positions each time in order to achieve precise ECG recordings.

ECGs may be sent to an ECG vendor for centralized reading at a later date if deemed necessary.

7.1.9 Eye Examination

All eye examinations should be carried out by an appropriately qualified professional. Best efforts should be made to ensure the same professional undertakes the slit lamp examinations for a particular subject. Each lens should be classified and graded against the Lens Opacity Classification System III, (Chylack et al, 1993).

In addition, best corrected distance visual acuity (including refraction) should also be assessed. A spherical equivalent change of ≥0.75 diopters will be considered to be a clinically significant change regardless of changes in lens clarity.

It is important that qualified professionals administering the examination are fully trained to reduce variability across Study Centers. The data should be collected in the separate eye examination workbook.

For scheduling reasons, the above assessments may be conducted any time between the initial Screening visit and Baseline for the pre-treatment assessment and ± 14 days of the visit window given for the eye assessments performed at the Week 12 and Week 24 visits. Note for re-screened subjects their original eye examination can be used and the examination does not need to be repeated if its within 6 months of the original eye examination date.

In addition, any subject who experiences any visual symptoms during the study should have an additional ophthalmic examination as appropriate.

7.1.10 12-Minute Walk Test

The 12MWT is a practical test that requires simple equipment to execute. This test measures the distance a subject can walk on a flat, hard surface in a period of 12 minutes. It is important that operators administering the test are fully trained to reduce variability across Study Centers. There must be a minimum of 4 weeks between the Screening and Baseline 12MWT to minimize any training effect. Subjects who walk further than 1000 m at Screening will be excluded from the study. Subjects who walk ≤ 1000 m at Screening but subsequently walk greater than 1000 m at Baseline will be allowed to continue into the study but will be excluded from the per protocol analysis. Further details are given in the REN001-201 Exercise Manual.

7.1.11 36 Item Health Survey V2.0® (SF-36)

The 36-Item Health Survey Version 2.0® asks questions which cover eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. It also includes a single item that provides an indication of perceived change in health. A one-week recall period will be used.

7.1.12 Modified Fatigue Impact Scale (MFIS)

The MFIS is a detailed tool, that is completed by the subject personally, rather than having an interview and thus, no training is required to deliver it. Scoring is simple, the score reflects functional limitation due to fatigue experienced within the previous 7 days rather than a measure of the current level of fatigue. It may be used in both the clinical and the research setting in people for whom fatigue is a predominant symptom.

There are 21 items, each of which is scored 0 (Never) to 4 (Almost always), providing a continuous scale of 0–84. It is composed of three subscales that describe the impact of fatigue on physical, cognitive and psychosocial functioning:

Physical functioning (9 items) reflects motivation, effort, stamina, and coordination. The physical subscale can range from 0 to 36. It is calculated by adding items 4+6+7+10+13+14+17+20+21.

Cognitive functioning (10 items) concerns concentration, memory, thinking and organization of thoughts. The cognitive subscale can range from 0 to 40. It is calculated by adding items 1+2+3+5+11+12+15+16+18+19.

Psychosocial functioning (2 items) describes the impact of fatigue upon isolation, emotions, workload, and coping. The psychosocial subscale can range from 0 to 8. It is calculated by adding items 8+9.

All items are scaled so that a higher score indicates a greater level of fatigue.

See Appendix 2.

7.1.13 Brief Pain Inventory (BPI)

The Brief Pain Inventory (Short Form) rapidly assesses the severity of pain and its impact on functioning. It is widely used in research and clinical settings.

Four severity items will be investigated by the responses to the worst, least and average pain in last 24 hours and the pain right now (Questions 3, 4, 5 and 6). The higher score indicates worse pain. A pain severity score will be calculated as the mean of the non-missing 4 severity items. A higher score indicates greater pain severity.

In addition, the average pain interference score will be derived as the average of the responses to the 7 components to Question 9. A higher score indicates the more pain interferes with daily functioning.

See Appendix 3.

7.1.14 Patient Global Impression of Severity (PGIS) – Muscle Symptoms

The patient will be asked to rate the severity of their PMM muscle symptoms over the past 7 days.

Overall, how would you rate the severity of your muscle symptoms related to your Primary Mitochondrial Myopathy over the past 7 days?

a. □ Absent
b. □ Mild
c. □ Moderate
d. □ Severe
e. □ Very Severe

7.1.15 Patient Global Impression of Severity (PGIS) – Fatigue Symptoms

The patient will be asked to rate the severity of their PMM fatigue symptoms over the past 7 days.

Overall, how would you rate the severity of your fatigue symptoms related to your Primary Mitochondrial Myopathy over the past 7 days?

a. □ Absent
b. □ Mild
c. □ Moderate
d. □ Severe
e. □ Very Severe

7.1.16 Patient Global Impression of Change (PGIC) – Muscle Symptoms

At the end of study treatment (Day 168) or Early Termination visit with respect to the last day of study treatment, the subject will be asked to rate their degree of improvement or worsening of PMM muscle symptoms compared to before the start of study drug, using a 7-point scale, standardized PGIC scale.

Overall, how would you rate the change in the severity of your muscle symptoms related to your Primary Mitochondrial Myopathy **since starting the study**?

a. ☐ Very much improved
b. ☐ Moderately improved
c. ☐ Minimally improved
d. ☐ No change
e. ☐ Minimally worse
f. ☐ Moderately worse
g. ☐ Very much worse

7.1.17 Patient Global Impression of Change (PGIC) – Fatigue Symptoms

At the end of study treatment (Day 168) or Early Termination visit with respect to the last day of study treatment, the subject will be asked to rate their degree of improvement or worsening of PMM fatigue symptoms compared to before the start of study drug, using a 7-point scale, standardized PGIC scale.

Overall, how would you rate the change in the severity of your fatigue symptoms related to your Primary Mitochondrial Myopathy **since starting the study**?

a. ☐ Very much improved
b. ☐ Moderately improved
c. ☐ Minimally improved
d. ☐ No change
e. ☐ Minimally worse
f. ☐ Moderately worse
g. ☐ Very much worse

7.1.18 Work Productivity and Activity Impairment Questionnaire: Specific Health Problem (WPAI:SHP)

The Work Productivity and Activity Impairment (WPAI) questionnaire is a well validated instrument to measure impairments in work and activities due to a specific disease. Outcomes are expressed as impairment percentages, with higher numbers indicating greater impairment and less productivity, i.e., worse outcomes. See calculation below:

Scores:

Percent work time missed due to PMM: 100 x Q2 / (Q2 + Q4)
Percent impairment while working due to PMM: 100 x Q5 / 10
Percent overall work impairment due to PMM: 100 x Q2 / (Q2 + Q4) + [(1-(Q2 / (Q2 + Q4))) x (Q5 / 10)]
Percent activity impairment due to PMM: 100 x Q6 / 10

See Appendix 4.

7.1.19 PROMIS Short Form v1.0 – Fatigue 13a (FACIT-Fatigue)

The PROMIS® (Patient-Reported Outcomes Measurement Information System) Fatigue items assess a range of self-reported symptoms, from mild subjective feelings of tiredness to an overwhelming, debilitating, and sustained sense of exhaustion that likely decreases one's ability to execute daily activities and function normally in

family or social roles. The fatigue short forms are universal rather than disease-specific. All assess fatigue over the past seven days. The score is calculated by adding all the individual question scores (scores of 1 to 5) together to give a total score. Hence a range of scores between 13 and 65 are possible with a higher score indicating a greater level of fatigue.

See Appendix 5.

7.1.20 30 Second Sit-To-Stand Test

The purpose of the 30 second sit-to-stand test is to assess leg strength and endurance. Subjects are required to sit and rise to full standing position as many times as they can in 30 seconds. A subject can only conduct the test if they are able to stand without using their arms to assist them out of the chair. A score of 0 and a reason for not completing the test must be noted in the eCRF in these circumstances. Details of any orthotics used should also be recorded in the eCRF. This test must be completed at least an hour after the 12MWT.

Further details are given in the REN001-201 Exercise Manual.

7.1.21 Pedometer Step Counts and eDiary

At the completion of the Screening visit each subject will be given a pedometer (3DFitBud model A420S or equivalent). The site staff will set-up the pedometer with the eDiary (Medrio formerly HMD Clinical Ltd) and train the subject on how and when to use it. Subjects will collect their daily step counts (from waking until bedtime) and should enter the total step count each day in the eDiary. Subjects will be asked to start using the pedometer for 14 days once they have returned home after their Screening visit. For the remainder of the study subjects will use the pedometer and collect the daily step counts for 7 consecutive days within a 2-week window before each study visit. Reminders will be sent to subjects who forget to record the step count data. In addition, the eDiary will allow subjects to enter missed data up until midday the next day. If subjects are unable or non-compliant in using either the pedometer or eDiary for any reason, data will not be analyzed for that subject.

7.1.22 PMM Phenotypic Description

To enable comparative descriptions of the phenotypic state of each subjects' disease, 3 questions, taken from the Newcastle Mitochondrial Disease Adult Scale (NMDAS) will be completed by the physician. A physician will complete the questions at Baseline and Week 24 (or at the Early Termination Visit).

The questions do not need to be read verbatim, but the subject should be questioned to determine the response which best describes the subject's function. To ensure consistency in interpretation the same physician (where possible) should assess subjects on both occasions.

Rate function over the preceding **4-week period, according to patient and/or caregiver** interview only. The clinician's subjective judgement of functional ability should not be taken into account.

Question 1 – Exercise Tolerance

0. Normal
1. Unlimited on flat – symptomatic on inclines or stairs.

2. Able to walk <1000m on the flat. Restricted on inclines or stairs - rest needed after 1 flight (12 steps).
3. Able to walk <500m on the flat. Rest needed after 8 steps on stairs.
4. Able to walk <100m on the flat. Rest needed after 4 steps on stairs.
5. Able to walk <25m on the flat. Unable to do stairs alone.

Question 2 – Gait Stability

0. Normal
1. Normal gait – occasional difficulties on turns, uneven ground, or if required to balance on narrow base.
2. Gait reasonably steady. Aware of impaired balance. **Occasionally** off balance when walking.
3. Unsteady gait. **Always** off balance when walking. **Occasional** falls. Gait steady with support of stick or person.
4. Gait grossly unsteady without support. **High likelihood** of falls. Can only walk short distances (< 10m) without support.
5. Unable to walk without support. Falls on standing.

Rate current status according to examination performed at **the time of** assessment

Question 3 – Myopathy

0. Normal
1. Minimal reduction in hip flexion and/or shoulder abduction **only** (e.g., MRC 4+/5).
2. Mild but clear proximal weakness in hip flexion and shoulder abduction (MRC 4/5). Minimal weakness in elbow flexion and knee extension (MRC 4+/5 – both examined with joint at 90 degrees).
3. Moderate proximal weakness including elbow flexion and knee extension (MRC 4/5 or 4 -/5) **or difficulty** rising from a 90-degree squat.
4. Waddling gait. **Unable** to rise from a 90-degree squat (=a chair) unaided.
5. Wheelchair dependent **primarily** due to proximal weakness.

7.1.23 Pharmacokinetic Blood Samples

Blood samples for determination of REN001 concentrations will be collected via a direct venipuncture or indwelling cannula.

A 4.0 mL blood sample, to provide approximately 2 mL of plasma for pharmacokinetic analysis, will be collected into an appropriately labelled tube. Sample handling instructions will be provided in the REN001-201 Laboratory Manual. The plasma samples will be stored at the central lab prior to analysis. Plasma samples will be transferred from the central lab to the bioanalytical laboratory on dry ice to maintain frozen conditions.

Analysis of REN001 concentrations in plasma will be performed using a validated analytical method.

After completion of the study any remaining PK samples may also be analyzed for metabolites.

7.1.24 Genotyping Blood Samples

If a subject meets all criteria for enrollment into the study but a definitive mitochondrial DNA genotype has not been established, the Study Center should seek Sponsor approval to conduct local genotype testing for the subject. The Sponsor will evaluate and approve this testing for the study on a case by case basis.

A 5.0 mL blood sample for the determination of mitochondrial DNA genotyping will be collected via a direct venipuncture or indwelling cannula. Samples should be handled and processed according to local recommendations.

Definitive results of mitochondrial DNA mutation must be available before commencement of the subjects Baseline visit. The Screening period may be extended to accommodate collection of genotyping results.

8 STATISTICAL METHODS

A formal Statistical Analysis Plan (SAP) will be developed and finalized prior to locking the study database. The full details of data presentations and analyses will be provided in the SAP. Additional statistical analyses other than those described in this section may be performed if deemed appropriate and included in the SAP. Any deviations from the final SAP will be discussed in the final study report.

8.1 Study Subjects

8.1.1 Disposition of Subjects

The number and percentage of subjects entering and completing the study will be presented overall and by treatment group. Reasons for withdrawal will also be summarized.

8.1.2 Protocol Deviations

Deviations from the protocol will be categorized as "minor" or "major" in cooperation with the Sponsor. Deviations will be assessed regularly and finalized prior to database lock.

8.1.3 Analysis Populations

Screened Set	All subjects who sign the informed consent form.
Randomized Set	All subjects who are randomized.
Full Analysis Set (FAS)	All randomized subjects who receive at least one dose of study treatment.
Modified Analysis Set	All randomized subjects who receive at least one dose of study treatment and at least one on-treatment measurement*.
Safety Set	All subjects who receive at least one dose of study treatment.
Per protocol (PP) Set	All subjects in the FAS who do not violate inclusion or exclusion criteria and/or deviate from the protocol, in a way that could influence their efficacy assessment. Subjects who walk more than 1000m in the Baseline 12MWT will also be excluded from the set.
PK Set	All subjects in the FAS who receive REN001 and have at least 1 evaluable post-dose PK measurement (even if this is <Level of Quantification (LOQ)).

* For the primary endpoint this will be 12MWD

The FAS will be used for reporting demographic and baseline characteristic data, and as the primary analysis population for efficacy. Subjects will be analyzed by planned treatment. The PP set will be used for sensitivity analyses of the primary and secondary endpoints.

All safety analyses will be based upon the Safety Set. Subjects will be analyzed by the treatment received.

After all the data have been verified/coded/entered into the database, a review will be performed. The purpose of this review will be to define the analysis populations. The review will also check the quality of the data, identify outliers, and make decisions on how to deal with any data issues (e.g., missing values, withdrawals, protocol

deviations). After the pre-analysis review, resolution of all issues and documentation of all decisions, the database will be locked.

8.2 General Considerations

8.2.1 Statistical Hypotheses

The null (H_0) and alternative (H_1) hypotheses for the primary endpoint, can be expressed as:

H_0: $\mu_{REN} = \mu_{PLA}$

H_1: $\mu_{REN} \neq \mu_{PLA}$

where μ_{REN} and μ_{PLA} are the Week 24 mean changes from baseline in 12MWD for REN001 and Placebo respectively.

All statistical testing will be at the 5% level of significance (2-sided) and all point estimates for the comparison between treatment groups will be accompanied by 2-sided 95% confidence intervals.

8.2.2 Determination of Sample Size

A sample size of 186 subjects (93 per treatment group) provides 90% power, with a two-sided significance level of 5%, of obtaining a statistically significant difference between two treatment groups for the distance walked during the 12MWT. This assumes a treatment effect (difference to placebo in mean change from baseline) size of 60 meters. The standard deviation for the changes from baseline observed in Study REN001-101 was approximately 100 meters. A higher standard deviation, 125 meters, has been assumed for this study due to the longer assessment period (24 weeks instead of 12 weeks) and the larger number of sites included in this study. Allowing for a reduction in power due to subject withdrawals a total of approximately 200 subjects will be randomized.

8.2.3 Control of the Type 1 Error Rate

To preserve the Type 1 error a fixed sequence testing procedure will be adopted. The order of testing will be:

1. Change from Baseline at Week 24 in distance walked during the 12MWT
2. Change from Baseline at Week 24 in the MFIS Physical subscale score
3. PGIC score (muscle symptoms) at the end of treatment

8.2.4 Data Summaries

All endpoints will be summarized by treatment group and visit. Continuous data will be summarized using descriptive statistics (e.g., mean and standard deviation) and categorical data will be summarized using frequency tables (counts and percentages).

8.3 Demographics, Baseline Characteristics and Concomitant Medications

Demographics, baseline characteristics, medical history and concomitant medication data will be summarized by treatment group and over all subjects.

WHODrug and MedDRA coding dictionaries will be used for the concomitant medications and medical histories respectively.

8.4 Treatment Compliance

Treatment compliance will be assessed through capsule counts and will be summarized by treatment group.

8.5 Efficacy Analyses

8.5.1 Primary Endpoint

The changes from baseline in distance walked during the 12MWT for the FAS will be analyzed using a MMRM. The model will include fixed terms for treatment, visit and the treatment-by-visit interaction. The model will also include the stratification mutation factor and continuous baseline distance walked during the 12MWT as covariates. An unstructured covariance structure will be used for the repeated visits within a subject. The Kenward-Rogers approximation (Kenward & Roger 1997) will be used to estimate the denominator degrees of freedom. The treatment comparison (REN001 100mg - placebo) for the change from baseline at both Weeks 12 and 24 will be estimated. The estimates, standard errors, 95% confidence intervals and associated p-values will be presented. The primary comparison is Week 24.

Intercurrent Events

The following potential intercurrent events are currently identified:

- Discontinuation of study drug
- Inability to perform the test or stopping the test prematurely due to reasons at least possibly related to PMM

A treatment policy strategy will be used; hence the distance walked at the relevant visit will be included in the analysis, regardless of intercurrent events. If the subject is unable to perform the test a distance of 0 meters will be imputed for the visit.

Using this treatment policy strategy, the estimand is defined as the difference in means between the treatments in the target patient population for the change from baseline at Week 24 in 12MWD, regardless of whether or not the subject continues with treatment or is unable to walk for 12 minutes.

Missing data

All possible efforts will be made to ensure that subjects complete all the required assessments. Subjects who discontinue study drug will be encouraged, where appropriate, to continue with reduced assessments at the Week 12 and Week 24 visits (see Section 3.6). However missing data may arise for the following reasons:

- Withdrawal from the study
- Unable to perform the test for reasons unrelated to their PMM (e.g., site unable to perform the test at the visit, or the subject suffers an accidental injury prior to the visit)
- Unable to walk for 12 minutes for reasons unrelated to their PMM (e.g., unexpected fire alarm at the study center means the test has to be aborted)

For the primary analysis the data will be considered as MAR. Missing scores (as opposed to intercurrent events) will be imputed using the technique of multiple imputations. The multiple imputation step will be performed prior to any fixed imputations.

Each dataset with complete data for each subject will be analyzed using the MMRM model described above and the results obtained will be combined using Rubin's imputation rules (Rubin, 1987) to produce pooled estimates of the treatment difference, along with their standard errors, 95% confidence intervals, and associated p-values.

As rules for handling intercurrent events and missing data may potentially bias the outcome of the statistical analyses and the subsequent estimation of the magnitude of the treatment effect, sensitivity analyses will be conducted to provide a balanced assessment of treatment efficacy.

The following sensitivity analyses will be conducted:

- a pattern-mixture multiple imputation approach where missing data from subjects who receive either treatment will be assumed to follow a similar trajectory to those in the placebo group.
- a tipping point analysis (O'Kelly & Ratitch, 2014) will be used, imputing different changes from baseline for the missing data.

In addition, the primary analysis will be replicated using the PP set.

A while on-treatment policy strategy will also be employed in a supplementary analysis utilizing only the on-treatment measurements for each subject. All missing data will be taken as MAR and so a MMRM will be used, without multiple imputation. As all subjects would be required to have at least one post-baseline on-treatment measurement this analysis will use the modified analysis set.

All model assumptions will be checked by evaluating model residuals. If the evaluation of residuals indicates a problem with the model assumptions, a transformation of the data or non-parametric analyses may be considered.

8.5.2 Secondary Endpoints

The MFIS Physical subscale score changes from baseline will be analyzed using a MMRM. The model will include fixed terms for treatment, visit and the treatment-by-visit interaction. The model will also include the stratification mutation factor, continuous baseline MFIS physical subscale score and continuous baseline distance walked during the 12MWT as covariates. An unstructured covariance structure will be used. The treatment comparison for the change from baseline at each visit will be estimated. The estimates, standard errors, 95% confidence intervals and associated p-values will be presented for each visit. The primary comparison is Week 24.

The MFIS assessment may also be impacted by intercurrent events. Similar to the primary endpoint a treatment policy strategy will be adopted. Pattern mixture and tipping point analyses will be included as sensitivity analyses and a while on treatment policy strategy will also be employed as a supplementary analysis.

The PGIC (muscle symptoms) data will be converted to a numerical scale from 3 (very much improved) to -3 (very much worse); hence a value of 0 would signify no change. This score will then be analyzed using ANCOVA, using a fixed term for treatment and the mutation stratification factor and continuous baseline distance walked during the 12MWT as covariates. The treatment comparison will be estimated, along with its standard error, 95% confidence interval and associated p-value.

As the PGIC is assessed at the end of study treatment (Week 24 or Early Termination) a while on treatment strategy will be employed.

The primary analyses for these endpoints will also be replicated using the PP set.

8.5.3 Exploratory Endpoints

Exploratory endpoints will be summarized and analyzed, using the FAS, to estimate the treatment effect and corresponding 95% confidence interval. Full details of analyses will be provided in the SAP.

8.6 Safety Analyses

No imputation will be used for handling missing data, with the exception of conservative approaches taken for missing adverse event information (e.g., intensity). Details of such conventions will be documented in the Statistical Analysis Plan (SAP).

Treatment-emergent adverse events will be summarized by treatment group and by SOC and Preferred Term, in accordance with the MedDRA coding dictionary. The number of subjects reporting each AE preferred term will be tabulated for all TEAEs and separately for those considered as at least possibly related to study treatment by the Investigator. Number of subjects reporting SAEs and AESI will also be tabulated.

Changes from baseline in laboratory parameters, vital signs and ECG data will be summarized by treatment group and by visit. Baseline will be taken as the last measurement prior to dosing.

A categorical shift table of change from baseline will be summarized by treatment group and by visit for the eye examination parameters.

The incidence of potentially clinically significant laboratory and ECG values (e.g., increase in QTcF ≥30 msec from baseline or an absolute QTcF value of ≥500 msec) will be flagged in listings and summarized by treatment group.

8.7 Pharmacokinetic Endpoints

Plasma concentrations of REN001 will be summarized over time using descriptive statistics for Day 1, Week 12 and Week 24.

Individual subject concentration-time data on Day 1, Week 12 and Week 24 will be plotted over actual PK sampling times. Median profiles of the concentration-time data, using nominal PK sampling times, will also be presented for Day 1, Week 12 and Week 24. Both linear-linear and linear-log plots will be presented.

The data will be used for a population PK analysis which will be reported as a standalone report to the Clinical Study Report.

8.8 Interim Analyses

No formal interim analyses are planned for the primary endpoint; however, an independently chaired Safety Review Committee (SRC) will review safety data at specified intervals for the duration of the study. The structure, function and operation of the SRC will be detailed in the REN001-201 SRC charter.

9 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

9.1 Data Quality Assurance

The Sponsor (or its delegate) will conduct a site visit/telephone call to verify the qualifications of each Investigator, inspect the site facilities, and inform the Investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded in the eCRFs for this study must be consistent with the subjects' source documentation (e.g., medical records).

9.2 Electronic Case Report Forms (eCRF) and Source Documentation

All data obtained during this study should be entered in the eCRFs promptly. All source documents from which eCRF entries are derived should be placed in the subject's medical records. Measurements for which source documents are usually available include laboratory assessments, and ECG recordings.

The original eCRFs for each subject will be checked against source documents at the study site by the site monitor.

After review by the site monitor, completed eCRFs will be marked as complete and verified. Data Management will review the eCRFs within the electronic data capture (EDC) system. Where data is discrepant, Data Management will raise queries for the site to resolve within the EDC.

9.3 Access to Source Data

During the course of the study, a monitor will make site visits to review protocol compliance, compare eCRFs and individual subject's medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents in the presence of the Investigator, will be required to monitor the progress of the study. Moreover, regulatory authorities of certain countries, IECs, and/or the Sponsor's Clinical Quality Assurance consultant may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality. The Investigator assures Orphan-Reach Ltd and the Sponsor of the necessary support at all times.

9.4 Data Processing

The site will be supplied with the following data collection tool: a web browser address for EDC that has been fully validated and conforms to 21CFR11 requirements. The trained Investigator site staff will enter the data required by the protocol into the eCRFs from source documents (e.g., medical records and study-specific data capture tools as needed) using the supplied data collection tool. All information in the eCRFs must be traceable to these source documents. Data recorded directly into the eCRFs will be defined before study start and the eCRFs will be considered the source data. Site monitors and data managers will review eCRFs

entered by investigational staff for completeness and accuracy. Automatic quality programs check for data discrepancies in the eCRFs and the resulting queries will be notified to the investigational site using an electronic data query process within the EDC. Designated Investigator site staff are required to respond to queries and make any necessary changes to the data promptly. Details of the data correction process will be specified in the Data Management Plan. After database lock, the Investigator will receive a compact disc (or equivalent) of the subjects' eCRFs (portable document format) for archiving at the investigational site.

A validated, electronic database will be employed from the EDC system. An audit trail of all changes to this database, including the date, reason for the data change and who made the change, will be maintained within the same database. The audit trail will be part of the archived data at the end of the study.

9.5 Archiving Study Records

Adequate records as required by ICH GCP and Food and Drug Administration (FDA) Code of Federal Regulations (CFR), will be maintained for the study. This will include subject medical records, Investigator logs, eCRFs, laboratory reports, work sheets, signed Informed Consent Forms, drug dispensing records, adverse experience reports, information regarding subjects' discontinuation and electronic data. Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the final discontinuation of clinical development of the investigational product. However, if required by the applicable regulatory requirements or by an agreement with the Sponsor these documents should be retained for a longer period. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

9.6 Good Clinical Practice

The procedures set out in this study protocol are designed to ensure that the Sponsor and Investigator abide by the principles of GCP guidelines of the ICH, and of the Declaration of Helsinki (2013). The study also will be carried out in keeping with local legal requirements.

9.7 Confidentiality

All study findings and documents will be regarded as confidential. The Investigator and members of his/her research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified in eCRFs and other documents submitted to Orphan-Reach Ltd by their subject number and/or birth year, not by name. Documents not to be submitted to Orphan-Reach Ltd that identify the subject (e.g., the signed informed consent) must be maintained in confidence by the Investigator.

9.8 Informed Consent

Before each subject is admitted to the study, a personally signed and dated informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country (i.e., the Declaration of Helsinki, ICH GCP, and other applicable local regulations). This consent form must also be signed by the person collecting the informed consent and the original will be retained in the site file

and a copy retained in the subject medical records. The subject will also receive a copy of the signed consent. The Investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB and signed by all subjects subsequently enrolled in the study as well as those currently enrolled in the study.

9.9 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/CA's, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/CA approval prior to implementation (if appropriate). All amendments will be distributed to all protocol recipients, with appropriate instructions.

9.10 Duration of the Study

The planned duration of the study for each subject will be approximately 36 weeks (assuming up to 8 weeks screening, 24 weeks treatment and 3-4 weeks follow-up). The study will close when all subjects have completed the Week 24 (or Early Termination) visit and the subsequent Follow Up Visit (21-28 days after last study treatment).

9.11 Liability and Insurance

The conduct of this trial will be under reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the Investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the Sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this study are governed by the applicable law.

Subjects participating in this study will have access to insurance against financial loss due to personal injury caused by the study drug being tested or by medical steps taken in the course of the study.

9.12 Publication Policy

By signing the study protocol, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the Investigator's name, address, qualifications and extent of involvement.

After study completion, data from the entire study will be considered for reporting at a scientific meeting and for publication in a scientific journal. The Sponsor will coordinate these activities and will work with the Investigator to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted and other related issues. Authorship will be based on

criteria stipulated by leading clinical journals (e.g., contribution to one or more areas of study design, data analysis and interpretation, manuscript preparation and review, etc.).

An Investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance.

10 REFERENCES

Chylack L. T. Jr., Wolfe J. K., Singer D. M., Leske M. C., Bullimore M. A., Bailey I. L., Friend J., McCarthy D., & Wu S. Y. (1993) The Lens Opacities Classification System III. The Longitudinal Study of Cataract Study Group. *Arch Ophthalmol. 111(6),* 831-836.

Clinical Trials Facilitation Group. (2014). *Recommendations related to contraception and pregnancy testing in clinical trials*.

Clinical Trials Facilitation Group. (2020). *Recommendations related to contraception and pregnancy testing in clinical trials version 1.1*.

European Commission. (2001, April 1). *Clinical trials - Directive 2001/20/EC*. Retrieved from European Commission: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf

European Medicines Agency. (2009). *ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals*.

European Medicines Agency (2020, July 30). *ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials Step 5* Retrieved 2020, from European Medicines Agency: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e9-r1-addendum-estimands-sensitivity-analysis-clinical-trials-guideline-statistical-principles_en.pdf

European Union. (2003, October 8). *Commission Directive 2003/94/EC*. Retrieved from Official Journal of the European Union: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/dir_2003_94/dir_2003_94_en.pdf

Gorman, G. S., Chinnery, P. F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., & Turnbull, D. M. (2016). Mitochondrial Diseases. *Nat Rev Dis Primers, 2*, 1-22.

Karaa, A., Haas, R., Goldstein, A., & Witting, H. (2018) Randomized dose-escalation trial of elamipretide in adults with primary mitochondrial myopathy. *Neurology 0,* 1-10.

Kenward, M. G., & Roger, J. H. (1997) Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. *Biometrics* 53:983–997.

Knak K. L, Andersen, L. K., Witting, N., & Vissing J. (2017) The 2- and 6-minute walk tests in neuromuscular diseases: effect on heart rate correction on the learning effect. *Int. J. Phys. Med, Rehabil, 5,* (4).

Knak K. L., Andersen, L. K., Witting, N., & Vissing J. (2017) Reliability of the 2- and 6-minute walk tests in neuromuscular diseases. *J Rehabil Med. 49, (4)* 362-366.

Kierkegaard M., & Tollback,A. (2007) Reliability and feasibility of the six minute walk test in subjects with myotonic dystrophy. *Neuromuscular disorders 17*, 943-949.

Lippi, G., Schena, F., Salvagno, G. L., Montagnana, M., Gelati, M., Tarperi, C., Banfi, G., & Guidi, G. C. (2008) Acute variation of biochemical markers of

muscle damage following a 21-km, half marathon run. *Scand J Clin & Lab Invest 68, (7)* 667–672.

Mancuso, M., McFarland, R., Klopstock, T., & Hirano, M. (2017) International Workshop: Outcome measures and clinical trial readiness in primary mitochondrial myopathies in children and adults. Consensus recommendations. 16–18 November 2016, Rome, Italy. *Neuromuscul Disord (12),* 1126-1137.

Millum, J., & Grady, C. (2013) The Ethics of Placebo-controlled trials: methodological justifications. *Contemp Clin Trials, 36*, 510-514.

Moghadam-Kia, S., Oddis, C. V., & Aggarwal, R. (2016, January). Approach to asymptomatic creatine kinase elevation. *Cleve Clin J Med, 83*, 37-42.

Nance, J. R., & Mammen, A. L. (2015) Diagnostic evaluation of rhabdomyolysis. *Muscle Nerve*. *51(6)*:793-810.

O'Kelly, M., & Ratitch, B. (editors). (2014) Clinical Trials with Missing Data: A Guide for Practitioners. Wiley.

Parikh, S., Goldstein, A., Koenig, M.K., Scaglia, F., Enns, G.M., Saneto, R., Anselm, I., Cohen, B. H., Falk, M. J., Greene, C., Gropman, A.L., Haas, R., Hirano, M., Morgan, P., Sims, K., Tarnopolsky, M., Van Hove, J. L. K., Wolke, L., & DiMauro, S. (2014) Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genetics in Medicine,* 1-13.

Pfeffer, G., & Chinnery, P. (2013, Feb). Diagnosis and treatment of mitochondrial myopathies. *Ann Med, 45*, 4-16.

Prahm, K. P., Witting, N., & Vising J. (2014) Decreased variability of the 6-minute walk test by heart rate correction in patients with neuromuscular disease. *PloS ONE, 9, (12)* 1-8.

Rubin, D.B multiple Imputation for Nonresponse in Surveys, New York, Wiley, 1987.

Sources of Energy Adapted from Human Physiology: An Integrated Approach, Sixth Edition (2013) Dee Unglaub Silverthorn.

Tarnopolsky MA. (2016) Metabolic Myopathies. Continuum (Minneap Minn) *22 (6)* 1829–1851.

US Code of Federal Regulations 21CFR211.165 retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.165

US Code of Federal Regulations 21CFR312.6 retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.6

Wu, G., Sanderson, B., & Bittner, V. (2003) The 6-minute walk test: How important is the learning effect? *Am Heart J 146*, 129-133.

11 APPENDICES

Appendix 1: International Workshop

Outcome measures and clinical trial readiness in primary mitochondrial myopathies in children and adults. Consensus recommendations. 16–18 November 2016, Rome, Italy

(Mancuso, McFarland, Klopstock, & Hirano, 2017)

Primary mitochondrial myopathies (PMM), as defined by this consortium of international experts in mitochondrial disease, are genetically defined disorders leading to defects of oxidative phosphorylation affecting predominantly, but not exclusively, skeletal muscle (see below for methodology). Thus, secondary involvement of mitochondria, frequently observed in multiple neuromuscular diseases (e.g., inclusion body myositis, Duchenne muscular dystrophy, Kennedy disease) is not considered PMM.

PMM may present at any age, patients with severe generalized muscle involvement typically present early in life, although individuals with milder forms of the disease, or symptoms confined to specific muscles tend to have later presentations. The most common presentation of PMM is chronic progressive external ophthalmoplegia (PEO). Chronic PEO is characterized by a slowly progressive, usually bilateral limitation of eye movements (ophthalmoplegia) in all directions of gaze so that patients turn their heads to see a target at the periphery of the visual field; patients sometimes report diplopia, especially when onset of ophthalmoplegia is asymmetric. Intrinsic ocular muscles are not involved. PEO is usually accompanied by bilateral eyelid ptosis, which is often the presenting symptom, associated with a compensatory frontalis muscle hyperactivity and, in severe cases, tilting of the head backwards. PEO is often associated with other signs of skeletal muscle involvement, typically a slowly progressive axial and proximal limb weakness affecting predominantly the hip and shoulder girdle as well as neck flexor muscles often with variable muscle wasting. Muscle weakness may also cause dysphagia and dysarthria due to oropharyngeal weakness, as well as respiratory failure. Distal myopathic weakness may be present but is rarely seen early in the disease.

From a genetic point of view, PEO may be autosomal dominant or recessive, sporadic (usually due to single largescale deletions of mtDNA), or maternally inherited. Autosomal PEO can be associated with multiple deletions and/or depletion of mtDNA, caused by nuclear gene defects and subsequent impairment of mtDNA maintenance. PEO is also the most frequent phenotype associated with a single sporadic largescale deletion of mtDNA. The “common deletion” is 4.9-kb and accounts for about one-third of all single large-scale deletions of mtDNA.

Myopathy can be the only clinical feature of a mitochondrial disease but may also be part of a component of other mitochondrial syndromes. For example, Kearns-Sayre syndrome is defined by the early onset of PEO before age 20 years in association with pigmentary retinopathy, and at least one of the following: cerebellar ataxia, cardiac conduction block, or cerebrospinal fluid protein levels >0.1 g/L.

Other manifestations of PMM are exercise intolerance often with myalgia, fatigue (defined as an overwhelming sense of tiredness, lack of energy, and feeling exhausted), muscle wasting, muscle cramps, and recurrent rhabdomyolysis with myoglobinuria triggered by exercise as seen in cytochrome b deficiency or in the myopathic form of CoQ10 deficiency. Exercise-induced symptoms are common in

PMM and reflect lack of energy production due to mitochondrial dysfunction in skeletal muscle, increased lactate production and phosphocreatine depletion.

Appendix 2: Example Modified Fatigue Impact Score (MFIS)

Patient's Name: _____ **Date:** _____/_____/_____ (day / month / year)

ID#: _____ **Test#:** 1 2 3 4

FATIGUE IMPACT SCALE (MODIFIED)

Below is a list of statements that describe how fatigue may affect a person. Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. In medical conditions like multiple sclerosis (MS), feelings of fatigue can occur more often and have a greater impact than usual.
Please read each statement carefully, and then circle the one number that best indicates how often fatigue has affected you in this way during the past 7 days. (If you need help in marking your responses, tell the interviewer the number of the best response.) Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

Because of my fatigue during the past 7 days…

		Never	Rarely	Sometimes	Often	Almost always
1.	I have been less alert.	0	1	2	3	4
2.	I have had difficulty paying attention for long periods of time.	0	1	2	3	4
3.	I have been unable to think clearly.	0	1	2	3	4
4.	I have been clumsy and uncoordinated.	0	1	2	3	4
5.	I have been forgetful.	0	1	2	3	4
6.	I have had to pace myself in my physical activities.	0	1	2	3	4
7.	I have been less motivated to do anything that requires physical effort.	0	1	2	3	4

Because of my fatigue during the past 7 days…

		Never	Rarely	Sometimes	Often	Almost always
8.	I have been less motivated to participate in social activities.	0	1	2	3	4
9.	I have been limited in my ability to do things away from home.	0	1	2	3	4
10.	I have had trouble maintaining physical effort for long periods.	0	1	2	3	4
11.	I have had difficulty making decisions.	0	1	2	3	4
12.	I have been less motivated to do anything that requires thinking.	0	1	2	3	4
13.	my muscles have felt weak.	0	1	2	3	4
14.	I have been physically uncomfortable.	0	1	2	3	4
15.	I have had trouble finishing tasks that require thinking.	0	1	2	3	4
16.	I have had difficulty organising my thoughts when doing things at home or at work.	0	1	2	3	4
17.	I have been less able to complete tasks that require physical effort.	0	1	2	3	4
18.	my thinking has been slowed down.	0	1	2	3	4
19.	I have had trouble concentrating.	0	1	2	3	4
20.	I have limited my physical activities.	0	1	2	3	4
21.	I have needed to rest more often or for longer periods.	0	1	2	3	4

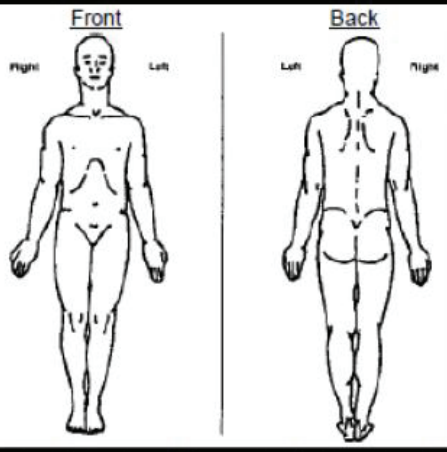
Appendix 3: Example Brief Pain Inventory (Short Form)

Brief Pain Inventory (Short Form)

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

☐ Yes ☐ No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by marking the box beside the number that best describes your pain at its worst in the last 24 hours.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

4. Please rate your pain by marking the box beside the number that best describes your pain at its least in the last 24 hours.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

5. Please rate your pain by marking the box beside the number that best describes your pain on the average.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

6. Please rate your pain by marking the box beside the number that tells how much pain you have right now.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please mark the box below the percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
☐ No Relief	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Complete Relief

9. Mark the box beside the number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

B. Mood

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

C. Walking ability

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

D. Normal Work (includes both work outside the home and housework)

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

E. Relations with other people

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

F. Sleep

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

G. Enjoyment of life

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

Appendix 4: Example Work Productivity and Activity Impairment Questionnaire: Specific Health Problem V2.0 (WPAI:SHP)

Work Productivity and Activity Impairment Questionnaire: Primary Mitochondrial Myopathy Disease V2.0 (WPAI:PMM)

The following questions ask about the effect of your Primary Mitochondrial Myopathy Disease (PMM) on your ability to work and perform regular activities. *Please fill in the blanks or circle a number, as indicated.*

1. Are you currently employed (working for pay)? _____ NO ___ YES
If NO, check "NO" and skip to question 6.

The next questions are about the **past seven days**, not including today.

2. During the past seven days, how many hours did you miss from work because of problems associated with your PMM? Include hours you missed on sick days, times you went in late, left early, etc., because of your PMM. Do not include time you missed to participate in this study.

_____ HOURS

3. During the past seven days, how many hours did you miss from work because of any other reason, such as vacation, holidays, time off to participate in this study?

_____HOURS

4. During the past seven days, how many hours did you actually work?

_____HOURS *(If "0", skip to question 6.)*

5. During the past seven days, how much did your PMM affect your productivity while you were working?

Think about days you were limited in the amount or kind of work you could do, days you accomplished less than you would like, or days you could not do your work as carefully as usual. If PMM affected your work only a little, choose a low number. Choose a high number if PMM affected your work a great deal.

Consider only how much PMM affected productivity <u>while you were working</u>.

PMM had no effect on my work	0 1 2 3 4 5 6 7 8 9 10	PMM completely prevented me from working

CIRCLE A NUMBER

6. During the past seven days, how much did your PMM affect your ability to do your regular daily activities, other than work at a job?

By regular activities, we mean the usual activities you do, such as work around the house, shopping, childcare, exercising, studying, etc. Think about times you were limited in the amount or kind of activities you could do and times you accomplished less than you would like. If PMM affected your activities only a little, choose a low number. Choose a high number if PMM affected your activities a great deal.

Consider only how much <u>PMM</u> affected your ability to do your regular daily activities, other than work at a job.

PMM had no effect on my daily activities	0 1 2 3 4 5 6 7 8 9 10	PMM completely prevented me from doing my daily activities

CIRCLE A NUMBER

WPAI:SHP V2.0 (US English)

Appendix 5: Example PROMIS Short Form - Fatigue 13a (FACIT-Fatigue)

PROMIS® Item Bank v1.0 – Fatigue – Short Form 13a (FACIT-Fatigue)

Fatigue – Short Form 13a (FACIT-Fatigue)

Please respond to each question or statement by marking one box per row.

	During the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
HI7	I feel fatigued	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
HI12	I feel weak all over	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN1	I feel listless ("washed out")	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN2	I feel tired	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN3	I have trouble starting things because I am tired	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN4	I have trouble finishing things because I am tired	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN5	I have energy	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
AN7	I am able to do my usual activities	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
AN8	I need to sleep during the day	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN12	I am too tired to eat	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN14	I need help doing my usual activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN15	I am frustrated by being too tired to do the things I want to do	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN16	I have to limit my social activity because I am tired	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Appendix 6: Amendment 3

Amendment Rationale

a) A global update to remove the requirement for at least 40% of subjects in study REN001-201 to have the m.3243A>G genotype. Reneo's Phase 1b study (REN001-101) was used as the indicator of the proportion of subjects with m.3243A>G point mutations in the general PMM population. However recent information from external and internal recruitment data, and discussion with experts indicates that the initial intent of recruiting 'at least 40% of subjects with the m.3243A>G' genotype is neither viable nor desirable. Requiring 40% of subjects to have m.3243A>G mutation would result in a genotypic mix in the REN001-201 study that is not representative of the primary mitochondrial myopathy population. Furthermore, this amendment to remove the requirement for 'at least 40% of subjects with the m.3243A>G' genotype is consistent with advice received from the European Medicines Agency to include a broader population than was studied in REN001-101. Review of baseline characteristics of subjects recruited into REN001-201 to date indicate that when grouped into m.3243 A>G, other single point mutations and single large deletions, these groups have comparable baseline characteristics. Thus, the intent of recruiting based on phenotype is effective. The Sponsor has therefore removed the requirement for at least 40% of subjects in study REN001-201 to have the m.3243A>G genotype.
b) A global update to Section 7.1.9 eye examination has been made to delete redundant text and clarify lens grading and visual acuity.
c) A global update to Section 8 Statistical Methods - The primary analysis model has been updated to a mixed effect model for repeated measures (MMRM) to allow the estimation of different between subject variances for the two visits. The model for the analysis of the secondary endpoint, MFIS physical subscale, has been updated to remove the random subject effect as the different sources of variability are accounted for within the covariance structure of the MMRM.
d) A global update to clarify that re-screened subjects are not required to repeat their Screening eye examinations or DXA scans repeated within 6 months of the original assessments.
e) A global update to reflect that the REN001 open-label extension study is now available, for subjects who complete REN001-201, in countries where the extension study is approved.
f) A global update to clarify that study centers can complete the Baseline, Week 12, Week 24 and Early Termination visits over two days at the Investigator's discretion due to the complexity of scheduling all the assessments on these visits.
g) A global update to clarify the postmenopausal definition for consistency with the central lab to women of 45 years and older and amenorrhoeic for 1 year in addition to an FSH level indicating postmenopausal state.
h) Belgium ethics committee requested that the reason for ethnicity being collected (regarding eGRF calculation) is mentioned in the protocol. This has been added to Section 6.1 in the Subject Demography bullet point.
i) Minor typographical errors and corrections have been updated throughout.

Details of Amended Text

The following changes to the study protocol have been made:

1) Synopsis – Design section introduction of DXA sub-study
2) Synopsis – Treatment section removal of 'At least 40% of subjects will be recruited with a mutational genotype of m.3243A>G'.
3) Synopsis – Exclusion Criteria 14 states '….long pulse rate (PR) interval…' this has been corrected to 'long PR interval'.
4) Synopsis – Exclusion Criteria 17 updated for clarity to 'Positive hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (HBcAb) at Screening, or positive for hepatitis C or human immunodeficiency virus (HIV) at Screening'.
5) Synopsis – Study Visits section addition of 'Re-screened subjects will not be required have either the eye exam or DXA scan (REN001-201 DXA sub-study subjects) repeated within 6 months of the original assessments.' Clarification that the Follow-Up visit '… This visit will not be required if a subject chooses to enter the REN001 open label extension study (REN001-202) at their Week 24 visit; applicable only for countries where the open label extension study is being conducted.' Clarification that the Baseline, Week 12 and Week 24 visits may be completed over two days if needed.
6) Synopsis – Statistical Methods section updated to replace the 'random effect model' to a 'mixed effects model for repeated measures'.
7) Section 1.4 Risk-Benefit Section – update to the indicate the non-clinical carcinogenicity studies have been initiated.
8) Section 3.1 Addition of 'Re-screened subjects will not be required have either the eye exam or DXA scan (REN001-201 DXA sub-study subjects) repeated within 6 months of the original assessments.' Clarification that the Follow-Up visit '… This visit will not be required if a subject chooses to enter the REN001 open label extension study (REN001-202) at their Week 24 visit; applicable only for countries where the open label extension study is being conducted.' Clarification that the Baseline, Week 12 and Week 24 visits may be completed over two days if needed.
9) Section 3.3 Justification of the Study Design text updated to the '… study will aim to recruit 1) subjects with the mt DNA defects (including the most common point mutation m.3243A>G) and 2) subjects with a primary phenotypic presentation of myopathy.
10) Section 4.2 Exclusion criteria 17: Updated for clarity to 'Positive HBsAg and HBcAb at Screening, or positive hepatitis C or HIV at Screening'.
11) Section 5.2 Allocation to Treatment – removal of sentence stating 'At least 40% of subjects will be recruited with a mutational genotype of m.3243A>G.
12) Section 5.4 Breaking the Blind – The break blind process has been clarified to indicate the Investigator may break the blind in a medical emergency without notifying the Sponsor prior to breaking the blind.
13) Section 5.10 – Age of collection of FSH sample clarified to 45 years and older and duration of amenorrhoeic reduced to 1 year from 2 years.
14) Section 5.11 - Table 1 Prohibited Medications – addition of anticoagulants and steroids to be reviewed on a case by case basis by medical monitor and Sponsor.
15) Section 6.1 Screening - Addition of 'Re-screened subjects will not be required have either the eye exam or DXA scan (REN001-201 DXA sub-study subjects)

repeated within 6 months of the original assessments.' Clarification that the collection of Subject Demography includes ethnicity for accurate calculation of eGFR.

16) Section 6.6 Weeks 12 and 24 Visits - Clarification that the Week 12 and Week 24 visits may be completed over two days if needed.
17) Section 6.7 Follow Up Visit - Clarification that the Follow-Up visit '… will not be required if a subject chooses to enter the REN001 open label extension in countries where the open label extension study is approved'.
18) Section 6.8 Early Termination Visit - Clarification that the Early Termination visit may be completed over two days if needed.
19) Table 2 Schedule of Activities footnote 2 – updated to indicate that in addition to the Baseline visit the Week 12, Week 24/Early Termination visits may also be conducted over 2 days at the Investigators discretion.
20) Table 2 Schedule of Activities footnote 9 – updated for consistency to 'Serum FSH testing for postmenopausal females 45 years and older'.
21) List of Study Personnel and Section 7.1.1.9 – As highlighted at site initiation visits SAE reporting email address corrected from 'Drug.safety@bionical-emas' to 'Drug.safety@bionical-emas.com'. Physical address also updated as office location has changed since last protocol release.
22) Section 7.1.2 - Table 3 biochemistry – additional note to indicate subject's ethnicity is required for accurate calculation of eGFR.
23) Section 7.1.2 - Table 3 hematology – HbA1c assessment moved from serology to hematology to correctly reflect collection of samples.
24) Section 7.1.2 - Table 3 Urinalysis – Collection of hCG at week 18 removed as an error in table and correct elsewhere in the protocol.
25) Section 7.1.8 ECG Recording – updated to fully reflect Exclusion Criteria 14.
26) Section 7.1.9 Eye examination – Redundant text removed for clarity to say 'Each lens should be classified and graded against the Lens Opacity Classification System III, (Chylack et al, 1993)' and 'best corrected distance visual acuity (including refraction) should also be assessed.
27) Section 7.1.12 MFIS – correction of the description of the scoring categories.
28) Section 8.5.1 Primary Endpoint – Random effects model updated to MMRM.
29) Section 8.5.2 Secondary Endpoints – removal of 'a random subject effect'.
30) Minor typographical errors updated throughout.