CLINICAL PROTOCOL

EFFICACY AND SAFETY STUDY OF VATIQUINONE FOR THE TREATMENT OF MITOCHONDRIAL DISEASE SUBJECTS WITH REFRACTORY EPILEPSY (MIT-E)

PTC743-MIT-001-EP

27 MAY 2022

VERSION 8.0

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applicable law) and should not be used for unauthorized purposes. In the event of an actual or suspected breach of this obligation, PTC Therapeutics, Inc. should be notified promptly.

PROTOCOL IDENTIFIERS AND STUDY PERSONNEL

Project Code	PTC743-MIT
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PTC [redacted]	[redacted]
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PTC [redacted]	[redacted]

PTC THERAPEUTICS PROTOCOL APPROVAL SIGNATURES

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The eSignature page is located on the last page.

PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

I have read the protocol document and, on behalf of my institution, agree to comply with the protocol and all applicable regulations.

Principal Investigator	**Date**

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialized Term	Explanation
15-LO	15-lipoxygenase
15-HETE	15-hydroxyeicosatetraenoic acid
15-HpETE	15-hydroperoxyeicosa-tetraenoic acid
15(S)-HpETE	15-OOH-arachindonic acid
AA	Arachidonic acid
AE	Adverse event
AEDs	Antiepileptic drugs
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSO	Buthionine sulfoximine
CBC	Complete blood count
COVID-19	Coronavirus disease 2019
CRF	Paper case report form
CRO	Contract research organization
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DSMB	Data Safety Monitoring Board
EC_{50}	Half maximal concentration
eCRF	Electronic case report form
ECG	Electrocardiogram
EEG	Electroencephalogram
GCP	Good Clinical Practices
Gpx4	Glutathione peroxidase 4
GSH	Glutathione
GSSG	Glutathione disulfide
G-Tube	Gastrostomy tube
IB	Investigator's Brochure
IC_{50}	Half maximal inhibitory concentration
ICF	Informed consent form
IEC	Institutional Ethics Committee
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
LC-MS/MS	Liquid chromatography with tandem mass spectroscopy
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MIT-E	Mitochondrial Disease Subjects with Refractory Epilepsy
mtDNA	mtDNA
MT-TK	mitochondrially encoded tRNA lysine
NF	National Formulary
NOAEL	No observed adverse effect level
PedsQL	Pediatric Quality of Life Inventory™
PCH6	Pontocerebellar hypoplasia type 6
PK	Pharmacokinetics
POLG	DNA polymerase subunit gamma
PT	Prothrombin time
PTT	Partial thromboplastin time
RSI	Reference Safety Information
RSL3	Oncogenic-RAS-selective lethal compound 3
SAE	Serious adverse event
SAP	Statistical analysis plan

Abbreviation or Specialized Term	Explanation
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
WOCBP	Women of childbearing potential

1. INTRODUCTION

1.1. Background Information

Vatiquinone is a novel small molecule therapeutic in development for the treatment of mitochondrial diseases and associated disorders of oxidative stress and inflammation. Vatiquinone targets oxidoreductase enzymes essential to inflammation, oxidation, and cell death. To date, vatiquinone has been evaluated in a number of clinical trials for the treatment of mitochondrial diseases and has demonstrated a reduction of seizure- and disease-related morbidity in clinical studies of patients with mitochondrial disease and associated refractory epilepsy. The purpose of this study is to evaluate the efficacy and safety of vatiquinone in patients with genetically defined mitochondrial disease and associated refractory epilepsy.

1.2. Name and Description of Investigational Product

Vatiquinone is 2-[(3R,6E,10E)-3-hydroxy-3,7,11,15-tetramethyl-6,10,14-hexadecatrienyl]-3,5,6-trimethyl-2,5-cyclohexadiene-1,4-dione. Vatiquinone is the quinone oxidation product of alpha-tocotrienol, one of the 8 naturally occurring forms of vitamin E. Vatiquinone is a viscous yellow to brown oil and will be administered as a mixture with sesame oil National Formulary (NF)/ United States Pharmacopeia.

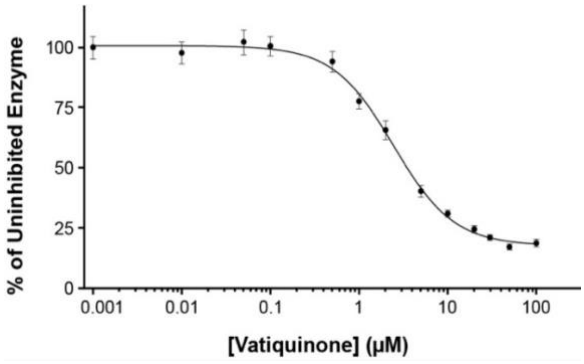
Vatiquinone is manufactured as a 100 mg/mL Oral Solution.

The investigator will ensure that all study drugs are stored and dispensed in accordance with the Food and Drug Administration and local regulations concerning the storage and administration of investigational drugs.

1.3. Vatiquinone Mechanism of Action

1. Vatiquinone targets 15-lipoxygenase (15-LO), a key governor of ferroptosis and inflammation.
    - 15-LO is a key enzyme governor of the biological process of ferroptosis, which has been implicated in epilepsy and a number of other central nervous system diseases (Joshi 2015, Guiney 2017).
    - In a cell-free enzymatic assay, we have demonstrated that Vatiquinone dose-dependently inhibits 15-LO enzyme activity (half maximal inhibitory concentration [IC_{50}]=1.9 μM) (Figure 1).

Figure 1: Inhibition of 15-LO by Vatiquinone



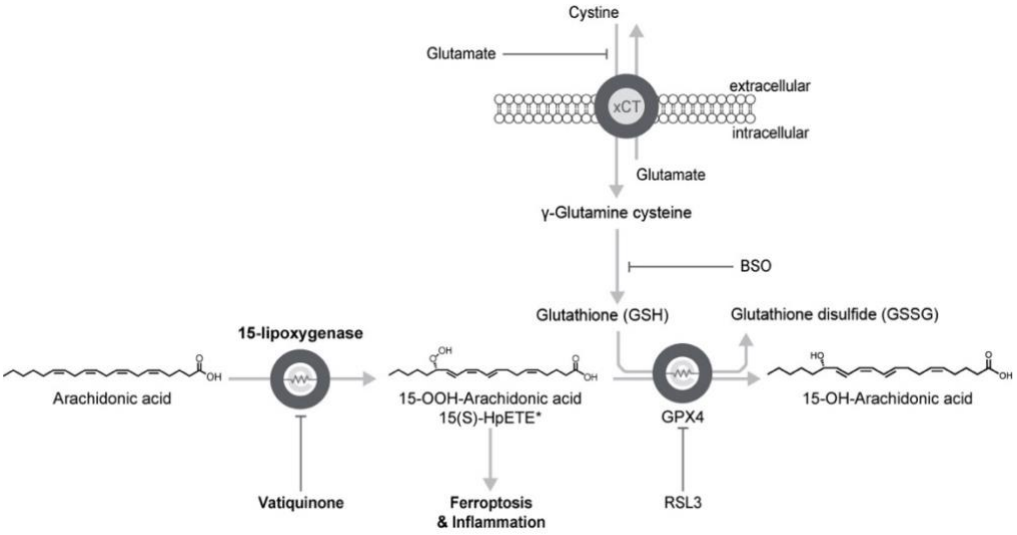
Abbreviations: 15-LO, 15-lipoxygenase

- 15-LO catalyzes the peroxidation of arachidonic acid (AA) to produce the lipid hydroperoxide 15-hydroperoxyeicosa-tetraenoic acid (15-HpETE), a pro-inflammatory and pro-ferroptotic lipid signaling molecule.
- 15-HpETE and downstream metabolites, such as HETEs and 4-HNE, are clinical biomarkers that have been associated with mitochondrial disease and seizure disorders (Menon 2012, Sanders 2013, Abeti 2016).
- *Net*: Vatiquinone is an inhibitor for the enzyme 15-LO, a key governor of fatty acid oxidation and inflammation that underpin neurological disease.

2. Ferroptosis (the biochemical process regulated by 15-LO) has been demonstrated to be a key mechanism underpinning mitochondrial and seizure disorders. The ferroptosis pathway is summarized in Figure 2.

   Ferroptosis is regulated by 15-LO, and glutathione peroxidase 4 (Gpx4). Activation of 15-LO leads to the production of the lipid signaling molecule 15-OOH-Arachindonic acid [15(S)-HpETE]. Glutathione peroxidase 4 reduces 15(S)-HpETE to 15-OOH-Arachindonic acid preventing inflammation and ferroptosis. Ferroptosis is experimentally induced by inhibition of Gpx4 (via oncogenic-*R*AS-*s*elective *l*ethal compound 3 [RSL3]), depletion of glutathione (via glutamate or buthionine sulfoximine [BSO]), or excess AA. Vatiquinone inhibits 15-LO and prevents ferroptosis under the 4 pro-ferroptotic conditions described above.

Figure 2: Ferroptosis Pathway



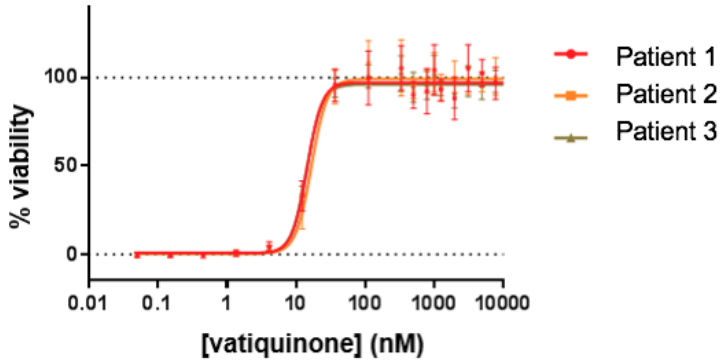
Abbreviations: 15(S)-HpETE, 15-OOH-arachindonic acid; BSO, buthionine sulfoximine; GPX4, glutathione peroxidase 4; GSH, glutathione; GSSG, glutathione disulfide; RSL3, oncogenic-RAS-selective lethal compound 3

- Ferroptosis is a form of regulated cell death (Stockwell 2017) mediated by lipid signaling molecules produced by 15-LO (Seiler 2008, Dixon 2012, Friedmann Angeli 2014).
- Under normal conditions, ferroptosis is prevented by the constitutive activity of the selenoenzyme, Gpx4, which utilizes glutathione (GSH) to reduce cellular lipid hydroperoxides [such as 15(S)-HpETE] to their benign secondary alcohols.
- Depletion of cellular GSH, as has been observed in mitochondrial disease and seizure disorders (Enns 2012, Milder 2012), or inhibition of Gpx4 leads to the accumulation of 15(S)-HpETE and rapidly induces ferroptotic cell death (Seiler 2008, Yang 2014, Kagan 2017).
- When neuronal cells are depleted of endogenous GSH by an irreversible inhibitor of GSH synthesis (BSO), the result is rapid cell death that can be rescued by 15-LO inhibition (Seiler 2008).
- Similarly, mice lacking Gpx4 activity fail to develop parvalbumin interneurons and consequently suffer fatal epileptic seizures (Seiler 2008, Wirth 2010, Ingold 2018).
- Neurons from Gpx4 null mice are protected by the administration of 15-LO inhibitors (Seiler 2008).
- *Net:* The ferroptosis biochemical pathway, regulated by 15-LO, underpins aspects of pontocerebellar hypoplasia type 6 (PCH6) disease pathology including epilepsy.

3. *In vitro validation:* Vatiquinone is a potent rescue agent of human cells in an in vitro test system mimicking ferroptosis-related disease pathology.
   - To determine the potential therapeutic benefit of vatiquinone and 15-LO inhibition, an in vitro ferroptosis assay was developed, employing PCH6 patient primary fibroblasts and Gpx4 inhibition.

- Primary fibroblasts were obtained from 3 PCH6 patients untreated with vatiquinone.
- Inhibition of cellular Gpx4 activity by RSL3, an irreversible small molecule inhibitor (Yang 2014), led to the rapid and nearly-complete death of PCH6 patient fibroblasts.

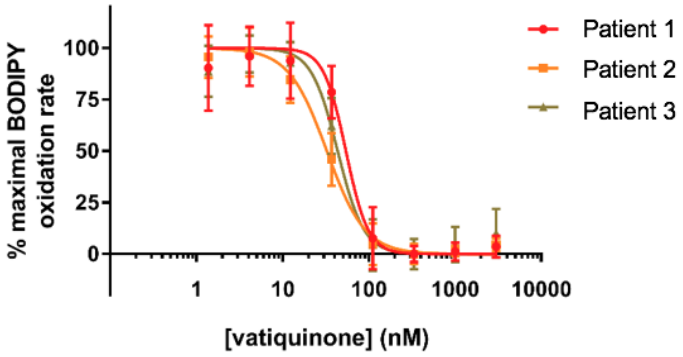
Figure 3: Vatiquinone Dose-dependently Protected Each PCH6 Patient Fibroblast Culture from RSL3-induced Cell Death (EC_{50} Range 17 to 22 nM)



Abbreviations: EC_{50}, half maximal concentration; PCH6, pontocerebellar hypoplasia type 6; RSL3, oncogenic-RAS-selective lethal compound 3

- Similar results were obtained with a second means of inducing ferroptosis by the inhibition of endogenous glutathione synthesis in the presence of excess iron (data not shown; vatiquinone half maximal concentration range 7-14 nM).
- To confirm the mechanism by which vatiquinone protected cells from ferroptosis, we demonstrated that vatiquinone dose-dependently prevented RSL3-induced formation of oxidized lipid signaling molecules in PCH6 patient primary fibroblasts measured using the BODIPY 581/591 C11 oxidation-sensitive fluorescent lipid probe (IC_{50} range 33-58 nM).

Figure 4: RSL3-Induced Formation of Oxidized Lipid Signaling Molecules in PCH6 Patient Primary Fibroblasts

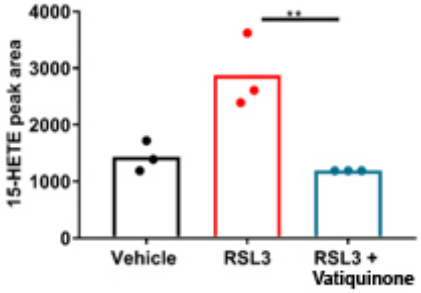


Abbreviations: PCH6, pontocerebellar hypoplasia type 6; RSL3, oncogenic-RAS-selective lethal compound 3

- Consistent with direct cellular inhibition of 15-LO, vatiquinone decreased 15-LO-dependent lipid oxidation products, including 15-hydroxyeicosatetraenoic acid

(15-HETE), in RSL3-treated PCH6 patient fibroblasts, as measured by liquid chromatography with tandem mass spectroscopy (LC-MS/MS).

Figure 5: 15 LO-Dependent Lipid Oxidation Products as Measured by LC-MS/MS



Abbreviations: 15-HETE, 15-hydroxyeicosatetraenoic acid; 15-LO, 15-lipoxygenase; LC-MS/MS liquid chromatography with tandem mass spectroscopy; RSL3, oncogenic-RAS-selective lethal compound 3

Net: Collectively, these in vitro mechanism of action studies in PCH6 patient primary fibroblasts establish that vatiquinone potently prevents lipid oxidation and ferroptotic cell death via *inhibition* of 15-LO and provides supportive rationale for use of vatiquinone to treat PCH6 patients.

4. *Clinical effect:* In compassionate use studies of patients with mitochondrial disease in the United States and European Union, vatiquinone treatment has been associated with the following clinical effects:
    - Arrest of status epilepticus
    - Decrease in seizure frequency
    - Decrease in seizure-related hospitalizations
    - Improvement in disease-related quality of life

Summary: Based on the vatiquinone mechanism of action, the understood biochemical mechanisms underpinning seizures in mitochondrial disease, the in vitro evidence of vatiquinone benefit and clinical data collected to date, there is a valid rationale for the study of vatiquinone in patients with mitochondrial disease and associated refractory epilepsy.

1.4. Findings from Nonclinical and Clinical Safety Studies

Vatiquinone has been evaluated in a comprehensive battery of nonclinical safety and toxicology studies. The clinical safety experience with vatiquinone has been collected in over 500 subjects over 523000 dosing days. Refer to the Investigator's Brochure (IB) for additional detail.

1.4.1. Nonclinical Safety Assessment Studies

Vatiquinone is an orally available compound that has been demonstrated in vitro and in vivo to cross the blood-brain-barrier. In 6-month (Study 1660-045) and 9-month (Study 1660-046) repeat-dose toxicity studies in rat and dog, respectively, the no observed adverse effect level (NOAEL) was 30 mg/kg/day, corresponding to Day 180 and Day 273 combined mean maximum observed plasma concentrations values of 8828 ng/mL (rat) and 12854 ng/mL (dog) and area under the plasma concentration-time curve from time zero to 24 hours values of 48,786 (rat) and

107,426 ng×h/mL (dog). The dose-limiting adverse effect in both rat and dog studies was anticoagulation as demonstrated by prolonged prothrombin time (PT) and activated partial thromboplastin time that most likely results from weak vitamin K antagonism exhibited by this class of compounds.

Vatiquinone was negative in the Good Laboratory Practice battery of genotoxicity assays including the bacterial reverse mutation Ames assay, the in vitro micronucleus assay in human peripheral blood lymphocytes, and the in vivo micronucleus assay in rats (Studies AD75SX.503.BTL, AD75SX.348ICH.BTL, and AD75SX.125012ICH.BT).

Safety pharmacology data collected in the 28-day toxicity studies in rat and dog and during the 6-month and 9-month toxicity studies in rat and dog, respectively, demonstrated that vatiquinone did not affect neurobehavioral or cardiovascular electrocardiogram (ECG) parameters (Studies 1660-027, 1660-028, 1660-045, and 1660-046). In addition, vatiquinone is not a potent inhibitor of human ether-à-go-go-related gene channel current (Study 130416.FEK). Vatiquinone did not influence respiratory function in rat (Study 1660-032).

An in vitro phototoxicity study conducted in BALB/c 3T3 mouse fibroblasts (Study PTC743-2020-003) to assess phototoxic potential demonstrated that vatiquinone had no phototoxic potential when administered to the fibroblasts at concentrations up to 17,800 ng/mL (approximately 16 times the C_{max} obtained at the highest clinical dose of 400 mg).

1.4.2. Clinical Safety Studies

To date, over 500 patients have been treated with vatiquinone, ranging in age at enrollment from <1 year to 70 years old. Total drug exposure to date is more than 523000 patient days, with the longest exposure being over 10 years in a pediatric subject with Leigh syndrome (surfeit locus protein 1 [SURF1] variant). There have been 7 serious adverse events (SAEs) deemed possibly related to vatiquinone:

- One subject experienced 3 SAEs (pneumonia, tachycardia, and increased unresponsiveness) assessed as possibly related to vatiquinone by the investigator
- One subject experienced hypoglycemia assessed as possibly related to vatiquinone by the investigator
- One subject experienced depression assessed as possibly related to vatiquinone by the investigator
- One subject developed pancreatitis assessed as possibly related to vatiquinone by the investigator
- One subject developed neutropenia assessed as possibly related by the investigator and the sponsor

No indications of adverse changes in laboratory liver function, renal function, or coagulation tests have been recorded in clinical studies that have been deemed associated with vatiquinone.

1.5. Risk/Benefit Assessment

1.5.1. Risk Assessment

At the dose levels selected for this study it is not foreseen that subjects will endure any risks greater than minimal. However, as the dose-limiting adverse effect in nonclinical toxicology studies was anticoagulation, to further minimize potential risks subjects will be frequently monitored for changes in coagulation parameters and other clinical pathology assessments. Administration of study drug will be adjusted or completely stopped if coagulation parameters (PT, partial thromboplastin time [PTT]) are found to be increased to more than 1.5×upper limit of normal (ULN).

1.5.2. Potential Benefits of Trial Participation

There are no effective and/or approved treatments available for seizures in the majority of patients with mitochondrial disease. In order to participate in this trial, subjects must have frequent seizure episodes despite treatment with existing antiepileptic therapies. In addition to improved seizure control, potential benefits may manifest in a variety of ways, such as in disease-related parameters, functioning, and quality of life.

1.5.3. Risk-Benefit Assessment

The screening criteria for this trial include that subjects must have seizures that are refractory to antiepileptic therapies. Therefore, the potential benefit to subjects is better control of their seizure activity. In order to further minimize risks, subjects will be closely monitored with a variety of safety measures.

2. STUDY OBJECTIVE AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objective

The primary objective of the study is to demonstrate the effect of vatiquinone (PTC743) on reduction in observable motor seizure frequency in subjects with genetically confirmed mitochondrial disease, as assessed by a seizure diary.

2.1.2. Secondary Objectives

Secondary objectives of the study are to:

- Demonstrate the effects of vatiquinone on seizure-related emergency room visits and hospitalizations
- Demonstrate the effects of vatiquinone on occurrence of status epilepticus
- Demonstrate the effects of vatiquinone in monthly total seizure count
- Demonstrate the effects of vatiquinone in responder rate in motor and non-motor seizures
- Demonstrate the effects of vatiquinone on number of rescue antiepileptic medications used
- Demonstrate the effects of vatiquinone on health-related quality of life (using the CarerQoL-7D questionnaire)
- Demonstrate the effects of vatiquinone on occurrence of seizure clusters
- Demonstrate the safety of vatiquinone as assessed by drug-related SAEs, drug-related adverse events (AEs), and dose modifications

2.1.3. Exploratory Objective

The exploratory objective of the study is to demonstrate the effects of vatiquinone on health-related quality of life (using the Pediatric Quality of Life Inventory™ [PedsQL] questionnaire).

2.2. Endpoints

2.2.1. Primary Endpoint

The primary efficacy endpoint of the study is the percent change from baseline in frequency of observable motor seizures per 28 days during the placebo-controlled phase.

2.2.2. Secondary Endpoints

Key secondary efficacy endpoints of the study are as follows:

- Number of disease-related hospitalization days
- Occurrence or recurrence of status epilepticus

Other Secondary endpoints are as follows:

- Number and percent of subjects with disease-related in-patient hospitalization/emergency room visits
- Number of disease-related in-patient hospitalization admissions/emergency room visits
- Percent change from baseline in total seizure frequency per 28 days of all types
- Proportion of subjects with ≥25%, ≥50%, ≥75%, and 100% reduction in motor seizures
- Proportion of subjects with ≥25%, ≥50%, ≥75%, and 100% reduction in total seizures
- Number of rescue seizure medications
- Health-related quality of life as measured by the CarerQoL-7D questionnaire
- Number of seizure clusters as defined by "too many to count" entries in the seizure diaries

2.2.3. Exploratory Endpoint

The exploratory efficacy endpoint of the study is health-related quality of life as measured by the PedsQL questionnaire.

2.2.4. Safety Endpoints

Safety endpoints include both clinical and laboratory variables.

2.2.4.1. Clinical Variables

- All AEs
- Drug-related SAEs
- Drug-related AEs

2.2.4.2. Laboratory Variables

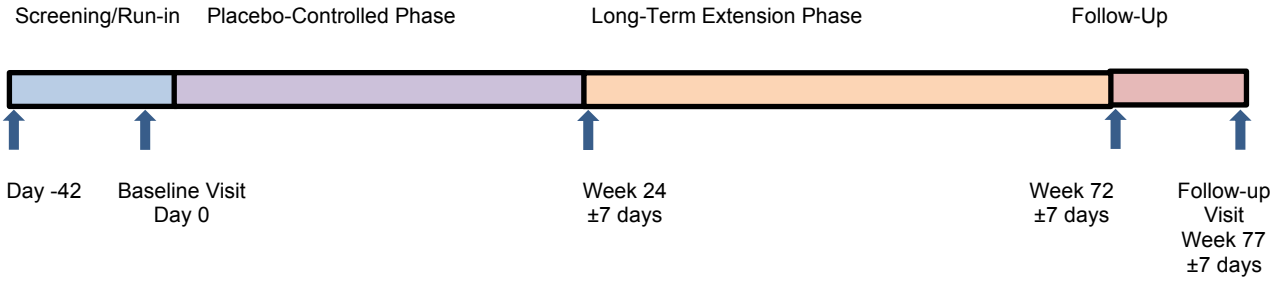
- Complete blood count with differential
- PT and PTT reported as international normalized ratio (INR) units
- Electrolytes, renal function, liver function

3. STUDY DESIGN

3.1. Overall Study Design

This study will be a parallel-arm, double-blind, placebo-controlled trial, with a screening phase that includes a 28-day Run-in phase to establish baseline seizure frequency. The 28 days immediately prior to the Baseline Visit will be considered the Run-in phase. Screening is followed by a 24-week randomized placebo-controlled phase during which subjects will have been randomized to receive either vatiquinone at a dose of 15 mg/kg if body weight <13 kg, and 200 mg if body weight ≥13 kg, TID or placebo TID. Following completion of the randomized placebo-controlled phase, all subjects will be offered entry into a long-term extension phase (48 weeks) during which they will receive open-label treatment with vatiquinone and then a safety follow-up as needed. The study design is summarized in Figure 6.

Figure 6: Study Design Schematic



3.1.1. Screening and Run-in Phase

During Screening, the investigator should inform each prospective subject of the nature of the study, explain the potential risks, and obtain written informed consent from the subject or parent(s)/legal guardian(s) prior to performing any study-related procedures and prior to the administration of study drug.

Screening will consist of up to 42 days. During the first part of Screening (up to 14 days), subjects will be instructed on how to complete the seizure diaries. To ensure the accuracy of diary entries during the study, subjects must complete at least 5 days of diary entries that will be reviewed prior to the Run-in period. Following diary review, at the discretion of the investigator and sponsor a further diary entry training period can be implemented, until the investigator is satisfied that the seizures are correctly captured in the diaries, in which case the duration of Screening will be longer. Diary entries during this period are for training purposes and will not be included in the seizure count.

The PTC Therapeutics (PTC) medical monitor will closely review the seizure entry during the seizure training phase of the study and notify the principal investigators via e-mail to begin "Run-in" phase of the study for the subject.

The last 28 days of screening will consist of a Run-in period for minimum number of seizure evaluation. If subjects are deemed eligible for all other entry criteria (except for seizure count) during the first period of Screening (up to 14 days), they will enter the Run-in period, in which the frequency of seizures will be collected daily via an electronic diary and the baseline frequency of seizures will be assessed. Subjects will be eligible to participate in the study if they:

- Have ≥6 observed motor seizures occurring during the 28 days prior to the Baseline Visit
- Have ≥2 observed motor seizures in each half of the 28-day Run-in period
- Do not have a consecutive 20-day seizure free period, and
- Have at least 80% of seizure diary data

Eligible subjects will then enter a 24-week placebo-controlled phase. Subjects who do not meet the above criteria for observed motor seizures during this period will be considered screen failures.

3.1.2. Randomization

Randomization will take place during the Baseline Visit. Instructions for randomization are provided in an operations manual. Randomization will be performed globally with 1:1 vatiquinone to placebo and will be stratified by disease subtype including: 1) Alpers/DNA polymerase subunit gamma (POLG); 2) Leigh syndrome; 3) Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); and 4) Other genetically confirmed mitochondrial disease secondary to mitochondrial mutation.

3.1.3. Placebo-controlled Phase

Subjects will be randomized to receive vatiquinone at a dose of 15 mg/kg if body weight <13 kg, and 200 mg if body weight ≥13 kg, TID or placebo TID for 24 weeks.

3.1.4. Long-term Extension Phase

Following the 24-week randomized, placebo-controlled phase, all subjects will be eligible to continue in a 48-week open-label treatment phase with vatiquinone at a dose of 15 mg/kg if body weight <13 kg, and 200 mg if body weight ≥13 kg, TID. During this phase, subjects and investigators will remain blinded to the treatment assignment of the placebo-controlled phase.

3.1.5. Follow-up Phase

The follow-up contact will be by telephone and should be completed 30 days (±7 days) post last study drug administration or termination visit, whichever is later. Adverse events and SAEs whether or not deemed study drug-related will be followed until resolution or until they have normalized. At the investigator's discretion an office visit and additional tests may be conducted in order to ensure proper follow-up of an AE/SAE.

3.2. Study Duration and Number of Sites

3.2.1. Duration of Subject Treatment

The maximum duration of subject treatment will be approximately 72 weeks (including placebo-controlled phase and extension phase). The overall study duration will be a maximum of approximately 78 weeks per subject.

3.2.2. Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at approximately 30 sites globally. Approximately 60 subjects will be enrolled. Additional sites and investigators may be added as needed to complete the study.

3.3. Scientific Rationale for Study Design

Seizures are a common symptom of mitochondrial disease and occur in up to 60% of patients with inherited mitochondrial disease. Over 90% of patients with mitochondrial disease-associated epilepsy have seizures that are refractory to traditional antiepileptic therapies (Saneto 2017). The refractory nature of seizures that occur in patients with inherited mitochondrial disease may be related to the fact that traditional antiepileptic therapies do not target the energetic pathways that underpin seizure pathology in these patients (Verrotti 2020). Furthermore, traditional antiepileptic therapies have been reported to increase oxidative stress, which could have the potential to exacerbate mitochondrial disease pathology (Hamed 2004, Aycicek 2007). There is increasing evidence that seizure pathology is associated with ferroptosis (a form of programmed cell death that is regulated by the enzymes 15-LO and Gpx4). Vatiquinone, a novel small molecule therapeutic in development for inherited mitochondrial disease, inactivates 15-LO and downregulates the production of oxidized lipid signaling molecules key to the mechanism of ferroptosis. In preclinical studies, vatiquinone has been demonstrated to rescue cells from patients with mitochondrial disease and associated epilepsy by targeting 15-LO. In previous clinical studies, vatiquinone therapy has been associated with a decrease in seizure frequency and seizure-related morbidity in patients with refractory epilepsy and mitochondrial disease.

Therefore, the development of vatiquinone for the treatment mitochondrial disease patients with refractory epilepsy is based on the following rationale:

1. Vatiquinone mechanism of action: Vatiquinone targets 15-LO, a key regulator of ferroptosis and inflammation.
2. Ferroptosis and seizure disorders: Ferroptosis and inflammation have been demonstrated to be fundamental biochemical mechanisms underpinning mitochondrial disease and seizure disorders.
3. Vatiquinone in vitro validation: Vatiquinone is a potent rescue agent of human cells in an in vitro ferroptosis test system mimicking mitochondrial disease and seizure disorders.
4. Reduction in seizure severity, resolution of status epilepticus, and reduction in disease-related hospitalizations were recorded in patients with pontocerebellar type 6, a mitochondrial epileptic encephalopathy.

3.4. Justification of Dose

The dose of 15 mg/kg if body weight <13 kg, and 200 mg if body weight ≥13 kg, TID was selected on the basis of clinical experience with vatiquinone and plasma concentration data in pediatric patients treated with doses of 100 to 200 mg TID. This dose range was well tolerated in all patients, showed biochemical evidence of target engagement, and was associated with clinical improvements in pediatric patients with mitochondrial disease. Using body weight-based dosing is standard of care for pediatric patients and eliminates the potential for overdose in low body weight patents, and also allows adequate plasma concentrations to be achieved in the face of significant variability in bioavailability.

3.5. End of Study Definition

The study will end when the last subject has completed the follow-up visit.

4. STUDY POPULATION

4.1. Overview

The study population consists of male and female subjects <21 years of age who have genetically determined mitochondrial disease and associated refractory epilepsy (defined as those who were unsatisfactorily treated with at least 2 antiepileptic drugs [AEDs]).

4.2. Inclusion Criteria

To be eligible for enrollment in the study, subjects must meet all of the following inclusion criteria:

1. Evidence of signed and dated informed consent/assent document(s) indicating that the subject (and/or his/her parent/legal guardian) has been informed of all pertinent aspects of the trial
2. Age <21 years at time of randomization
3. Subject or parent/legal guardian is able and willing to complete seizure diaries for the duration of the study
4. Subjects with genetic confirmation of inherited mitochondrial disease with associated epilepsy phenotype (Alpers/POLG, Leigh syndrome, MELAS), or other genetically confirmed mitochondrial diseases secondary to mitochondrial mutations (Pontocerebellar Hypoplasia Type 6 [PCH6], nuclear DNA RARS2 mutation) or myoclonic epilepsy with ragged red fibers (MERRF, mitochondrial DNA [mtDNA] mitochondrially encoded tRNA lysine [MT-TK] mutation) are eligible.
5. Despite treatment with at least 2 AEDs:
    - Have ≥6 observed motor seizures occurring during the 28 days prior to the Baseline Visit
    - Have ≥2 observed motor seizures in the first 14 days and ≥2 in the second 14 days of the Run-in period
    - Do not have a consecutive 20-day seizure free period and
    - Have at least 80% of seizure diary data
    - No changes to the AED regimen will be allowed (except weight-based dose adjustments) during the first 24-week period.
6. Documented medical history of epilepsy associated with mitochondrial disease for at least 6 months prior to screening except for subjects who are <2 years of age at the time of screening (subjects <2 years of age can be considered for enrollment if all other screening criteria are met due to the potential for rapid progression in these subjects).
7. Consent to abstain from non-approved therapies for 30 days prior to the Screening Visit and for the duration of the study
8. Stable dose regimen of antiepileptic therapies 30 days prior to the Screening Visit

9. Stable regimen of dietary supplements 30 days prior and, if on a ketogenic diet, stable ketogenic diet 90 days prior to the Screening Visit and for duration of the study
10. Electroencephalogram (EEG) at Screening or historical EEG up to 6 months prior to screening for diagnostic confirmation of epilepsy

4.3. Exclusion Criteria

Subjects will be excluded from enrollment if any of the following exclusion criteria apply:

1. Allergy to vatiquinone or sesame oil
2. Aspartate transaminase (AST) or alanine transaminase (ALT) ≥3×ULN at time of screening
3. INR >ULN at time of screening
4. Serum creatinine ≥1.5×ULN at time of screening
5. Participation in another interventional clinical trial 60 days prior to randomization or for the duration of this clinical trial
6. Previously received vatiquinone
7. Concomitant treatment with drug(s) that have not received regulatory agency approval for the treatment of mitochondrial diseases and use of artisanal (non-Epidiolex cannabidiol) cannabidiol therapies
8. Concomitant treatment with idebenone
9. Ongoing treatment with strong cytochrome P450 (CYP) inhibitors such as itraconazole or strong CYP inducers such as rifampin. Treatment with these agents must be completed at least 4 weeks prior to enrollment. During the study, subjects should not use grapefruit/grapefruit juice or St John's wort extract.
10. Pregnant or lactating subjects or those male or female sexually active subjects who are unwilling to comply with proper birth control methods as defined in Section 7.5.10 from the time consent is signed until 30 days after treatment discontinuation. Females of childbearing potential must have a negative pregnancy test at Screening and during the Baseline Visit.
11. Comorbidities that may confound study results (eg, fat malabsorption syndrome, other mitochondrial disorders) in the opinion of the investigator.

4.4. Screen Failures

Any subject that does not meet inclusion or exclusion criteria within the defined screening window prior to randomization, will be considered a screen failure. Screen failures can be rescreened after consultation with the medical monitor.

4.5. Strategies for Recruitment and Retention

Subjects will be recruited via existing populations with mitochondrial diseases at identified investigational sites or via referrals.

4.6. Home Care Services

If for unforeseen reasons subjects are unable to travel to the study site, with approval from both the Principal Investigator and PTC medical monitor, they may be offered an opportunity to have study visits performed in their home.

In order to conduct the home visits, the subject must agree to utilize home care services. A licensed nurse will then contact the subject to schedule the visits.

The home care nurse, the home care agency, and the home care services provider may have access to the subject's personal data including their individually identifiable protected health information, such as the subject's name, address, or phone number. This type of information will only be used as necessary to schedule and conduct the home visits and will not be provided to the sponsor.

5. STUDY INTERVENTION

5.1. Study Intervention(s) Administration

5.1.1. Study Intervention Description

Vatiquinone oral solution is manufactured at 100 mg/mL for oral administration. Subjects will receive vatiquinone at a dose of 15 mg/kg if body weight <13 kg, and 200 mg if body weight ≥13 kg, TID (Table 1). At each study visit the subjects are weighed; any change in weight of ≥10% from baseline should result in dose adjustment. Weights ending in decimals ≥0.5 should be rounded up to the next whole number for dosing calculation.

For this study, placebo will consist of sesame oil NF, with a colorant added to match vatiquinone oral solution. During the phase of the study where subjects are to receive placebo, a volume of placebo equal to the volume of active received when assigned to vatiquinone treatment will be provided to each subject.

Table 1: Weight-Based Dosing in mL of the 100 mg/mL Solution

Subject Weight (kg)	Dose (mg/kg) TID	Dose Volume (mL)
4	15	0.6
5	15	0.8
6	15	0.9
7	15	1.1
8	15	1.2
9	15	1.4
10	15	1.5
11	15	1.7
12	15	1.8
13	15	2.0
Above 13 kg	200 mg per dose	2.0

5.1.2. Dosing and Administration

Drug dosing will occur according to the schedule described in Sections 7.1.2 and 7.1.3. Food is required for appropriate absorption of vatiquinone from the gastrointestinal tract. For subjects who have a gastrostomy tube (G-tube) installed, the study drug will be instilled into the G-tube using an oral dispenser. For subjects who are receiving continuous feed with liquid nutritional preparation such as PediaSure, the feed is interrupted to allow instillation of the drug solution into the G-tube using an oral dispenser, followed by resumption of the continuous feed to flush the drug into the stomach. For subjects who received G-tube feeding as bolus, the study drug is instilled into the G-tube using an oral dispenser, followed by the feed bolus to flush the drug into the stomach. The same procedures are to be followed in case the subject has a nasogastric tube installed. Additional detail on dosing can be found in the Pharmacy Manual.

For subjects who do not have a G-tube installed and who can swallow, the study drug is given orally using an oral dispenser along with a meal that includes foods such as cream cheese, peanut butter, or other fatty components. The dose of study drug needs to be followed by eating yogurt, ice cream (no non-fat), or any other food with at least 25% calories from fat.

5.1.3. Treatment of Missed Doses

If a dose is missed, or if the subject vomits after receiving a dose, the dose should not be "made up." The subject will be required to skip that dose and wait until the next scheduled dose.

5.2. Preparation/Handling/Storage/Accountability

5.2.1. Accountability

Adequate records of study drug receipt and disposition will be maintained by the (Institution's) Pharmacy Records of receipts, investigational drug orders, dispensing records, and disposition forms. The study monitor will assess drug accountability and will request to review the Pharmacy Records and investigator study file to assure the study medication is prescribed by the investigator or designee for the purpose of this study.

At study completion, all drug supplies including partially used and empty containers must be either returned to the sponsor or designee or destroyed per your institution's guidelines for destruction of investigational product after drug accountability has been performed by the study monitor.

5.2.2. Formulation, Appearance, Packaging, and Labeling

Vatiquinone oral solution, 100 mg/mL, is a solution of vatiquinone drug substance in super refined, preservative free sesame oil. At room temperature, it is a yellow to orange clear liquid. Fifty-four mL of the solution is filled into a 60 mL amber glass bottle and closed with child resistant cap suitable for multiple dose administration. The placebo oral solution is packaged in the same container/closure as the active substance and is also a yellow to orange clear liquid.

Each bottle will be labeled with the following but not limited to: Product name, concentration, batch number, expiration date, storage conditions, and a caution limiting it for Clinical Trial Use Only.

5.2.3. Preparation

No preparation is required; vatiquinone is provided as a ready-to use oral solution.

5.3. Measures to Minimize Bias: Randomization and Blinding

Subjects meeting the eligibility criteria for the randomized placebo-controlled phase will be randomized at the Baseline Visit to either vatiquinone treatment or placebo group. Subjects who withdraw from the study after being assigned a subject number will retain that subject number. Randomization will be stratified by disease subtype including: 1) Alpers/POLG, 2) Leigh syndrome, 3) MELAS, and 4) Other genetically confirmed mitochondrial disease secondary to either nuclear or mitochondrial DNA mutation.

The investigators, study subjects, and sponsor will be blinded as follows: the sponsor will be blinded to the randomized treatment until the study is unblinded after all subjects complete the placebo-controlled phase; subjects and investigators remain blinded to the treatment received during the placebo-controlled phase until after all subjects complete the open-label phase. An independent Data Safety Monitoring Board (DSMB) will monitor the study until completion of the placebo-controlled phase of the study.

Under normal circumstances, the blind will not be broken. The blind may be broken by the principal investigator only if specific emergency treatment would be dictated by knowing the treatment assignment. The investigators have the ability to unblind via the Interactive Response Technology system.

5.4. Study Intervention and Compliance

Study medication will be reviewed to assess compliance on an ongoing basis. Subjects who take >80% of the prescribed doses and no more than 120% of the prescribed doses will be considered compliant. Subjects who fall outside of this threshold will be counseled and instructed on dosing procedures. Medication compliance will be recorded in the diary.

5.5. Concomitant Therapy

Any medication taken by a subject 30 days prior to Baseline Visit and during the course of the study and the reason for use of the medication will be recorded on the electronic case report form (eCRF)/paper case report form (CRF). During Screening, each subject will be instructed to report the use of any medication to the investigator. Subjects will also be instructed about the importance of not taking any medication throughout the study (including over-the-counter medications) without consulting the investigator.

All immunizations will be considered as concomitant medications and will be recorded in the CRF. Subjects will be instructed to consult the investigator before immunization. The final decision on any immunization should be made by the treating physician in consultation with the patient and/or caregiver.

6. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1. Discontinuation of Study Intervention

Subjects receive study treatment until treatment discontinuation for one of the reasons listed below. However, subjects may discontinue study treatment or withdraw their consent to participate in the study at any time without prejudice.

The following conditions require subject discontinuation from all study treatment:

1. At their own request or at the request of their legally authorized representative
2. If a subject experiences an AE that is deemed related to treatment with vatiquinone and, in the investigator's or the sponsor's medical judgment, continuation of treatment would be detrimental to the subject
3. Spontaneous bleeding in a subject
4. At the specific request of a regulatory agency for termination of treatment of an individual subject or all subjects under the protocol
5. Subject participation in another clinical study using an investigational agent or investigational medical device
6. Refusal of sexually active fertile subjects (excluding subjects who have been sterilized) to use medically accepted methods of contraception (Section 7.5.10)
7. If a subject becomes pregnant
8. Significant noncompliance with the protocol in the opinion of the investigator or the sponsor
9. Use of prohibited concomitant medication

6.2. Participant Discontinuation/Withdrawal from the Study

In all cases of subject withdrawal or discontinuation, the reason for withdrawal must be recorded in the eCRF/CRF and in the subject's medical records. If the reason is not known, the subject must be followed to establish whether the reason was due to an AE, and, if so, this must be reported in accordance with the procedures in Section 7.5.8.

Evaluations should be performed on all subjects who participate but do not complete the study according to protocol. Dropouts may be replaced at the request of the sponsor. The investigator will make every effort to contact subjects lost to follow-up. Subjects who have an ongoing AE at the time of study completion will be followed until the event resolves, or until the sponsor and the Investigator agree that further follow-up is not medically necessary.

6.3. Lost to Follow-up

Subjects are considered lost to follow-up if the subject does not return to the clinic and attempts to contact the subject are unsuccessful. Efforts must be made on the part of the site to avoid any subject being lost to follow-up during the study. Before subjects are considered lost to follow-up, a minimum of 2 documented telephone contact attempts and 1 certified letter within 6 weeks of the most recent planned study visit must be sent in efforts to contact the subject. After being considered lost to follow-up, a subject's status may be changed if the subject makes contact at a later time provided the trial is ongoing.

7. STUDY ASSESSMENT AND PROCEDURES

7.1. Schedule of Events and Study Parameters

Table 2: Schedule of Events

Procedures	**Screening[a]**		**Baseline**	**Placebo-controlled Phase**					**Long-term Extension Phase**								**Follow-up Phase[b]**
	Day -42 to 0	Day -28	Day 0 up to +7 days	Week 2[c]±7 days	Week 4 & 8 ±7 days	Week 12 ±7 days	Week 16 & 20[c] ±7 days	Week 24 ±7 days	Week 28 & 32[c] ±7 days	Week 36 ±7 days	Week 40 & 44[c] ±7 days	Week 48 ±7 days	Week 52 & 56[c] ±7 days	Week 60 ±7 days	Week 64 & 68[c] ±7 days	Week 72 ±7 days	Week 77 ±7 days
Informed consent	X							X									
Medical history	X																
Genetic confirmation of mitochondrial disease	X																
Inclusion/exclusion criteria	X																
12-lead ECG	X		X[d]					X[d]				X[d]				X[d]	
EEG	X[e]							X								X	
Height, weight, oxygen saturation	X[f]		X		X	X		X		X		X		X		X	
Physical exam and vital signs	X		X		X	X		X		X		X		X		X	
Pregnancy test[g]	X		X		X	X		X		X		X		X		X	X
Hematology (including CBC w/diffs)			X		X	X		X		X		X		X		X	
Coagulation panel	X		X		X	X		X		X		X		X		X	
Serum chemistry	X[h]		X		X	X		X		X		X		X		X	
Urinalysis	X		X		X	X		X		X		X		X		X	
Blood samples for PK analysis[i]			X					X				X				X	
Train caregiver on diary completion and hospitalization log	X[j]																
AE/SAE assessment[k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[l]
Concomitant medications	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Randomization			X														

Procedures	Screening[a]		Baseline	Placebo-controlled Phase					Long-term Extension Phase								Follow-up Phase[b]
	Day -42 to 0	Day -28	Day 0 up to +7 days	Week 2[c]±7 days	Week 4 & 8 ±7 days	Week 12 ±7 days	Week 16 & 20[c] ±7 days	Week 24 ±7 days	Week 28 & 32[c] ±7 days	Week 36 ±7 days	Week 40 & 44[c] ±7 days	Week 48 ±7 days	Week 52 & 56[c] ±7 days	Week 60 ±7 days	Week 64 & 68[c] ±7 days	Week 72 ±7 days	Week 77 ±7 days
Seizure and dosing diary distribution	X																
Seizure and dosing diary collection/review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Total hospital admissions and total hospital days for disease-related issues log	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CarerQoL-7D			X		X	X		X				X				X	
PedsQL			X					X								X	
Initiate dosing diary entry			X														
In-office dosing PO with food			X[m]					X[m]				X[m]				X[m]	
Dispense study drug			X[m]			X		X[n]		X		X		X			
Return of study drug bottles and drug reconciliation with dosing diary						X		X		X		X		X		X	

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood count; COVID-19, coronavirus disease 2019; ECG, electrocardiogram; EEG, electroencephalogram; eCRF, electronic case report form; PedsQL, Pediatric Quality of Life Inventory™; PK, pharmacokinetics; SAE, serious adverse event; w/diffs, with differentials

[a] Screening will be conducted and completed in a maximum of 42 days (up to 14 days Screening and 28 days Run-in period) prior to the Baseline Visit. Before the Run-in period subjects will be trained in diary completion. The Run-in phase and initiation of the seizure diary completed during this phase of the study, will contain a minimum of 28 consecutive days of seizure data collected prior to the Baseline Visit. Ideally the subject will be instructed to start entering diary information the day after Screening, so that the requisite number of days can be collected prior to the Baseline Visit. Laboratory tests from within 30 days of Screening are acceptable for entry to the study.

[b] The follow-up visit will be conducted via telephone 30 days (±7 days) after last dose or termination visit.

[c] Telephone call only.

[d] ECG measurement will be performed at 4 hours postdose during each visit, time-matched to PK blood collection. Electrocardiogram should be performed approximately within 10 minutes preceding the 4-hour PK blood collection.

[e] EEG for epilepsy confirmation if historical EEG up to 6 months prior to screening for confirmation of epilepsy is not provided.

[f] Height will only be collected at the Screening Visit; all subsequent visits will collect weight and oxygen saturation only. Any change in weight of ≥10% from Baseline should result in dose adjustment. Weights ending in decimals ≥0.5 should be rounded up to the next whole number for dosing calculation.

[g] Female subjects of childbearing potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy text at all other indicated timepoints. If a subject cannot perform a urine pregnancy test at any timepoint, a serum pregnancy test should be performed. At-home urine pregnancy test kits will be provided; subjects will be required to perform the test at home 30 days after the last dose of study drug and self-report the results via telephone.
[h] ALT, AST, and creatinine are the only chemistries required at the Screening Visit.
[i] Blood will be drawn for PK assessment predose and 1, 3, 4, and 8 hours postdose (prior to the next dose of vatiquinone) at Baseline and Week 24, and only 4 hours postdose at Weeks 48 and 72. For subjects weighing less than 10 kg, PK blood draw can be reduced to 2 timepoints per visit at the Baseline and Week 24 visits. Recommended timepoints are 0 (predose) hour and 4 hours postdose.
[j] During the Screening Visit, the subject's caregiver will be trained on how to capture information in the seizure diary and hospital admissions log.
[k] Serious adverse events will be assessed from the Screening Visit through 30 days following the last dose of study drug should be reported via the SAE report form to PTC within 24 hours of becoming aware of the event(s). In addition, the AE portion of the eCRF must also be completed.
[l] The follow-up contact will be by telephone and should be completed 30 days (±7 days) post last study drug administration or termination visit, whichever is later. Adverse events and SAEs will be followed until resolution or until they have normalized. At the investigator's discretion an office visit and additional tests may be conducted in order to ensure proper follow-up of an AE/SAE.
[m] First dose for the placebo-controlled phase of the study will be taken the same day as the Baseline Visit. Study drug will be dispensed during the Baseline Visit and at each subsequent in-person visit. Drug may be delivered via delivery service if needed.
[n] From Week 24 onwards, vatiquinone will be dispensed to all subjects entering the long-term extension phase. Vatiquinone will be dispensed after completing the Week 24 assessments. The subjects will enter the open-label phase of the study and receive vatiquinone for the dose after the visit.

7.1.1. Screening Visit/Run-in Phase

Screening evaluations will be performed within a 42-day period (up to 14 days initial screening then 28 days Run-in) prior to the Baseline Visit. The investigator should inform each prospective subject of the nature of the study, explain the potential risks, and obtain written informed consent from the subject or parent(s)/legal guardian(s) prior to performing any study-related procedures and prior to the administration of study drug.

Screening (Day -42 to Day 0) evaluations will consist of the following:

1. Administer informed consent (Assent)
2. Assessment of eligibility
3. Chart review- previous medical history
4. Genetic confirmation of mitochondrial disease
5. Review inclusion and exclusion criteria
6. 12-lead ECG
7. EEG-diagnostic confirmation of epilepsy (historical EEG, up to 6 months prior to screening for, is acceptable)
8. Height, weight, and oxygen saturation
9. Physical Examination including vital signs
10. Serum pregnancy test: women of childbearing potential (WOCBP) must have a negative serum pregnancy test (see Section 7.4.3)
11. Laboratory assessments for coagulation, serum chemistry (ALT, AST, and creatinine only), and urinalysis (see Section 7.4.2)
12. The subject's caregiver will be trained on how to capture information in dosing diary, seizure diary, and hospital admissions log. The subjects will begin entering information in seizure diary daily and hospital admissions log during the Run-in phase. However, the subjects will begin entering information in dosing diary only after first dose.
13. SAE assessment
14. Concomitant medications assessment (last 30 days prior to screening)

During the first Screening Visit (occurring before Run-in), subjects will be instructed on how to complete the seizure diaries. To ensure the accuracy of diary entries during the study, subjects must complete at least 5 days of diary entries that will be reviewed prior to the Run-in period. Following diary review, at the discretion of the investigator and sponsor, a further diary entry training period can be implemented, until the investigator is satisfied that the seizures are correctly captured in the diaries, in which case the duration of Screening will be longer. Diary entries during this period are for training purposes and will not be included in the seizure count. The PTC medical monitor will closely review the seizure entry during the seizure training phase of the study and notify the principal investigators via e-mail to begin "Run-in" phase of the study for the subject. Seizures will be documented in an electronic seizure diary provided to each subject. Run-in will be a minimum of 28 days prior to the Baseline Visit.

7.1.2. Placebo-controlled Treatment Phase

If the subject has the required frequency of observable motor seizures during Run-in, meets all inclusion/exclusion criteria, and is willing to participate in the study, the subject will return for the baseline assessment and initiation of treatment with vatiquinone at a dose of 15 mg/kg if body weight <13 kg, and 200 mg if body weight ≥13 kg, administered TID or placebo TID. In addition to the assessments listed below, all hospitalizations, total number of hospital days, total intensive care unit days and use of rescue antiepileptic medications will be tracked and recorded.

7.1.2.1. Baseline Visit/Randomization

The following assessments and procedures will be performed at Baseline Visit:

1. Review of all previous screening testing performed on the subject
2. 12-lead ECG at 4 hours postdose time-matched to pharmacokinetic (PK) blood collection; ECG should be performed approximately within 10 minutes preceding the 4-hour PK blood collection
3. Weight and oxygen saturation
4. Physical Examination including vital signs
5. Urine or serum pregnancy test: WOCBP must have a negative urine or serum pregnancy test (see Section 7.4.3)
6. Laboratory assessments for hematology, coagulation, serum chemistry, and urinalysis (see Section 7.4.2)
7. Randomization
8. The study drug will be administered at Baseline Visit by site staff or caregiver will be trained to administer the first dose on site
9. Blood samples drawn for PK analysis predose and 1, 3, 4, and 8 hours postdose (prior to the next dose of vatiquinone). For subjects weighing less than 10 kg, PK blood draw can be reduced to 2 timepoints per visit. Recommended timepoints are 0 (predose) hour and 4 hours postdose.
10. AE/SAE assessment
11. Concomitant medications assessment
12. Review of seizure diary
13. Calculation of total hospital admissions and total hospital days for disease-related issues from the time of the Screening Visit
14. CarerQoL-7D questionnaire
15. PedsQL questionnaire
16. Dispense study drug

During the Baseline Visit, study medication will be dispensed to the subject according to the randomization. First dose for the study drug will be taken the same day as Baseline Visit and PK samples collected during the Baseline Visit.

Subjects randomized to the treatment arm will be required to take study drug in a formulation mixed with sesame oil NF. Subjects in the placebo group will receive sesame oil with a colorant, which will have similar appearance and consistency as vatiquinone. In case the subject has a gastrostomy tube or nasogastric tube (G-tube or nasogastric tube) installed, vatiquinone or placebo will be administered using an amber oral dispenser into the G-tube with liquid food. If the subject is able to swallow, vatiquinone or placebo can be administered using an amber oral dispenser along with food.

7.1.2.2. Week 2 Visit (Day 14±1 week)

1. AE/SAE assessment
2. Concomitant medications assessment
3. Review of seizure and drug diaries
4. Calculation of total hospital admissions and total hospital days for disease-related issues

7.1.2.3. Week 4 Visit (Day 28 ± 1 week)

The following assessments and procedures will be performed at Week 4. A home health visit option may be performed at Week 4 if an in-person visit is not feasible due to travel or coronavirus disease 2019 (COVID-19) restrictions, etc.

1. Weight and oxygen saturation
2. Physical Examination including vital signs
3. Urine pregnancy test: WOCBP must have a negative urine pregnancy test (see Section 7.4.3). If a subject cannot perform a urine pregnancy test, a serum pregnancy test should be performed.
4. Laboratory assessments for hematology, coagulation, serum chemistry, and urinalysis (see Section 7.4.2)
5. AE/SAE assessment
6. Concomitant medications assessment
7. Review of seizure and dosing drug diaries
8. Calculation of total hospital admissions and total hospital days for disease-related issues
9. CarerQoL-7D questionnaire

7.1.2.4. Week 8 Visit (Day 56 ± 1 week)

The following assessments and procedures will be performed at Week 8. A home health visit option may be performed at Week 8 if an in-person visit is not feasible due to travel or COVID-19 restrictions, etc.

1. Weight and oxygen saturation
2. Physical Examination including vital signs

3. Urine pregnancy test: WOCBP must have a negative urine pregnancy test (see Section 7.4.3). If a subject cannot perform a urine pregnancy test, a serum pregnancy test should be performed.
4. Laboratory assessments for hematology, coagulation, serum chemistry, and urinalysis (see Section 7.4.2)
5. AE/SAE assessment
6. Concomitant medications assessment
7. Review of seizure and dosing drug diaries
8. Calculation of total hospital admissions and total hospital days for disease-related issues
9. CarerQoL-7D questionnaire

7.1.2.5. Week 12 Clinic Visit (Day 84 ± 1 week)

The following assessments and procedures will be performed at Week 12 (± 1 week):

1. Weight and oxygen saturation
2. Physical Examination including vital signs
3. Urine pregnancy test: WOCBP must have a negative urine pregnancy test (see Section 7.4.3). If a subject cannot perform a urine pregnancy test, a serum pregnancy test should be performed.
4. Laboratory assessments for hematology, coagulation, serum chemistry, and urinalysis (see Section 7.4.2)
5. AE/SAE assessment
6. Concomitant medications assessment
7. Review of seizure and dosing drug diaries
8. Calculation of total hospital admissions and total hospital days for disease-related issues
9. CarerQoL-7D questionnaire
10. Drug reconciliation of returned study drug bottles with dosing diaries
11. Dispense study drug

7.1.2.6. Week 16 Visit (Days 112 ± 1 week)

Week 16 assessments can be conducted by telephone and must include review of:

1. AE/SAE assessment
2. Concomitant medications assessment
3. Review of seizure and dosing drug diaries
4. Calculation of total hospital admissions and total hospital days for disease-related issues

7.1.2.7. Week 20 Visit (Days 140 ± 1 week)

Week 20 assessments can be conducted by telephone and must include review of:

1. AE/SAE assessment
2. Concomitant medications assessment
3. Review of seizure and dosing drug diaries
4. Calculation of total hospital admissions and total hospital days for disease-related issues

7.1.2.8. Week 24 Clinic Visit (Days 168 ± 1 week)

The following assessments and procedures will be performed at Week 24 (±1 week):

1. 12-lead ECG at 4 hours postdose time-matched to PK blood collection; ECG should be performed approximately within 10 minutes preceding the 4-hour PK blood collection
2. Weight and oxygen saturation
3. Physical Examination including vital signs
4. Urine pregnancy test: WOCBP must have a negative urine pregnancy test (see Section 7.4.3). If a subject cannot perform a urine pregnancy test, a serum pregnancy test should be performed.
5. Laboratory assessments for hematology, coagulation, serum chemistry, and urinalysis (See Section 7.4.2)
6. Blood samples drawn for PK analysis predose and 1, 3, 4, and 8 hours postdose (prior to the next dose of vatiquinone). For subjects weighing less than 10 kg, PK blood draw can be reduced to 2 timepoints per visit. Recommended timepoints are 0 (predose) hour and 4 hours
7. EEG
8. AE/SAE assessment
9. Concomitant medications assessment
10. Review of seizure and dosing drug diaries
11. Calculation of total hospital admissions and total hospital days for disease-related issues
12. CarerQoL-7D questionnaire
13. PedsQL questionnaire
14. Drug reconciliation of returned study drug bottles with dosing diaries
15. Informed consent if subject is continuing in Long-Term Extension phase
16. Dispense study drug if subject is continuing in Long-Term Extension phase

7.1.3. Long-Term Extension Phase (Study Weeks 36, 48, 60, and 72 are In-Person Visits and Study Weeks 28, 32, 40, 44, 52, 56, 64, and 68 are Telephone contact ±1 week)

The subjects who are interested in continued treatment with vatiquinone will continue in the long-term extension phase of the study. In-person visits will occur every 12 weeks (±1 week) and there will be every 4 weeks (±1 week) telephone contact with the subject in between the in-person visits. Study medication will be dispensed at each in-person visit and will be sufficient to reach the next 12-week in-person visit.

The following assessments and procedures will be performed at each of the in-person visits under the long-term extension phase:

1. 12-lead ECG at 4 hours postdose time-matched to PK blood collection (Weeks 48 and 72 only; ECG should be performed approximately within 10 minutes preceding the 4-hour PK blood collection)
2. Weight and oxygen saturation
3. Physical examination including vital signs
4. Urine pregnancy test: WOCBP must have a negative urine pregnancy test (see Section 7.4.3). If a subject cannot perform a urine pregnancy test, a serum pregnancy test should be performed.
5. Laboratory assessments for hematology, coagulation, serum chemistry, and urinalysis (see Section 7.4.2)
6. Blood samples drawn for PK analysis 4 hours postdose at Study Weeks 48 and 72
7. EEG at Week 72
8. AE/SAE assessment
9. Concomitant medications assessment
10. Review of seizure and dosing drug diaries
11. Calculation of total hospital admissions and total hospital days for disease-related issues
12. CarerQoL-7D questionnaire will be collected at Study Week 48 and Study Week 72
13. PedsQL questionnaire (Week 72)
14. Drug reconciliation of returned study drug bottles with dosing diary (Study Weeks 36, 48, 60, and 72)
15. Dispense study drug (Weeks 36, 48, and 60)

The following assessments will be conducted at each of the telephone contacts and must include review of:

1. AE/SAE assessment
2. Concomitant medications assessment
3. Seizure and drug diaries

4. Calculation of total hospital admissions and total hospital days for disease-related issues

7.1.4. Post Treatment Follow-up Phase

The follow-up contact will be by telephone and should be completed 30 days (±7 days) post last study drug administration or termination visit, whichever is later. Adverse events and SAEs whether or not deemed study drug-related will be followed until resolution or until they have normalized. At the investigator's discretion an office visit and additional tests may be conducted in order to ensure proper follow-up of an AE/SAE. Subjects will be closely monitored for possible rebound effects following discontinuation of study drug. Additionally, subjects who are WOCBP will be required to perform an at-home pregnancy test (study-provided) 30 days after the last dose of study drug and self-report the results via telephone.

7.1.5. Unscheduled Visits

If a subject returns to the clinic outside of the normal study visit windows, assessments will be made at the investigator's discretion. All study relevant unscheduled visit assessments will be recorded in the eCRF using the supplemental visit eCRF pages.

7.1.6. Sample Collection Procedures

Blood samples for clinical laboratory measurements should be collected and processed according to the central laboratory collection manual guidance or if collection is for local laboratory according to study site laboratory's standard procedure.

7.1.7. Safety Considerations

Based on the known clinical manifestations of mitochondrial diseases, PTC expects that many AEs could occur during the course of the trial that are known to be associated with disease natural history.

Both the preclinical and clinical safety profiles of vatiquinone have been favorable. To date, in over 523000 patient treatment days with vatiquinone there have been 7 SAEs assessed by the investigators to be probably or possibly drug-related.

Specific dose modification guidelines for INR are provided in Section 7.4.6.

Nonetheless, specific or supportive care as chosen by the investigator may be considered.

7.1.8. Rescue Medication Administration for Epilepsy

Use of rescue medications for epilepsy will be collected throughout the study and analyzed as a secondary study endpoint. The following standardized criteria should be used for use of rescue medications:

- Seizure lasting longer than 4 minutes
- Occurrence of status epilepticus
- Cluster of 15 or more seizures

7.1.9. Prohibited Medications

Subjects will be required to maintain a stable regimen of mitochondrial disease supplements from screening throughout the trial.

Anticoagulants such as vitamin K antagonists, Factor Xa inhibitors are not permitted during the course of the study because of the potential interaction with vatiquinone on vitamin K associated coagulation factor synthesis.

Cytochrome P450 inhibitors such as itraconazole have been demonstrated to significantly increase the plasma concentrations of vatiquinone (up to 2-fold). Cytochrome P450 inducers such as rifampin significantly reduce the plasma concentrations of vatiquinone (up to 50% less). Therefore, strong CYP inducers and inhibitors, including grapefruit/grapefruit juice and St John's wort extract, should be avoided during the course of the study.

- In case treatment with a CYP inhibitor is required during the course of the study, coagulation parameters should be monitored and vatiquinone dose should be reduced by 33% in case INR increases over 1.5×ULN or Common Terminology Criteria for Adverse Event (CTCAE) Grade 2.
- In the event that a CYP inducer is used, no adjustment of the study drug dose will be required because there are no safety concerns with CYP inducers.

No changes to the AED regimen (except for weight-based dose adjustments) will be allowed during the first 24-week period.

The use of artisanal (non-Epidiolex cannabidiol) cannabidiol therapies should continue to be excluded due to uncertainty regarding the quality and quantity of cannabidiol in the artisanal product.

7.1.10. End of Treatment Visits

End of treatment visit takes place at Study Week 72 and includes the assessments listed in Section 7.1.2.1. Subjects who have an ongoing AE/SAE at the time of study completion will be followed for up to 30 days, until the event resolves, or until the sponsor and the Investigator agree that further follow-up is not medically necessary.

7.1.11. Early Termination Study Visit

Subjects who terminate from the study after receiving first dose and prior to the completion of all scheduled visits should have the end of treatment visit assessments completed as soon as possible. Early termination study visit will include the assessments listed in Section 7.1.2.1. Subjects who have an ongoing AE/SAE at the time of study completion will be followed for up to 30 days, until the event resolves, or until the sponsor and the Investigator agree that further follow-up is not medically necessary.

7.2. Efficacy Assessments

Efficacy assessments will include numbers of motor and non-motor seizures, which should be recorded at least daily in the electronic diary.

The number of emergency room visits, use of rescue seizure medications, and health-related quality of life as measured by the CarerQoL-7D and PedsQL questionnaires, will also be captured in the electronic diary or recorded on the appropriate page of the eCRF.

7.3. PK and/or Other Assessments

Blood samples for PK assessments will be drawn predose and 1, 3, 4, and 8 hours postdose (prior to the next dose of study drug) at Baseline and Week 24, and 4 hours postdose at Week 48 and Week 72. For subjects weighing less than 10 kg, PK blood draw can be reduced to 2 timepoints per visit at the Baseline and Week 24 visits. Recommended timepoints are 0 (predose) hour and 4 hours postdose. The collection of these drug concentration values will serve 2 purposes: (1) to assess the consistency of systemic drug concentrations at specific timepoints around the time to maximum plasma concentration to confirm the durability of drug exposure and (2) be potentially incorporated in a structural model (from prior PK profiles) that can help to quantitate selected pharmacodynamic relationships such as safety and disease improving effects.

7.4. Safety Evaluations

Safety will be evaluated by physical examinations, vital signs assessments, 12-lead ECGs, EEGs, routine clinical laboratory tests (including blood chemistry, hematology, coagulation, and urinalysis as described below), and AE assessments from baseline through study completion. Serious adverse events will be collected from the time of obtaining informed consent through the end of the follow-up phase.

If at any time during the treatment phase of the study, in the opinion of the investigator, a finding of an AE precludes continuation of treatment with study drug at the dose level at which the AE was observed, the dose and/or dose regimen for the study drug may be reduced or terminated altogether at the discretion of the investigator. The investigator will inform the sponsor of any discontinuations of treatment with vatiquinone and the DSMB will be notified of all AEs and SAEs. The investigator may solicit advice of the sponsor at any time to ensure the safety and well-being of participating subjects.

7.4.1. Physical Examination, Height, Weight, and Vital Signs

Complete physical exams will be conducted (excluding genital/rectal exam) at protocol specified study visits. The physical examination will consist of an examination of the following: general appearance, skin, head, eyes, ears, nose, throat, neck (including thyroid), lymph nodes, chest, heart, abdomen, and extremities.

Medical history and demographics including age, gender, and race, will be collected at the Screening Visit. Weight (lb or kg) will be measured in ordinary indoor clothing (ie, street clothes, scrubs, etc) with shoes off. Height will be recorded in centimeters or inches.

If clinically significant findings, as determined by the investigator, are recorded for a particular symptom, sign, or abnormal measurement, that measurement will be repeated at medically appropriate intervals until the value returns to an acceptable range, a specific diagnosis is established, or the condition is otherwise explained.

Vital sign measurements (pulse rate, blood pressure, and respiration rate) will be obtained in the sitting position (after the subject has been sitting for 5 minutes, if the subject is capable of sitting for 5 minutes).

7.4.2. Laboratory Evaluations

The maximum total quantity of blood that will be collected during a subject's participation in the MIT-E study will be approximately 100 mL for subjects weighing 10 kg or more and approximately 88 mL for subjects weighing less than 10 kg. The maximum volume that will be collected per visit will be approximately 8 mL for safety laboratory assessments and 18 mL during PK visits for subjects weighing 10 kg and over.

The maximum volume that will be collected per visit will be approximately 8 mL for safety laboratory assessments and approximately 12 mL during PK visit for subjects weighing less than 10 kg.

The maximum total volume (if a subject is rescreened) that could be withdrawn is approximately 107 mL in the case of a subject weighing 10 kg or more and approximately 95 mL in the case of a subject weighing less than 10 kg.

Refer to Section 7.1 (Table 2), Section 7.1.2.1, Section 7.1.2.8 , and Section 7.3 for modified PK collection for subjects weighing less than 10 kg. Urine will also be collected at specified timepoints for analysis.

The following variables will be collected at various times to assess safety.

7.4.2.1. Hematology

1. Erythrocytes: red blood cell count, hematocrit, hemoglobin, mean corpuscular volume
2. Leukocytes: white blood cell count and differential (basophils, eosinophils, lymphocytes, monocytes, and neutrophils)
3. Platelets: platelet count

7.4.2.2. Coagulation Panel

1. PT with INR and PTT

7.4.2.3. Serum Chemistry

1. Liver: Alkaline phosphatase, ALT (serum glutamic-pyruvic transaminase), AST (serum glutamic-oxaloacetic transaminase), bilirubin (total, direct), gamma-glutamyl transferase, and lactic dehydrogenase
2. Renal: blood urea nitrogen and creatinine
3. Electrolytes: sodium, potassium, chloride, carbon dioxide, calcium, magnesium, phosphate
4. General: total protein, albumin, glucose
5. Lipids: cholesterol (total) and triglycerides

7.4.2.4. Urinalysis

1. pH
2. Protein
3. Glucose
4. Ketones
5. Blood
6. Bilirubin

7.4.3. Urine or Serum Pregnancy Test

Both men and women should be counseled to abstain from sexual activity or use an acceptable method of birth control (Section 7.5.10) while participating in this clinical research study and for 30 days afterwards. They are required to use acceptable methods of birth control. Female subjects who become pregnant should be immediately discontinued from the study if they have not yet received study drug. If a subject is found to be pregnant after she has received study drug, she should discontinue dosing, complete all end of study procedures, and be followed to determine the outcome of the pregnancy. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. See Section 7.5.11 for rules regarding reporting pregnancies.

Human chorionic gonadotropin beta subunit will be measured on all female subjects of childbearing potential at Screening, Baseline, Weeks 4, 8, and 12 and every 12 weeks thereafter throughout the study. If a subject cannot perform a urine pregnancy test at any timepoint, a serum pregnancy test should be performed. At-home urine pregnancy test kits will be provided; subjects will be required to perform the test at home 30 days after the last dose of study drug and self-report the results via telephone.

7.4.4. Laboratory Abnormalities

Laboratory values will be collected throughout the study to assess for safety. The investigator must review and assess all laboratory results in a timely manner, and determine whether the abnormal laboratory values, if any, are clinically significant or not clinically significant, and whether there are associated signs and symptoms. The investigator must make the determination whether the clinically significant abnormal laboratory values are AEs.

An abnormal laboratory finding in absence of any other signs or symptoms is not necessarily an AE. If the abnormal laboratory finding is accompanied by signs or symptoms, report the signs and symptoms as the AE in lieu of the abnormal laboratory value. If a diagnosis is available, report the diagnosis. Clinically significant laboratory abnormalities after taking study medication that reflect a meaningful change from the screening value(s) and that require active management are to be considered by the investigator as AEs (eg, abnormalities that require study treatment dose modification, discontinuation, more frequent follow-up assessments, etc).

7.4.4.1. Grade 3 or 4 Laboratory Abnormalities

Diagnoses associated with any CTCAE Version 5.0 Grade 3 or 4 laboratory abnormalities will be recorded as AEs on the eCRF. Repeated and verified laboratory tests that meet at least

Grade 3 AE requirements will be reported to the Institutional Review Board/Institutional Ethics Committee (IRB/IEC) per institutional requirements. The recorded AEs should indicate the underlying abnormality or diagnosis (eg, renal insufficiency) if known, as opposed to the observed deviation in laboratory results (eg, elevated creatinine). Any additional relevant laboratory results obtained by the investigator during the study will be supplied to the sponsor.

7.4.5. Dose-Limiting Toxicity

No dose-limiting toxicities or other drug-related laboratory abnormalities were reported in prior clinical studies. In preclinical studies, prolongation of PT was identified as the NOAEL. Coagulation parameters (PT [can be measured as INR] and PTT) will be monitored throughout this study.

7.4.6. Dose Modification Guidelines and Stopping Rules

In the event that any subject develops any AE ≥Grade 3 per CTCAE criteria version 5.0 in severity that is deemed related to treatment with study drug and not related to the underlying disease, the Principal Investigator should contact PTC's medical monitor to discuss dose modification options up to and including discontinuation of the administration of study drug.

In the event that any subject develops or is diagnosed with an allergy to vatiquinone or sesame oil after initiating treatment with study drug, administration of study drug will be discontinued.

In the event that any subject develops elevated INR ≥Grade 2 in severity (>1.5×ULN) per CTCAE criteria version 5.0 thought to be related to treatment with study drug, administration of study drug will be reduced to 66%. Should the INR toxicity not resolve to at least Grade 1 in severity within 2 weeks of the initial dose reduction, administration of study drug may either be further reduced or discontinued (Table 3).

Once a subject's dose is reduced due to elevated INR thought to be related to study drug administration, their dose should not be re-escalated.

Table 3: Dose Modification and Stopping Rules for Vatiquinone

Qualifying Event	Example Guidelines for Management		
	Recommendation	**If Resolved to ≤Grade 1**	**If *Not* Resolved to ≤Grade 2 After 14 Days**
INR ≥Grade 2 or >1.5xULN thought to be related to study drug and confirmed within 24-48 hours	Reduce dose by 1/3 from TID to BID Re-evaluate 7 days after dose reduction	Continue on-study at 2/3 dose.* *Once a subject is dose reduced, do not re-escalate.	Consider withdrawing the subject after consultation with the study medical monitor.

Abbreviations: AE, adverse event; DSMB, Data Safety Monitoring Board; INR, International Normalized Ratio; ULN, upper limit of normal
The investigator is responsible for decisions on dose holds, reductions, or interruptions for any clinically significant AE. The DSMB is responsible for determining dose modification or stopping actions for any other safety event.

7.4.7. Safety Guidance

PTC may request that the investigator perform or arrange for the conduct of supplemental measurements and/or evaluations. If a subject dies during participation in the study or during a recognized follow-up period, PTC should be provided with a copy of any post-mortem findings, including histopathology.

7.5. Adverse Events and Serious Adverse Events

7.5.1. Definition of Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered related to the drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease in a study subject who is administered study drug in this study.

For this protocol, untoward medical occurrences that should be reported as AEs include the following:

- All AEs during the course of treatment with study drug administration
- All AEs resulting from medication misuse, abuse, withdrawal, or overdose, of study drug
- All AEs resulting from medication errors such as dispensing or administration error outside of what is described in the protocol
- Apparently unrelated illnesses, including worsening of a preexisting illness
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as 2 separate AEs. The outcome of the accident (hip fracture secondary to the fall) should be recorded in source documents.
- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test)
- Laboratory or ECG abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities associated with a clinical event should be captured in the source documents. Laboratory abnormalities not requiring clinical intervention or further investigation will be captured as part of overall laboratory monitoring, and should not be reported as AEs.
- A preexisting condition (eg, allergic rhinitis) must be noted on the appropriate eCRF, but should not be reported as an AE unless the condition worsens or episodes increase in frequency during the AE reporting period. Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that occurs during the treatment with study drug should be reported as the AE and the resulting appendectomy should be recorded in the source documents and eCRF. If a surgical procedure was planned prior to entry into the study, and the surgery is not performed because of a worsening of a baseline condition, this should not be reported as an AE. Note that, as described in Section 7.5.2 any hospitalization occurring as the consequence of an AE during the study period should be reported as an SAE.

Each AE is to be classified as serious or nonserious by the investigator using medical and scientific judgment.

7.5.2. Definition of Serious Adverse Events

An SAE is an untoward medical occurrence or effect associated with the use of a study drug at any dose, regardless of whether it is considered to be related to the study drug, which results in one of the following:

- Results in death. This includes all deaths on treatment or within 30 days after last study drug administration, including deaths due to disease progression. Any death occurring later than 30 days following the last dose need not be reported as an SAE unless it is a result of an event that started within the period covered by the on-study definition. The reported AE should be the event that caused the death. In addition, any AE resulting in death that occurs subsequent to the AE reporting period and that the investigator assesses as possibly related to the study drug should also be reported as serious.
- Is life-threatening. This refers to an event in which the subject was at risk of death at the time of the event. It does not include an event that, had it occurred in a more severe form, hypothetically might have caused death.
- Requires hospitalization or prolongation of existing hospitalization (excluding hospitalizations for administration of the study drug, procedures required by the study protocol, or treatment-related diagnostic procedures; other planned hospitalizations; or hospitalizations related only to progression of disease).

  Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

  Additionally, hospitalization should be used as an indicator of the seriousness of the AE and should only be used for situations where the AE truly fits this definition and not for hospitalizations associated with less serious events (eg, a hospital visit where a subject is admitted for observation or minor treatment [eg, hydration] and released in less than 24 hours). Furthermore, hospitalization for pharmacokinetic sampling, is not an AE, and therefore is not to be reported either as a routine AE or in an expedited report.
- Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions.
- Any other medically important event that the investigator or the sponsor judges to be serious or which is defined as serious by the regulatory agency in the local country. These are AEs that might not be immediately life-threatening or result in death or

hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Medical judgment should be exercised in deciding whether an AE is serious based on above definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

- A pregnancy resulting in spontaneous abortion, stillbirth, neonatal death, or congenital anomaly (including that in an aborted fetus).

Serious adverse events will be assessed from the time of the Screening Visit through 30 days after the end of the subject's participation in the study (last dose) and should be reported to the sponsor if the investigator becomes aware of them.

7.5.3. Unexpected Adverse Events

The IB contains the Reference Safety Information (RSI), which will be used for assessing expectedness. If an event is not listed in the RSI, it should be considered unexpected or if the AE occurs at a greater severity, specificity, or frequency, it should be considered unexpected.

7.5.4. Eliciting Adverse Event Information

The investigator is to report all directly observed AEs and all AEs spontaneously reported by the study subject/parent(s)/legal guardian/legally acceptable representative. In addition, each study subject/parent(s)/legal guardian/legally acceptable representative will be questioned about AEs at each scheduled clinic visit after study drug administration or during any telephone contact with the subject/parent(s)/legal guardian/legally acceptable representative. The type of question asked should be open-ended, for example, "*How have you been feeling*?" or a similar type of query.

7.5.5. Recording Nonserious AEs and SAEs

All AEs (both serious and nonserious) that occur in subjects during the AE reporting period must be recorded, whether or not the event is considered drug-related. In addition, any known untoward event that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational drug/product should also be recorded as an AE.

All AEs are to be recorded in the source documents and on the eCRF using concise medical terminology; whenever possible, terms contained in the Medical Dictionary for Regulatory Activities should be employed. In addition, the following information should be recorded:

- Indication of whether the event is serious or nonserious (see Section 7.5.2)
- Relationship to study drug (see Section 7.5.6)
- Severity of the event (see Section 7.5.7)
- Onset date
- Resolution date, or date of death
- Action taken
- Outcome of the event

Classification of the event as serious or nonserious determines the reporting procedures to be followed.

7.5.6. Describing Adverse Event Relationship to Study Drug

The investigator should provide an assessment of the relationship of the AE to the study drug, ie, whether there is a reasonable possibility that the study drug caused the AE, using the considerations outlined in Table 4.

Table 4: Relationship of Study Drug to Adverse Event Relationship

Relationship	Description
Probable	A clinical event in which a relationship to the study drug seems probable because of such factors as consistency with known effects of the drug; a clear temporal association with the use of the drug; improvement upon withdrawal of the drug; recurrence upon rechallenge with the drug; lack of alternative explanations for the event.
Possible	A clinical event occurring coincident with administration of the study drug and which may or may not be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal or rechallenge may be lacking.
Unlikely	A clinical event with a temporal relationship to the study drug exposure that does not preclude causality but for which there is a clear alternate cause that is more likely to have caused the AE than study drug. Such alternatives include a concomitantly administered drug, the subject's disease state, other medical conditions, or environmental factors.
Unrelated	A clinical event, for which a relationship to the study drug seems improbable because of factors such as inconsistency with known effects of the study drug, lack of a temporal association with study drug administration, lack of association of the event with study drug withdrawal or rechallenge, and/or presence of alternative explanations for the event. Alternative explanations might include a known relationship of the AE to a concomitant drug, medical history of a similar event, the subject's disease state, other medical conditions, or environmental factors.

Abbreviation: AE, adverse event

7.5.7. Grading of Severity of Adverse Event

The severity of AE will be graded using CTCAE Version 5.0 (refer to the Study Manual). For each episode, the highest severity grade attained should be reported.

If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the AE. For purposes of consistency with the CTCAE, these intensity grades are defined in Table 5.

Table 5: Grading of Adverse Event Severity Grade

Grade	Adjective	Description
Grade 1	Mild	Sign or symptom is present, but it is easily tolerated, is not expected to have a clinically significant effect on the subject's overall health and well-being, does not interfere with the subject's usual function, and is not likely to require medical attention
Grade 2	Moderate	Sign or symptom causes interference with usual activity or affects clinical status, and may require medical intervention
Grade 3	Severe	Sign or symptom is incapacitating or significantly affects clinical status and likely requires medical intervention and/or close follow-up
Grade 4	Life-threatening	Sign or symptom results in a potential threat to life

Grade 5	Fatal	Sign or symptom results in death

7.5.8. Adverse Event Reporting

Investigator site reporting requirements for AEs are summarized in Table 6.

Table 6: Investigator Site Requirements for Reporting Adverse Events

Event	Recorded on the eCRF	Reported on the SAE/Pregnancy Report Form to PTC Pharmacovigilance Within 24 Hours of Awareness
Serious AE	All	All
Non-Serious AE	All	None
Exposure to the study drug during pregnancy or breastfeeding, and occupational exposure	All (regardless of whether associated with an AE), except occupational exposure	Exposure during pregnancy, exposure via breastfeeding, occupational exposure (regardless of whether associated with an AE)

Abbreviations: AE, adverse event; eCRF, electronic case report form; SAE, serious adverse event

All AEs should be followed up by the investigator until they are resolved, or the investigator assesses them as chronic or stable. The investigator should consider protocol guidelines and use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. In the event of additional investigations, the PTC Pharmacovigilance Department or designee should be informed via e-mail or fax. A subject withdrawn from the study because of an AE must be followed by the investigator until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. Follow-up may need to continue after the subject has discontinued from the study, and additional investigations may be requested by the PTC medical monitoring team.

The first day of AE reporting will coincide with the date of signing of informed consent and including a minimum of 30 calendar days after the last administration of study drug.

7.5.9. Serious Adverse Event Reporting

All SAEs occurring from the Screening Visit through 30 days following the last dose of study drug should be reported via the SAE report form to PTC within 24 hours of becoming aware of the event(s). In addition, the AE portion of the eCRF must also be completed.

The SAE report form should be signed by the investigator; however, if the investigator is unable to sign at the time of the event or within 24 hours, the form should be signed by the clinical staff member reporting the SAE (eg, the study coordinator). The SAE report form must be faxed or e-mailed to the PTC Pharmacovigilance Department or designee and to the site IRB/IEC (if required by local regulations) within 24 hours.

Follow-up information to the SAE should be clearly documented as “follow-up” in the SAE report form and must also be faxed or e-mailed to the same party. All follow-up SAE report forms for the event must be signed by the investigator. Any source documents (eg, progress notes, nurses’ notes, laboratory and diagnostic test results, discharge summaries) provided to the sponsor should be redacted so that the subject’s name, address, and other personal identity information are obscured. Only the subject’s study number and initials are to be provided (in regions where the provision of such information is permitted). The information in the AE portion of the eCRF and the SAE report form(s) must match or be reconciled. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should be used on both forms.

In the rare event that the investigator does not become aware of the occurrence of an SAE immediately (for example, if a subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and to document his/her first awareness of the AE.

The PTC Pharmacovigilance Department contact information for reporting SAEs is provided below. This information is also provided in the Study Manual and in the SAE report form.

PTC Therapeutics Safety Department
Attention: Pharmacovigilance
E-mail: Pharmacovigilance@ptcbio.com
Facsimile: 1 (908) 325-0355

7.5.10. Contraception

Women of childbearing potential includes any female who has experienced menarche and who has not undergone successful surgical sterilization (eg, hysterectomy, bilateral tubal ligation, bilateral oophorectomy) or is not postmenopausal. Postmenopausal is defined as ≥12 months with no menses without an alternative medical cause. Women who are using an active method of birth control, who are practicing abstinence or whose partner is sterile (eg, vasectomy), are considered to be WOCBP.

Males subjects and subjects who are WOCBP must use 2 forms of effective contraception simultaneously from the time consent is signed until 30 days after treatment discontinuation in a manner such that risk of failure is minimized. Periodic and/or temporary abstinence such as declaration of abstinence during study participation or fertility awareness- based methods to prevent pregnancy (including but not limited to symptothermal and ovulation estimation by either calendar day or salivary/cervical secretions) are not considered effective methods of birth control; however, true (absolute) sexual abstinence (ie, in line with the preferred and usual lifestyle of the subject) may be permitted. Effective methods of birth control approved for use in this study are as follows:

- Implants (eg, Norplant® system)
- Injectable (eg, Depo-Provera®)
- Transdermal patch
- Combined oral contraceptives
- Barrier methods (condoms and diaphram with spermicide) - note: double barrier method is required if no other methods of birth control are in use.
- Intrauterine devices (eg, ParaGard®, Mirena®)

Prior to study enrollment, male subjects and WOCBP must be advised of the importance of avoiding pregnancy during trial participation. During the trial, all WOCBP will be instructed to contact the investigator immediately if they suspect they might be pregnant (eg, missed or late menstrual period).

7.5.11. Reporting Pregnancy

PTC should be notified in the event that a female subject in the study, or a female partner of a male subject in the study, becomes pregnant on-study or within 30 days of the last administration of study drug and must be reported on a Pregnancy Notification Form (see Study Manual for details).

This must be done whether or not an AE has occurred and within 24 hours of awareness of the pregnancy. The information submitted should include the anticipated date of birth or pregnancy termination.

Written consent is required prior to collecting and reporting any information on a female partner of a male subject in the study.

If possible, the investigator should follow the subject, or the pregnant female partner of a male subject, until completion of the pregnancy and notify the PTC medical monitor of the outcome within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial Pregnancy Notification Form via the Pregnancy Outcome Form (see the Study Manual for details).

If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (ie, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus]), the investigator should follow the procedures for reporting SAEs, ie, report the event to the PTC Safety Department or designee and follow-up by submission of appropriate AE eCRFs (see Section 7.5.9).

7.5.12. PTC Therapeutics Safety Reporting Requirement

In compliance with local requirements, PTC shall immediately notify the investigators of any new or emerging safety information, which impacts the benefit-risk ratio of the drug, once confirmed by the executive safety review board. This will include any urgent measures that need to be taken with respect to the protocol.

As the sponsor of the study, PTC is responsible for reporting certain safety information, particularly suspected unexpected serious adverse reaction (SUSAR) and other significant findings, as appropriate per local reporting requirements, to each investigator in an expedited manner. If notification of a SUSAR requiring expedited reporting to investigators is received, PTC or its designated representative will contact each investigational site participating in this study by e-mail, fax, and/or overnight mail such that the investigator can promptly notify the site IRB/IEC per their local requirements. The initial expedited safety report will be provided as required according to local regulations (eg, within 15 days) after the earliest date PTC or an agent of PTC (eg, a site monitor) becomes aware of an SAE. This awareness date is the date the regulatory reporting clock begins, and the date is considered Day 0.

8. STATISTICAL CONSIDERATIONS

This is a randomized, placebo-controlled trial of vatiquinone in pediatric subjects with genetically confirmed mitochondrial disease and associated refractory epilepsy. The primary endpoint of this study will be the percent change from baseline in the frequency of observable motor seizures per 28 days in the placebo-controlled phase.

A formal statistical analysis plan (SAP) will be finalized before the unblinding of the study. Detailed discussion of statistical methods, including estimand and missing data handling, will be specified in the SAP.

8.1. Statistical Hypotheses

The null hypothesis is that there is no difference in the effect of vatiquinone versus placebo in percent change from baseline in observable motor seizures per 28 days during the double-blind period.

8.2. Sample Size Determination

It is planned to randomize 30 subjects per arm in this study. Sixty subjects (30 subjects in each treatment group) will provide a power of 81% to detect a 40% treatment difference with common standard deviation of 50% at a two-sided significance level of 0.05 based on the Wilcoxon Rank-Sum Test. The 40% treatment difference is based on 10% and 50% decrease in the placebo treatment group and PTC743 treatment group, respectively, for percent change from baseline in motor seizure frequency per 28 days.

8.3. Population for Analyses

- Intent-to-treat (ITT) population will include all subjects who were randomized and received at least one dose of treatment. In the event that a subject received study drug different from the one to which he/she was randomized, the subject's efficacy data will be analyzed "as randomized." ITT Analysis population will be used in the statistical analyses of the efficacy endpoints.
- Safety population will include subjects who were randomized and received at least one dose of treatment. In the event that a subject received study drug different from the one to which he/she was randomized, the subject's safety data will be analyzed "as treated." The Safety Analysis population will be used in the statistical analyses for safety.

8.4. Statistical Analyses

8.4.1. General Approach

For continuous variables, median, mean, standard deviation, minimum, maximum, and number of subjects with non-missing data will be provided for each treatment group, and for overall treatment group for assessment prior to randomization. For categorical variables, the number (percent) of subjects in each category will be provided.

In general, data will be presented by treatment groups separately for double-blind period and open-label extension period. In addition, vatiquinone experience from both double-blind and open-label extension period will be combined and summarized. In this summary, baseline for subjects on placebo in the double-blind period will be the last observation prior to the first vatiquinone dose.

8.4.2. Analysis of Primary Efficacy Endpoints

Efficacy analyses will be based on the ITT population. Seizure frequency will be based on the number of seizures per 28 days, calculated as (the number of seizures over the time interval multiplied by 28) and divided by the number of days in the interval. Only "valid days" will be used in the calculations of seizures frequency per 28 days. A "valid day" is defined as the day where seizure counts information is present.

The percent change in the motor seizure frequency per 28 days in the double-blind period will be the primary efficacy endpoint.

For this analysis, first both the pre-randomization period motor seizure frequencies per 28 days as well as percent change in the double-blind period per 28 days will be rank transformed separately. The analysis of covariance will then be conducted on this rank transformed data with treatment, and stratifying factor (4 levels) and the ranked pre-randomization phase seizure frequency per 28 days as a covariate. P values will be computed using contrasts between active and placebo treatment groups.

Due to an expected irregular distribution of seizure frequency, median will be the primary statistic of interest for the primary endpoint. Hodges-Lehmann estimator and 95% confidence interval for this estimator will be displayed for understanding the treatment effect size.

A sensitivity analysis will be performed excluding all subjects with <25 days of valid days entered in the diary.

8.4.3. Analysis of Secondary Efficacy Endpoints

Change from baseline in number of disease-related hospitalization days per 28 days and change from baseline in occurrence/recurrence of status epilepticus per 28 days are the two key secondary endpoints. The treatment groups will be compared using Wilcoxon Rank-Sum Test. Number of disease-related hospitalization days and number of occurrence/recurrence of status epilepticus will be tabulated by frequency and percent as well.

Percent change from baseline in total seizure frequency per 28 days will be analyzed in a similar way to the primary endpoint.

Response rates (25%, 50%, 75%, and 100%) for motor seizure and total seizure will be tabulated by treatment groups and treatment will be compared by Cochran-Mantel-Haenszel test adjusting for stratifying factor.

Number of disease-related emergency room visits and in-patient hospitalizations, number of rescue seizure medications, and seizure clusters will be tabulated by frequency and percent. In addition, change from baseline in disease-related emergency room visits and in-patient hospitalizations per 28 days, and change from baseline in seizure clusters per 28 days will be summarized.

Health-related quality of life, as measured by the CarerQoL-7D questionnaire, will be summarized descriptively.

8.4.4. Analysis of Exploratory Endpoint

Health-related quality of life, as measured by the PedsQL, will be summarized descriptively

8.4.5. Multiplicity Adjustment

A sequential method will be used to adjust for multiple comparison. The primary endpoint will be first tested at 0.05 level. If the p-value is less than 0.05 the first key secondary endpoint, number of hospitalization days, will be tested at 0.05 level. If the p-value for the primary endpoint is >0.05 then the number of hospitalization days will not be tested. The same procedure will be applied to the endpoint of occurrence or recurrence of status epilepticus.

8.4.6. Safety Analyses

8.4.6.1. Subject Disposition

The disposition of subjects, including the number of subjects screened, the number of randomized subjects, the number of randomized subjects who received at least 1 dose of study drug, and the number of subjects who prematurely discontinue study drug as well as the reason for the premature termination will be tabulated. The number of subjects enrolled in the open-label extension study will also be tabulated.

8.4.6.2. Medical History and Prior Medication

Medical history and prior medication information will be summarized.

8.4.6.3. Extent of Exposure and Treatment Compliance

The extent of exposure to vatiquinone treatment is defined as the last dose date minus the first dose date +1 day. Compliance will be assessed in terms of the percentage of drug actually taken relative to the amount that should have been taken during the study. Exposure and compliance will be summarized descriptively.

8.4.6.4. Adverse Events

Summary information (the number and percent of subjects by treatment) will be tabulated for:

- Treatment-emergent adverse events (TEAEs)
- Treatment-related AEs
- TEAEs by severity
- SAEs
- AEs leading to discontinuation

Summaries will be presented by treatment groups and categorized by System Organ Class and Preferred Term. The frequencies of AEs displayed will be the crude rates that represent the number of subjects experiencing AEs divided by the total number of subjects.

8.4.6.5. Laboratory Parameters

Changes in clinical laboratory tests from Baseline (last measurement prior to randomization) and laboratory marked abnormalities using pre-defined abnormality criteria will be descriptively summarized.

8.4.7. Baseline Descriptive Statistics

Demographic and baseline characteristics of subjects will be summarized descriptively by means and standard deviations for continuous variables, and frequency distribution for categorical variables. Summaries will be performed based on all randomized subjects.

8.4.8. Planned Analyses

Two analyses will be conducted, Period 1 at Week 24 (the end of placebo-controlled period) and Period 2 at Week 72 (the end of long-term extension period), respectively. All formal statistical conclusions will be drawn from data collected in the double-blind treatment period, and all statistical type I error will be spent on the hypothesis tests performed on these data.

8.4.9. Sub-group Analyses

Percent change from baseline in motor seizure frequency per 28 days, percent change from baseline in total seizure frequency per 28 days, and response rates (25%, 50%, 75%, and 100%) for motor seizure and total seizure will be summarized descriptively by disease subtype (Alpers/POLG; Leigh syndrome; MELAS; and Other).

8.4.10. Exploratory Analyses

No exploratory analyses are planned.

9. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1. Regulatory, Ethical, and Study Oversight Considerations

9.1.1. Informed Consent Process

By signing the protocol, the investigator assures that informed consent/assent will be obtained from each subject prior to study entry and that the informed consent/assent will be obtained in accordance with current regulations.

The investigator or sub-investigator will give each subject full and adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. An informed consent/assent document will be provided to each subject in a language in which the subject is fluent. This information must be provided to the subject prior to undertaking any study-related procedure. Adequate time should be provided for the subject to read the informed consent, to understand the risks and benefits of participating in the study, and to ask any questions that the subject may have about the study. The subject should be able to ask additional questions as and when needed during the conduct of the study. The subject's signature on the informed consent form should be obtained at the investigator site in the presence of the investigator or a qualified representative (eg, sub investigator). Where applicable, the subject will sign an age-appropriate assent form.

Each subject will be given a copy of the signed consent/assent form. The original signed informed consent forms will be retained by the investigator with the study records.

The written subject information must not be changed without prior approval by PTC and the IRB/IEC.

9.1.2. Study Discontinuation and Closure

PTC reserves the right to discontinue the study prior to inclusion of the intended number of subjects. The investigator, after consultation with the PTC medical monitor, reserves the right to discontinue the study at the investigator site for safety reasons at any time.

After a decision to terminate the study, investigators must contact all subjects who are continuing their participation in the study and must do so within a time period set by PTC. As directed by PTC, all study materials must be collected and all electronic data entry forms completed to the greatest extent possible.

9.1.3. Confidentiality and Privacy

Research records will be collected and stored in a manner that protects the confidentiality of subject information. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs, paper CRFs, or other records provided to or retained by PTC (or its authorized designee). The names and identities of the subjects need not be divulged; however, the records must nevertheless be inspected. This will be accomplished by blanking out the subject's name and replacing the name with the subject's study identification number on any record provided to or retained by PTC. The informed consent form must include appropriate statements explaining these requirements.

By signing this protocol, the investigator affirms to PTC that the investigator will maintain, in confidence, information furnished by PTC and will divulge such information to the IRB/IEC under an appropriate understanding of confidentiality with such board.

9.1.4. Future use of Stored Specimens and Data

As part of the current study, blood, plasma, and serum will be collected and stored.

Sample processing will be performed by a laboratory under the direction of the sponsor. Processing, analysis, and storage will be performed at a secure laboratory facility to protect the validity of the data and maintain subject privacy.

Samples will only be used for the purposes described in this protocol. Laboratories contracted to perform the analysis on behalf of the sponsor will not retain rights to the samples beyond those necessary to perform the specified analysis and will not transfer or sell those samples. The sponsor will not sell the samples to a third party.

Samples will be stored until the completion of the study (defined as submission of the clinical study report to the appropriate regulatory agencies). At the end of the storage period, samples will be destroyed. Samples may be stored longer if a health authority (or medicinal product approval agency) has active questions about the study. In this special circumstance, the samples will be stored until the questions have been adequately addressed.

No subject-identifying information (eg, initials, date of birth, government identifying number) will be associated with any of the biologic samples. All samples will be single coded. The sponsor will take steps to ensure that data are protected accordingly, and confidentiality is maintained as far as possible. Data from subjects enrolled in this study may be analyzed worldwide, regardless of location of collection.

The sponsor and its representatives and agents may share coded data with persons and organizations involved in the conduct or oversight of this research. These include:

- Contract Research Organizations (CROs) retained by the sponsor
- IECs or IRBs that have responsibility for this research study
- National regulatory authorities or equivalent government agencies

At the end of the analysis, results may be presented in a final report which can include part or all of the coded data, in listing or summary format. Other publication (eg, in peer-reviewed scientific journals) or public presentation of the study results will only include summaries of the population in the study, and no identified individual results will be disclosed.

Given the research nature of the planned analyses, it will not be possible to return individual data to subjects. The results that may be generated are not currently anticipated to have clinical relevance to the subjects or their family members. Therefore, these results will not be disclosed to the subjects or their physicians.

9.1.5. Safety Oversight

External oversight for this trial will be provided by a DSMB. The primary responsibility of the DSMB is to protect the safety and welfare of subjects participating in this clinical trial and to ensure the integrity of the clinical trial.

Specifically, for this study, the DSMB will be responsible for:

- Examining accumulated safety data, PK, and compliance data in order to make recommendations concerning continuation, termination, or modification of the trial based on the safety of the interventions under study
- Reviewing major study design modifications proposed by PTC or the investigators prior to implementation of those modifications
- Reviewing the general progress of the study as regards accrual, protocol violations, and study conduct

The DSMB may review the safety data at any time as warranted by emerging results. Based on review of the safety data, the DSMB can recommend continuation of the study unchanged, study interruption, study termination, modification of the trial, or alteration in the DSMB monitoring plan. Further information regarding the DSMB review process is provided in the DSMB charter (see Study Manual).

9.1.6. Clinical Monitoring

In accordance with 21 Code of Federal Regulations Part 312.56 and/or relevant International Council for Harmonisation guidelines, PTC or a designee will periodically inspect all eCRFs, study documents, research facilities, and clinical laboratory facilities associated with this study at mutually convenient times, before, during, and after completion of the study. As required by applicable regulations (Responsibilities of Sponsors and Investigators), the monitoring visits provide PTC with the opportunity to evaluate the progress of the study; verify the accuracy and completeness of data in the eCRFs; ensure that all protocol requirements, relevant regulations, and investigator's obligations are being fulfilled; and resolve any inconsistencies in the study records. This includes inspection of all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the subjects in this study. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs or other records provided to or retained by PTC. The investigator/institution guarantees direct access to source documents by PTC and appropriate regulatory authorities.

It is important that the investigator and relevant institutional personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

9.1.7. Quality Assurance and Quality Control

To ensure compliance with Good Clinical Practices (GCP) and all applicable regulatory requirements, PTC, PTC's representatives, a regulatory authority and/or IRB may conduct a quality assurance audit. Reasons for quality assurance audit may include but are not limited to: random selection, geographic proximity, suspected GCP violation, high enrolling site, recurring protocol deviations, etc. The purpose of a sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the International Council for Harmonisation, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

9.1.8. Data Handling and Record Keeping

To enable evaluations and/or audits from regulatory authorities or PTC, the investigator agrees to keep accurate and complete records, including the identity of all participating subjects (sufficient information to link eCRFs and clinic records/source documents), all original signed informed consent forms, electronic copies (ie, CD-ROM, USB, etc.) or paper copies of the data that have been captured in the electronic data capture for each subject (eCRFs), and detailed records of study drug disposition. All records and documents pertaining to the study will be maintained by the investigator until notification is received from PTC that the records no longer need to be retained.

The investigator must obtain written permission from PTC before disposing of any records. The investigator will promptly notify PTC in the event of accidental loss or destruction of any study records. If the investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to PTC as applicable.

9.1.9. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, investigator, or site staff. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc.-either tests not done, incorrect tests done, or not done within the time frame specified in the protocol

- Procedural deviations such as incorrect storage of study drug, failure to update the Informed Consent Form (ICF) when new risks become known, or failure to obtain IRB approvals for the protocol and ICF revisions

Major deviations are any deviations that impact subject eligibility (ie, protocol inclusion/exclusion violations), subject safety or a subject's ability to continue in the clinical trial.

At the outset of the study, a process for defining and handling protocol deviations will be established with the CRO. This will include determining which deviations will be designated major; thus, requiring immediate notification to the PTC medical monitor and the sponsor.

Prospective deviations (eg, protocol waivers) are prohibited per PTC policy.

The investigator is responsible for seeing that any known protocol deviations are recorded handled as agreed.

9.1.10. Publication and Data Sharing Policy

The information developed during the conduct of this clinical study is considered confidential by PTC. This information may be disclosed as deemed necessary by PTC.

PTC intends that the data from this study will be presented and published. The PTC staff under the direction of the PTC Chief Medical Officer or designee in collaboration with the investigator will be responsible for writing presentations and manuscripts for publication. Investigators will not be allowed to publish or present the data from this study without prior agreement with PTC.

The investigator is obliged to provide the sponsor with complete test results and all data derived by the investigator from the study. During the study, only the sponsor may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the Clinical Study Site Agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the sponsor.

The sponsor may publish any data and information from the study (including data and information generated by the investigator) without the consent of the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

Data from all sites participating in the study will be pooled and analyzed by the sponsor or the sponsor's designee. The first publication of the study results shall be made in conjunction with the results from other study sites as a multicenter publication. If a multicenter publication is not forthcoming within 24 months of completion of the study at all sites, the investigator may publish or present the results generated at his or her site.

The investigator will provide the sponsor with a copy of any proposed publication or presentation for review and comment at least 60 days prior to such presentation or submission for publication. The sponsor shall inform the investigator in writing of any changes or deletions in such presentation or publication required to protect the sponsor's confidential and proprietary technical information and to address inaccurate data or inappropriate interpretations in the context of any pooled multicenter results. At the expiration of such 60-day period, the investigator may proceed with the presentation or submission for publication unless the sponsor has notified the institution or the investigator in writing that such proposed publication or presentation discloses the sponsor's confidential and proprietary technical information. Furthermore, upon the request of the sponsor, the investigator will delay the publication or presentation for an additional 90 days to permit the sponsor to take necessary actions to protect its intellectual property interests.

9.2. Additional Considerations

Not applicable.

9.3. Protocol Amendment History

Version 1.0: 26 March 2019

Version 2.0: 27 March 2020

Version 3.0: 22 May 2020

Version 4.0: 30 September 2020

Version 4.1: 12 January 2021

Version 5.0: 31 March 2021

Version 6.0: 04 June 2021

Version 7.0: 06 January 2022

Version 8.0: 27 May 2022

9.3.1. Version 2.0: 27 March 2020

Overall reason for Version 2.0: The overall reason for Version 2.0 of the protocol was to change the name of the study sponsor, subsequently the drug name, and to incorporate health authorities' feedback on the previous version of the protocol.

Item No.	Protocol Section	Version 2/Update	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. Editorial revisions (eg, typographical error, punctuation, tenses, abbreviations) were incorporated to provide clarity. The sponsor name was updated from BioElectron to PTC and the protocol was moved into the PTC template. Standard template sections in the BioElectron template were replaced with those from the PTC template. The synopsis was updated to be consistent with changes in the protocol.	Update
2	Section 1.5.1	Anticoagulation in the nonclinical toxicology studies was added to explain the reason for monitoring coagulation parameters.	Clarification
3	Section 2.1.2	The secondary objectives were expanded to include the effects of vatiquinone on the monthly seizure count, the responder rate in motor and non-motor seizures, seizure clusters, and to change the quality of life questionnaire to the CarerQoL-7D.	Update
4	Section 2.2	Endpoints were updated to align with the objectives and 2 endpoints were designated key endpoints.	Update
5	Section 3.1.1	The Screening and Run-in Phase were combined, and clarification was added that the 28 days prior to Baseline Visit is the Run-in phase. Clarification was added that if subjects do not meet the entry criteria regarding number of seizures, they will be considered screen failures.	Clarification
6	Section 3.2.2	The number of study sites was increased from 10 to 12.	Update
7	Section 3.4	A section justifying the chosen dose was added.	Update
8	Section 4.2	Inclusion criteria were expanded for clarification.	Clarification
9	Section 4.3	INR ≥1.5 ULN and ongoing treatment with CYP inhibitors were included in the exclusion criteria.	Update
10	Section 4.4	Section added per PTC template.	Update
11	Section 4.5	Section added per PTC template.	Update
12	Section 6.3	Section added per PTC template.	Update
13	Section 7	The requirement for laboratory assessments to be performed under fasting conditions was removed.	Update
14	Section 7.3	Blood samples for PK assessments were detailed in this section and added to the schedule of events (Section 7.1) and wherever appropriate throughout the protocol.	Update
15	Section 7.5	This section was updated per PTC template, including definitions and standard reporting procedures for AEs and SAEs.	Update
16	Section 8	Section added per PTC template.	Update

Abbreviations: AE, adverse events; CYP, cytochrome P450; INR, International Normalized Ratio; PTC, PTC Therapeutics; SAE, serious adverse events; ULN, upper limit of normal

9.3.2. Version 3.0: 22 May 2020

Overall reason for Version 3.0: The overall reasons for Version 3.0 of the protocol were to add urinalysis assessment and an additional PK timepoint and to give additional training for seizure diaries, if needed.

Item No.	Protocol Section	Version 3/Update	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. Editorial revisions (eg, typographical error, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis was updated to be consistent with changes in the protocol. The study name (MIT-E) was added to the study title.	Update
2	Protocol Identifiers and Study Personnel	The EudraCT number was added.	Update
3	Section 2.1.2 and 2.2.3.1	Dose-limiting toxicities were removed from the secondary objectives and endpoints.	Update
4	Sections 3.1, 3.4, 5.1.1 and 7.1.2	The dose regimen description was clarified.	Clarification
5	Sections 3.1.1 and 7.1.1	An extension to the Screening period, at the discretion of the investigator, was added, if further training on seizure diaries in necessary.	Update
6	Section 4.2	Inclusion criterion “Subject or parent/legal guardian is able and willing to complete seizure diaries for the duration of the study” was added. Inclusion criterion #6 was amended to remove the word “symptoms” after epilepsy. Inclusion criterion #10 was amended to remove the 30-day window for historical EEG.	Update and clarification
7	Section 4.3	Exclusion criterion #9 was amended to prohibit the use of grapefruit juice and St John’s wort extract. Exclusion criterion #8 was added prohibiting treatment with idebenone.	Clarification and update
8	Section 4.6	Home Care Services section was added.	Update
9	Sections 7.1 and 7.4.2	Urinalysis assessments were added.	Update
10	Sections 7.1 and 7.3	PK assessment timepoints were changed to Predose, 1h, 3h, 4h, and 8h postdose.	Update
11	Section 7.1	ECG and EEG assessments were reduced. EEG will only be required at Screening if no historical EEG is available. A +7-day window was added to the Screening Visit.	Update
12	Section 7.1.9	Prohibition of cannabidiol containing therapies was removed. Grapefruit juice and St John’s wort extract were specifically added as CYP inducers or inhibitors.	Update
13	Section 7.4.2.4	Urinalysis parameters were detailed.	Update

Item No.	Protocol Section	Version 3/Update	Reason/ Rationale
14	Sections 7.4.5 and 7.4.6	Dose-limiting toxicity text was clarified and a window for confirmation of elevated INR was added.	Clarification

Abbreviations: CYP, cytochrome P450; ECG, electrocardiogram; EEG, electroencephalogram; INR, International Normalized Ratio; MIT-E, Mitochondrial Disease Subjects with Refractory Epilepsy

9.3.3. Version 4.0: 30 September 2020

Overall reason for Version 4.0: The overall reasons for Version 4.0 of the protocol were to revise the ECG collection schedule so as to time match it with the 4-hour postdose PK, to add PK samples to 4 hours postdose at Weeks 48 and 72, to revise the definition of compliance with study drug dosing, to separate the secondary endpoints into key and other endpoints, and refinement of statistical considerations.

Item No.	Protocol Section	Version 4/Update	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. Editorial revisions (eg, typographical error, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis was updated to be consistent with changes in the protocol. The study name (MIT-E) was added to the study title.	Update
2	Section 3.1.2	Number of subjects to be allocated to the Alpers/POLG (DNA polymerase subunit gamma); Leigh syndrome; and Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes categories revised from 15 subjects to 6 subjects each.	Update
3	Section 4.2	Clarification of inclusion criterion 4 to include mitochondrial disease secondary to either nuclear or mitochondrial DNA mutation with associated epilepsy phenotype. Clarification added to exclusion criterion 5 indicating that no changes to the AED regimen will be allowed during the first 24-week period of the study. Revision of inclusion criterion 8 to indicate stable dose regimen of antiepileptic therapies for 30 days (revised from 60 days) prior to the Baseline Visit Clarification that electroencephalogram at Screening or historical EEG for diagnostic confirmation of “epilepsy” (revised from “seizures”) will be required in inclusion criterion 10.	Update and clarifications
4	Section 4.3	Clarification that, in addition to grapefruit juice, grapefruit will also be excluded.	Clarification
5	Section 5.4	Revision of compliance from 80% - 100% to 80% - 120%.	Update

Item No.	Protocol Section	Version 4/Update	Reason/ Rationale
6	Section 7.1	Update to include return of study drug bottles and reconciliation with dosing diary at each visit from Weeks 4 to 72 Addition of new footnote (“d”): ECG measurement will be performed at 4h postdose, time-matched to PK blood collection. Revision of the EEG footnote (“e” to reflect EEG for epilepsy confirmation must be obtained if historical EEG confirmation of epilepsy is not provided. Addition to PK footnote for 4h postdose sample at Weeks 48 and 72.	Updates and clarification
7	Section 7.1, Section 7.1.2.1, Section 7.1.2.7, Section 7.3, and Section 7.4.2	Revisions to note modified PK sample collection for subjects weighing less than 10 kg	Update
8	Section 7.1.2.1, Section 7.1.2.7, and Section 7.1.3	Addition to ECG collection indicating that ECG will be obtained at 4 hours postdose during each visit, time-matched to PK blood collection.	Update
9	Section 7.1.2.2, Section 7.1.2.3, Section 7.1.2.4, Section 7.1.2.5, Section 7.1.2.6, Section 7.1.2.7, and Section 7.1.3	Addition of review of study drug diary to occur along with review of seizure diary	
10	Sections 7.1.3 and Sections 7.3	Addition to PK collection indicating that 4h postdose sample will be collected at Weeks 48 and 72.	Update
11	Section 7.1.9	Clarification that, in addition to grapefruit juice, grapefruit will also be excluded Addition of statement that no changes to the AED regimen will be allowed during the first 24-week period.	Update and clarification
12	Section 8	Revision of primary endpoint description to reflect percent change from baseline in the motor seizure frequency per 28 days in the double-blind period for consistency with Section 8.4.2. Revision to SAP language to indicate analysis to be completed prior to database lock instead of prior to last subject completing the placebo-controlled phase.	Update and clarification
13	Section 8.4.1	Addition of text regarding presentation of data by treatment groups for the double-blind and open-label extension periods.	Clarification
14	Section 8.4.2	Removal of text regarding other seizure frequency based continuous endpoints (to focus on primary endpoint only).	Clarification
15	Section 8.4.3	Revision of language regarding analysis of secondary efficacy endpoints to separate key secondary endpoints from the other secondary endpoints.	Clarification
16	Section 8.4.8	Addition of text to indicate that response rates for motor seizure and total seizure will be summarized descriptively by disease subtype.	Clarification

Abbreviations: AED, antiepileptic drug; ECG, electrocardiogram; EEG, electroencephalogram; PK, pharmacokinetics; POLG, polymerase subunit gamma; SAP, statistical analysis plan

9.3.4. Version 4.1: 12 January 2021

Overall reason for Version 4.1: The overall reasons for Version 4.1 of the protocol were to incorporate administrative letters, add a dedicated contraception section, and revise the study calendar to add in-person visits.

Item No.	Protocol Section	Version 4.1/Update	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. Editorial revisions (eg, typographical error, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis was updated to be consistent with changes in the protocol.	Update
2	Section 3.1.1 Section 7.1.1	Clarification added that the sponsor/PTC medical monitor will review patient seizure diary entries prior to the run-in phase	Clarification
3	Section 4.3	Revision of exclusion criterion 3 from INR ≥1.5×ULN to INR ≥ULN at the time of screening Addition to exclusion criterion 10 of cross-reference to Section 7.5.10	Update
4	Section 6.1	Addition of spontaneous bleeding and use of prohibited concomitant medications as criteria for discontinuation of study intervention Addition of cross-reference to Section 7.5.10 to discontinuation criterion 6 (refusal of sexually active fertile subjects [excluding subjects who have been sterilized] to use medically accepted methods of contraception)	Update
5	Section 7.1	Addition of a week 2 telephone visit and change from telephone to in-person visits at weeks 4 and 8 Removal of return of study drug bottles and reconciliation with dosing diary from visits at Weeks 28 and 32, Weeks 40 and 44, Weeks 52 and 56, and Weeks 64 and 68 (telephone visits) Revision of footnote d to clarify window for ECG collection Added clarification in footnote f that weights ending in decimals >0.5 should be rounded up to the next whole number for dosing calculation Addition of statement in footnote g that at-home pregnancy test will be provided to be taken at 30 day follow-up and self-reported via telephone Footnote j added to indicate that SAEs will be assessed from the time of the Screening Visit Footnotes k and l re-lettered to footnotes l and m, respectively	Updates and clarifications
6	Section 7.1.2.1	Addition to ECG assessment to indicate that ECG should be performed approximately within 10 minutes preceding the 4-hour PK blood collection Clarification that hospitalizations and hospital days for disease-related issues will be collected from the time of the Screening Visit	Clarification
7	Section 7.1.2.2	Week 2 visit added	Update
8	Section 7.1.2.3 Section 7.1.2.4	Revisions of Week 4 and Week 8 from telephone to in-person visits and related revisions of assessments	Updates
9	Section 7.1.2.2 through Section 7.1.2.7	Section numbers revised to 7.1.2.3 through 7.1.2.8 due to addition of new Section 7.1.2.2	Updates

Item No.	Protocol Section	Version 4.1/Update	Reason/ Rationale
10	Section 7.1.2.8	Addition to ECG assessment to indicate that ECG should be performed approximately within 10 minutes preceding the 4-hour PK blood collection	Clarification
11	Section 7.1.3	Addition to ECG assessment to indicate that ECG should be performed approximately within 10 minutes preceding the 4-hour PK blood collection Clarification that drug reconciliation of returned study drug bottles with dosing diary will occur every 12 weeks during the long-term extension phase	Clarification
12	Section 7.1.4	Addition of statement indicating that WOCBP will be required to perform a study-provided at-home pregnancy test 30 days after the last dose of study drug and self-report the results via telephone	Update
13	Section 7.1.9	Addition of the guidelines for use of CYP inducers during study treatment	Clarification
14	Section 7.4.3	Cross-reference to newly added Section 7.5.10 added Cross-reference to previous Section 7.5.10 updated to Section 7.5.11 Clarification added that pregnancy test will be conducted at Weeks 4.8, 12 and every 12 weeks thereafter. Addition of statement that at-home urine pregnancy test kits will be provided and subjects will be required to perform the test at home 30 days after the last dose of study drug and self-report the results via telephone	Updates
15	Section 7.5.2	Revision of language regarding reporting adverse events leading to hospitalization as SAEs Addition of statement that SAEs will be assessed from the time of the Screening Visit	Updates
16	Section 7.5.9	Clarification that SAEs will be collected from the Screening Visit through 30 days following the last dose of study drug	Clarification
17	Section 7.5.10	Addition of new section (Contraception)	Update
18	Section 7.5.12	Section 7.5.11 renumbered to Section 7.5.12 due to addition of new Section 7.5.10	Update

Abbreviations: CYP, cytochrome P450; ECG, electrocardiogram; INR, international normalized ratio; SAE, serious adverse event; ULN, upper limit of normal; WOCBP, women of childbearing potential

9.3.5. Version 5.0: 31 March 2021

Overall reason for Version 5.0: The overall reasons for Version 5.0 of the protocol were to add PedsQL questionnaire at Weeks 24 and 72; revise the number of study sites from approximately 12 to approximately 30; clarify that historical EEG may be within 6 months prior to the Screening Visit; revise inclusion and exclusion criteria to clarify genetic confirmation of mitochondrial disease, clarify use of AEDs, clarify AST and ALT range for subjects with underlying Alpers-Huttenlocher syndrome/POLG subtypes, and clarify exclusion of artisanal (non-Epidiolex cannabidiol) cannabidiol; add a weight-based dosing table for study drug; and clarify that the SAP will be finalized prior to unblinding of the study and revise the text regarding the sample size and statistical powering.

Item No.	Protocol Section	Version 5/Update	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. Editorial revisions (eg, typographical error, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis was updated to be consistent with changes in the protocol. Renumbering of Tables 1 through 6 to Tables 2 through 7 due to addition of a weight-based dosing table as Table 1.	Update
2	Section 1.4	Update of number of subjects who have been treated with vatiquinone and exposure (in dosing days)	Update
3	Section 1.4.1	Addition of brief summary of in vitro phototoxicity study	Update
4	Section 1.4.2	Update of number of patients who have been treated with vatiquinone and exposure (in dosing days) Revision of age range from 22 days to 70 years to <1 year to 70 years Revision of largest duration of vatiquinone exposure	Update and clarification
5	Section 2.1.3	Addition of section to add an exploratory objective (PedsQL)	Update
6	Section 2.2.3	Addition of section to add an exploratory endpoint (PedsQL)	Update
7	Section 3.2.2	Revision of number of study sites from approximately 12 to 30	Update
8	Section 4.2	Revision of Inclusion Criterion #4 to include subjects with genetic confirmation of inherited mitochondrial disease with associated epilepsy phenotype (Alpers/POLG, Leigh syndrome, MELAS), or other genetically confirmed mitochondrial disease secondary to mitochondrial mutations (PCH6, nuclear DNA RARS2 mutation), or MERRF, mtDNA MT-TK mutation are eligible. Removal of the word “ongoing” from the phrase “Despite ongoing treatment with at least 2 AEDS” from Inclusion Criterion #5 per administrative letter dated 23 February 2021and clarification that weight-based adjustments to the AED regimen will be allowed Update of “Baseline Visit” to “Screening Visit” in Inclusion Criteria 7, 8, and 9 Clarification added to inclusion Criterion #10 that historical EEG may be up to 6 months prior to screening	Update and clarification
9	Section 4.3	Revision to exclusion Criterion #2 per administrative letter dated 09 February 2021 to clarify that AST and ALT can be ≥2×the ULN if hepatic transaminases may be associated with the underlying mitochondrial disorders like Alpers-Huttenlocher syndrome/POLG subtypes and that, in these cases, the AST or ALT ≥3×ULN at time of screening will be exclusionary Addition to exclusion Criterion #7 to exclude use of artisanal (non-Epidiolex cannabidiol) cannabidiol as per administrative letter dated 25 November 2020	Update
10	Section 5.1.1	Addition of Table 1 (Weight-Based Dosing in mL of the 100 mg/mL Solution)	Update
11	Section 5.1.3	Addition of section for treatment of missed doses	Update
12	Section 5.3	Clarification that the study will be unblinded after all subjects complete the placebo-controlled phase of the study Updated disease subtype #4 to “Other genetically confirmed mitochondrial disease secondary to either nuclear or mitochondrial DNA mutation”	Clarification
13	Section 5.5	Addition of guidelines for immunizations as concomitant therapy	Update

Item No.	Protocol Section	Version 5/Update	Reason/ Rationale
14	Section 7.1 (Table 2)	Addition of PedsQL at baseline and Weeks 24 and 72 Revision to indicated that EEG will only be done at screening and Week 72 New row inserted to include coagulation panel separately from hematology Revision to indicate that seizure diary distribution will only occur at screening Revision to footnote "e" to indicate that historical EEG may be up to 6 months prior to screening Clarification added in footnote "j" for consistency with SAE reporting requirements in Section 7.5.9 Clarification to footnote "m" to indicate that vatiquinone will be dispensed after completing the Week 24 assessments and that the subjects will enter the open-label of the study and receive vatiquinone for the dose after the visit	Update and clarification
15	Section 7.1.1	Addition of clarification that historical EEG may be done within 6 months prior to screening	Clarification
16	Section 7.1.2.1 Section 7.1.2.8 Section 7.1.3	Addition of PedsQL questionnaire at baseline and Weeks 24 and 72	Update
17	Section 7.1.2.3 Section 7.1.2.4	Deletion of drug reconciliation of returned study drug bottles with dosing diaries for consistency with return of study drug bottles being returned every 12 weeks only	Clarification
18	Section 7.1.3	Clarification that dispense study drug will occur during the long-term extension phase will occur on weeks 36, 48, and 60	Clarification
19	Section 7.1.7	Update of vatiquinone exposure in patient dosing days	Update
20	Section 7.1.9	Addition of exclusion of the use of artisanal (non-Epidiolex cannabidiol) cannabidiol per administrative letter dated 25 November 2020 Clarification that weight-based adjustments to the AED regimen will be allowed	Update and clarification
21	Section 7.2	Addition of the PedsQL as an efficacy assessment	Update
22	Section 7.4.2	Separation of coagulation panel into its own subsection from the hematology subsection	Clarification
23	Section 7.5.12	Revision of adverse event reporting requirements to include SUSAR language	Clarification
24	Section 8	Revision of paragraph regarding the SAP to indicate that the SAP will be finalized prior to unblinding of the study	Clarification
25	Section 8.2	Revision of text regarding sample size and statistical powering Removal of statistical power table	Clarification and update
26	Section 8.4.4	Addition of section for Analysis of Exploratory Endpoint due to the addition of PedsQL questionnaire	Update

Abbreviations: AED, antiepileptic drug; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EEG, Electroencephalogram; MELAS, Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; mtDNA, mitochondrial DNA; MT-TK, mitochondrially encoded tRNA lysine; PedsQL, Pediatric Quality of Life Inventory™; PCH6, Pontocerebellar Hypoplasia Type 6; POLG, DNA polymerase subunit gamma; SAE, serious adverse event; SAP, Statistical Analysis Plan; SUSAR, suspected unexpected serious adverse reaction; ULN, upper limit of normal

9.3.6. Version 6.0: 04 June 2021

Overall reason for Version 6.0: The overall reasons for Version 6.0 were to clarify that male subjects must use contraception and that all subjects must use contraceptive measures from the time consent is signed until 30 days after treatment discontinuation and to add an appendix of prohibited medications.

Section	Version 6.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. Editorial revisions (eg, typographical error, punctuation, tenses, and abbreviations) were incorporated to provide clarity. The synopsis was updated to be consistent with changes in the protocol.	Update
Section 4.3	Revision to exclusion criterion 2 to increase aspartate transaminase and alanine transaminase to ≥3×upper limit of normal and remove exception for underlying diseases Revision of exclusion criterion 9 to specify that “strong” cytochrome P450 inhibitors or inducers are prohibited Revision of exclusion criterion 10 to specify that male subjects must also use contraception and addition of requirement for use of contraception for all subjects from the time consent is signed until 30 days after treatment discontinuation Addition of exclusion criterion 11 to indicate that subjects with comorbidities that may confound study results (eg, fat malabsorption syndrome, other mitochondrial disorders) in the opinion of the investigator will be excluded	Update and clarification
Section 7.1	Removal of the option of choosing the time for the post-dose sample and specify 4 hours postdose to be done with the 4-hour ECG (for subjects weighing <10 kg) Removal of hematology panel at the Screening Visit Revision of chemistry laboratory assessments to include only alanine aminotransferase, aspartate aminotransferase, and creatinine at the Screening Visit Added electroencephalogram at Week 24	Update
Section 7.1.2.1 Section 7.1.2.8 Section 7.3	Removal of the option of choosing the time for the post-dose sample and specify 4 hours postdose to be done with the 4-hour ECG (for subjects weighing <10 kg)	Clarification
Section 7.1.2.8	Added electroencephalogram at Week 24	Update
Section 7.1.3	Removed all timepoints except for 4 hours postdose for PK at Weeks 48 and 72	Clarification
Section 7.4.2	Revision of total blood volume to be drawn over the course of the study and addition of weight-based volumes Addition of cross-reference to Table 2 and revision of cross-reference from Section 7.1.2.7 to Section 7.1.2.8	Update and Clarification
Section 7.5.10	Revision to specify that male subjects must also use contraception and addition of requirement for use of contraception for all subjects from the time consent is signed until 30 days after treatment discontinuation	Clarification
Section 7.5.12	Revision of section title to indicate “Safety Reporting Requirement” Addition of text to indicate notification by PTC to investigators of any new or emerging safety information	Clarification
Section 9.3.4	Revision of item numbers 4 through 18 due to previously missing number 4	Clarification
Appendix 1	Addition of appendix of prohibited medications	Update

9.3.7. Version 7.0: 06 January 2022

Overall reason for Version 7.0: The overall reason for Version 7.0 was to incorporate feedback from health authorities.

Section	Version 7.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with changes in the protocol. Editorial revisions (eg, typographical errors, punctuation, tenses, and abbreviations) were incorporated to provide clarity.	Update
Section 4.2	Inclusion criterion #2 was updated to include subjects <21 years of age. Inclusion criterion #6 was updated to "Documented medical history of epilepsy associated with mitochondrial disease for at least 6 months prior to screening **except for subjects who are <2 years of age at the time of screening (Subjects <2 years of age can be considered for enrollment if all other screening criteria are met due to the potential for rapid progression in these subjects)**".	Update
Section 4.3	Exclusion criterion #3 which read, "INR ≥ULN at time of screening", was revised to read "INR >ULN at time of screening".	Update
Section 5.1.1 Section 7.1	It was corrected that weights ending in decimals ≥0.5 (instead of >0.5, as stated previously) should be rounded up to the next whole number for dosing calculation.	Update
Section 7.1 Section 7.1.2.8	It was specified that informed consent for subjects entering the long-term extension phase would be completed at Week 24.	Update
Section 7.1.3	It was clarified that EEG is to be collected at Week 72, as already specified in Table 2.	Clarification
Section 7.4.1	The text was updated to specify that weight could also be collected in lb and height could also be collected in inches.	Update
Section 7.4.2	The total volume of blood collected as written in V6.0 is incorrect and has now been corrected.	Update

9.3.8. Version 8.0: 27 May 2022

Overall reason for Version 8.0: The overall reason for Version 8.0 was to remove "a minimum number of 6 subjects" per mitochondrial disease subtype category and clarify the blinding of the investigators, study subjects, and sponsor during the placebo-controlled and open-label phases of the study.

Section	Version 8.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with changes in the protocol. Editorial revisions (eg, typographical errors, punctuation, tenses, and abbreviations) were incorporated to provide clarity. Relevant updates to the study personnel and approval signatures pages were made.	Update
Section 3.1.2	The following text was removed: "A minimum of 6 subjects (approximately) will be allocated to category 1), 2), and 3), respectively."	Update

Section	Version 8.0/Update	Reason/ Rationale
Section 5.3	The blinding of the investigators, study subjects, and sponsor was clarified with the following text: "The investigators, study subjects, and sponsor will be blinded as follows: the sponsor will be blinded to the randomized treatment until the study is unblinded after all subjects complete the placebo-controlled phase; subjects and investigators remain blinded to the treatment received during the placebo-controlled phase until after all subjects complete the open-label phase. An independent Data Safety Monitoring Board (DSMB) will monitor the study until completion of the placebo-controlled phase of the study."	Clarification
Section 7.1	The following clarifying text was added to footnote "g" of Table 2 : "If a subject cannot perform a urine pregnancy test at any timepoint, a serum pregnancy test should be performed."	Clarification
Section 7.1.2 Section 7.1.3	The following clarifying text was added to the relevant timepoints: "If a subject cannot perform a urine pregnancy test, a serum pregnancy test should be performed."	Clarification
Section 7.4.3	The following text was added for clarification: "If a subject cannot perform a urine pregnancy test at any timepoint, a serum pregnancy test should be performed."	Clarification

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APPENDIX 1. PTC743-MIT-001-EP PROHIBITED MEDICATION LIST

Table 7: PTC743-MIT-001-EP Prohibited Medication List

Prohibited Medications	Examples
Anticoagulants	• heparin • warfarin • aspirin • clopidogrel • apixaban • dabigatran • edoxaban • enoxaparin • rivaroxaban
CYP3A4 strong inducers	• apalutamide • carbamazepine • enzalutamide • mitotane • phenytoin • rifampin • St. John's wort
CYP3A4 strong inhibitors	• boceprevir • cobicistat • danoprevir and ritonavir • elvitegravir and ritonavir • grapefruit juice (any grapefruit product) • indinavir and ritonavir • itraconazole • ketoconazole • lopinavir and ritonavir • paritaprevir and ritonavir and (ombitasvir and/or dasabuvir) • posaconazole • ritonavir • saquinavir and ritonavir • telaprevir • tipranavir and ritonavir • telithromycin • troleandomycin • voriconazole • clarithromycin • idelalisib • nefazodone • nelfinavir
Investigational medications for MIT-E	
Idebenone	

Prohibited Medications	Examples
Artisanal (non-Epidiolex cannabidol)	

Abbreviations: CYP3A4, cytochrome P450 3A4

Note: This list is neither comprehensive, nor final. In general, study subjects should not be taking any strong CYP3A4 inhibitors or inducers, anticoagulants, or any investigational medications.