STATISTICAL ANALYSIS PLAN

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

PTC743-MIT-001-EP

15 JUNE 2023

VERSION 2.0

PTC THERAPEUTICS, INC.
100 CORPORATE COURT
SOUTH PLAINFIELD, NJ 07080 USA

APPROVAL SIGNATURES

[redacted]
PTC Therapeutics, Inc.

Date

[redacted]
PTC Therapeutics, Inc.

Date

[redacted]
PTC Therapeutics, Inc.

Date

The eSignature page is located on the last page.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Term
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
CarerQoL-7D	Care-Related Quality of Life-7 Dimensions
CI	confidence interval
COVID-19	coronavirus disease of 2019
CRF	case report form
DNR	do not resuscitate
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
EEG	electroencephalogram
FDA	United States Food and Drug Administration
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
INR	international normal ratio
ITT	intention-to-treat
LLoQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MELAS	mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
mt-aaRS2	mitochondrial aminoacyl tRNA synthetase 2 genes
PCH6	pontocerebellar hypoplasia type 6
PedsQL	Pediatric Quality of Life Inventory™
PK	pharmacokinetics
POLG	polymerase subunit gamma
PP	per-protocol
PT	prothrombin time
PT	preferred term
PTT	partial thromboplastin time

Abbreviation	Term
QTc	corrected QT interval
SAE	serious adverse event
SAP	statistical analysis plan
SOC	system organ class
TEAE	treatment emergent adverse event
TFL	table, figure, and listing
TID	ter in die (Three times a day)
VAS	visual analogue scale
WHO	World Health Organization

1. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures and the statistical methods that will be used to analyze data and report results for PTC743-MIT-001-EP (Protocol version 8.0 dated on 27 May 2022). Table, figure, and listing (TFL) specifications are contained in a separate document.

There will be two database locks for this study. The first database lock (soft lock) will occur when all subjects complete the double-blind treatment period. The final database lock will occur when all subjects have completed the open-label treatment period.

This SAP outlines the statistical methods that will be used to analyze the data collected during the double-blind period and will be finalized and approved prior to the first database lock. A separate SAP for the open-label treatment period will be finalized and approved prior to the final database lock.

1.1. Study Design

This study is a parallel-arm, double-blind, placebo-controlled trial, with a screening phase that includes a 28-day run-in phase to establish baseline observable motor seizure frequency. The 28 days immediately prior to the Baseline Visit is considered the run-in phase. Screening is followed by a 24-week randomized double-blind placebo-controlled phase during which subjects will be 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, 3 times daily (TID) or placebo TID. Following completion of the randomized double-blind 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.

Subjects who meet eligibility criteria will be randomized to vatiquinone or placebo in 1:1 ratio, with approximately 30 subjects in each arm. The randomization included stratification 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. These strata were decided prior to initiation of enrollment based on the fact they are three mitochondrial disease subtype with relatively high prevalence (though still quite rare) and seizures as a prominent aspect of disease pathology.

1.2. Study Objectives

1.2.1. Primary Objective

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

1.2.2. 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 Care-Related Quality of Life-7 Dimensions [CarerQoL-7D] questionnaire).
- Demonstrate the effects of vatiquinone on occurrence of seizure clusters.
- Demonstrate the safety of vatiquinone as assessed by drug-related serious adverse events (SAEs), drug-related adverse events (AEs), and dose modifications.

1.2.3. 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).

1.3. Endpoints

1.3.1. 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 double-blind phase.

1.3.2. 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.
- Each of the CarerQoL-7D dimensions will be summarized descriptively by visit for double-blind period and open-label period respectively.
- Number of rescue seizure medications.

- Number of seizure clusters as defined by "too many to count" entries in the seizure diaries.

1.3.3. Exploratory Endpoint

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

1.3.4. Safety Endpoints

Safety endpoints include both clinical and laboratory variables.

1.3.4.1. Clinical Variables

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

1.3.4.2. Physical Examination, Vital Signs, 12-lead Electrocardiogram (ECG) and Electroencephalogram (EEG)

- Vital signs include height, weight, temperature, respiratory rate, systolic blood pressure, diastolic blood pressure, heart rate and oxygen saturation.
- Physical examination includes general appearance, skin, head, eyes, ears, nose, throat, neck (including thyroid), lymph nodes, chest, heart, abdomen, and extremities.

1.3.4.3. Laboratory Variables

- Hematology includes erythrocytes, leukocytes, and platelets.
- Coagulation includes PT and PTT reported as international normalized ratio (INR) units.
- Serum Chemistry includes liver function, renal function, electrolytes, general chemistry and lipids.
- Urinalysis includes pH, protein, glucose, ketones, blood and bilirubin.

1.4. 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 80% power 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 vatiquinone treatment group, respectively, for percent change from baseline in motor seizure frequency per 28 days.

2. STUDY POPULATIONS AND PERIODS FOR ANALYSIS

This is a basket trial of mitochondrial disease enrolling several specific, ultrarare, and potentially heterogeneous disease subtypes where refractory epilepsy is a significant and highly morbid aspect of disease pathology. Due to this, this trial will be analyzed overall and in predefined disease subtypes.

Considering the patient population currently enrolled into the study we are pre-specifying three specific mitochondrial disease subtypes of interest in whom we seek to understand treatment effect independent of those recorded in the overall population. These disease subtypes are:

- Leigh syndrome
- Pontocerebellar Hypoplasia Type 6 (PCH6)
- Mitochondrial aminoacyl tRNA synthetase (mt aaRS2) disorders inclusive of PCH6

All analyses planned for the overall population will be repeated in these disease subtypes.

2.1. Study Populations

2.1.1. Intention-to-Treat Population

Intention-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.

2.1.2. Safety Population

Safety 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 safety data will be analyzed "as treated". The Safety Analysis population will be used in the statistical analyses for safety.

2.1.3. Per-Protocol Population

The per-protocol (PP) population will include subjects in the ITT population who do not have any important protocol deviations leading to exclusion from the PP population.

The protocol deviations that may have major effect on efficacy which will lead to exclusion of the subjects from per-protocol population include:

- Subjects that had significant inclusion and exclusion criteria violations.
- Subjects that had protocol deviations which may impact effectiveness of study treatment.
- Subjects that received study treatment different from the randomized treatment throughout the double-blind period.
- Subjects with <80% of valid days entered in the seizure diary during the run-in period.

- Subjects with non-compliance to study drug administration with compliance <80% and >120%.
- Subjects with less than 80% compliance in the seizure diary of valid days entered in the diary during either run-in period or during double-blind period.
- Subjects who change anti-seizure medication or increase dose of anti-seizure medication not related to weight or begin/change in ketogenic diet during the study during double-blind period.
- Subjects that discontinued study treatment due to withdrawal, noncompliance, and death during the double-blind period.

All protocol deviations leading to exclusion from the PP population will be reviewed and approved by the study team prior to treatment unblinding. The list of PP population subjects will be finalized prior to treatment unblinding.

This population will be used as supportive efficacy analysis for the primary efficacy endpoint.

2.2. Study Periods

2.2.1. Double-blind Period

Double-blind period for efficacy or safety endpoints is defined as the period from randomization or first dose date, respectively, to:

- the last dose of double-blind study drug if the subject continues to the open-label extension period

  or

- the last study visit if the subject does not continue to the open-label extension period.

Double-blind period will be used for summarizing efficacy and safety endpoints.

2.2.2. On-vatiquinone Period

On-vatiquinone period is defined as the period from the first dose of vatiquinone, regardless of double-blind or open-label vatiquinone, to the end of the study.

On-vatiquinone period will be used for summarizing safety endpoints.

3. GENERAL CONSIDERATIONS

3.1. Definition of Estimand for the Primary Objective

The primary estimand of the study is the percent change from baseline in frequency of observable motor seizures per 28 days during the double-blind period. The attributes of the primary estimand are defined in Table 1.

Summary Measure:

The median of the variable of interest will be estimated for each treatment group. The difference in median of the variable of interest between two treatment groups will be estimated using Hodges-Lehmann estimator and 95% confidence interval (CI). The p-value will be computed from ranked ANCOVA detailed in Section 5.1.2.

Table 1: Primary Estimands

Population	Variable	Intercurrent Events	Population-level Summary	Analysis
The population of subjects <21 years of age with genetically confirmed mitochondrial disease as defined by the protocol inclusion/exclusion criteria.	Percent change from baseline of motor seizure frequency per 28 days during the double-blind period.	Rescue medication status: Study treatment will not be modified based on rescue medication status in line with a treatment-policy strategy. Emergency room visits and hospitalization: The days which patient has emergency room visits, or is hospitalized, will not be excluded from the calculation of primary endpoint as long as the diary is available for such days in line with a treatment-policy strategy. Changes in anti-seizure medications: Study treatment will not be modified and patient's will not be excluded as a result of changes in anti-seizure medications that are a medical necessity and in line with a treatment-policy strategy Early termination or discontinuation: Data will be used as collected	Treatment difference between vatiquinone and placebo in percent change from baseline to Week 24 will be estimated using the ANCOVA based on rank transformed data.	Primary analysis for primary efficacy endpoint

Abbreviations: ANCOVA, analysis of covariance

3.1.1. Intercurrent Events

The following events will be considered intercurrent events for the MIT-E study:

- Use of rescue medications for seizures
- Emergency room visits and hospitalizations
- Changes in anti-seizure medications, except weight-based adjustments
- Discontinuations due to withdrawal, noncompliance, and death during the double-blind period.

Subjects with changes in anti-seizure medications and subjects who discontinued from the study will be excluded from the Per Protocol population and will be assessed in separate sensitivity analyses.

Sensitivity analyses will not be performed for subjects that have use of rescue medications, visits to the emergency room, or hospitalizations, as these events will not have long term impact on the effectiveness of study treatment.

Sensitivity analysis performed regarding these intercurrent events are described in Section 5.1.3.

3.2. General Considerations

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.

Day 1 is defined as first dose date relative to double-blind period. Relative days after day 1 are calculated as: assessment date - first dose date + 1. Relative days prior to day 1 are calculated as assessment date - first dose day.

All study days will be calculated as relative to the first dose day of double-blind period.

All analyses will be based on observed data only, and no missing values will be imputed except specifically indicated, for example, components of a date.

Measurements collected from unscheduled visits, or repeated assessments will be included in the listings only unless otherwise specified. Source data for the summary tables and statistical analyses will be presented as subject data listings.

In general, statistical inferential statistics will only be performed for the double-blind period using 2-sided α of 0.05 level of significance. The descriptive summaries will be presented by treatment groups (placebo, vatiquinone) and in total (where applicable) during double-blind period for both efficacy and safety endpoints. In addition, on-vatiquinone experience from both double-blind and open-label extension period will be combined and summarized for safety endpoints. In On-vatiquinone summaries, baseline for subjects on placebo in the double-blind period will be the last observation prior to the first vatiquinone dose.

3.3. Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) has been set up for this study. The primary responsibility of the DSMB is to protect the safety and welfare of subjects participating in this clinical trial.

Periodic DSMB meetings are planned to monitor subject safety. Unblinded outputs on subject disposition, demographic and baseline characteristic, efficacy, safety, and PK will be reviewed by DSMB members. Details are provided in the DSMB charter and DSMB SAP.

3.4. Interim Analyses

No formal interim analyses are planned for this study.

However, there will be 2 database locks for this study. The first database lock (soft lock) will occur when all subjects complete the double-blind treatment (Week 24). The study will be unblinded after soft lock. The final database lock will occur when all subjects have completed open-label treatment period. The subjects and investigators will remain blinded to the treatment assignment until the final data base lock.

3.5. Baseline

For safety endpoints, baseline for double-blind period and open-label period will be defined as the last measurement prior to or on the date of first dose date.

For efficacy endpoints during double-blind period, baseline is defined as the 28 days prior to randomization. Baseline definition for the seizure frequency, disease-related hospitalization days, and occurrence/recurrence of status epilepticus per 28 days will be calculated as the number of seizures, hospitalization days, and status epilepticus, respectively, during the run-in period, divided by the number of days in the run-in period, and multiplied by 28. Only “valid days” will be used in the calculation of seizure frequency per 28 days. A “valid day” is defined as a day where seizure counts information is present.

For summary, based on on-vatiquinone period, the baseline will be defined as the last measurement prior to the first dose of vatiquinone. ie, the last assessment prior to first dose in subjects with treatment sequence vatiquinone/vatiquinone, or the last assessment prior to first open-label vatiquinone in subjects with treatment sequence placebo/vatiquinone. For subjects who are randomized to placebo group in double-blind period and enter into the open-label period, all assessments conducted on the day they take last dose of double-blind period drug will be considered as baseline for the on-vatiquinone period.

3.6. Multiplicity Adjustment

To control the family-wise error rate for the primary and key secondary efficacy endpoints, a fixed sequence procedure will be used. Testing orders of the primary endpoint and the key secondary endpoints are listed as below:

- Primary endpoint: percentage change from baseline in the seizure frequency in motor seizures per 28 days during the double-blind period
- The first key secondary endpoint: number of disease-related hospitalization days

- The second key secondary endpoint: occurrence or recurrence of status epilepticus

The primary endpoint will be tested at the significance level of 0.05 (two-sided). If $p<0.05$, then the primary endpoint will be considered statistically significant, and the study will be declared positive. If the test of the primary endpoint is statistically significant, then the key secondary endpoints will be tested in the order specified above, each at the 0.05 (two-sided) significance level. Only if the first key secondary endpoint is statistically significant at the 0.05 significance level, the second key secondary endpoint will be tested at the 0.05 significance level.

3.7. Missing Data Handling

In general, missing data will not be imputed, unless otherwise specified.

To minimize the impact from the missing data, the seizure frequency will be based on number of seizures on "valid days". A "valid day" is defined as the day where seizure counts information is present.

In addition, a sensitivity analysis will be performed excluding all subjects with < 80% compliance in the seizure diary of valid days entered in the diary either during run-in period, or during double-blind period.

For analysis purposes, lab or PK values preceded by a "<" sign, ie, below the limits of quantification, will be considered as half of the limit of quantification. Lab or PK values preceded by a ">" sign, ie, above the limits of quantifications, will be considered as plus one unit of the minimum digit of the limit of quantifications. The original value will be included in the listing.

Per study design, last dose during double-blind period will be taken in the office the same day as first dose during open-label period, which will be taken at home later that day. When the dose time for first dose during open-label period is missing, all labs, vital signs taken on that day will be considered as prior to open-label period. All seizures, AE and concomitant medications that occurred on that day will be considered as occurred during the open-label period to be conservative, as all subjects will take vatiquinone during open-label period.

3.8. Merging Strata

If treatment arms have less or equal to three subjects in one of the strata in ITT populations, the stratum cell will be combined to stratum "Other". Based on enrollment, all strata with the exception of Leigh Syndrome will be combined in "Other".

3.9. Visits During On-vatiquinone Period

The nominal visits during on-vatiquinone period will be reassigned for subjects randomized to placebo/vatiquinone treatment sequence as in Table 2.

Table 2: Study Visits for On-vatiquinone Period

Study Visit	Nominal visit during on-vatiquinone period for treatment sequence vatiquinone/vatiquinone	Nominal visit during on-vatiquinone period for treatment sequence placebo/vatiquinone
Baseline	Baseline	

Study Visit	Nominal visit during on-vatiquinone period for treatment sequence vatiquinone/vatiquinone	Nominal visit during on-vatiquinone period for treatment sequence placebo/vatiquinone
Week 4	Week 4	
Week 8	Week 8	
Week 12	Week 12	
Week 24	Week 24	Baseline
Week 36	Week 36	Week 12
Week 48	Week 48	Week 24
Week 60	Week 60	Week 36
Week 72	Week 72	Week 48
Follow-up	Follow-up	Follow-up

3.10. Changes of Analysis from Protocol

There are no major changes in analysis from study protocol.

At the time of study design and enrollment, the initial plan of randomization and stratification was planned based on the disease subtype to include the following:

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.

However, due to the challenges in identifying and enrolling participants who meet the inclusion and exclusion criteria for the study, there was a disparity in enrollment of subjects with the prespecified subtypes mentioned in the protocol (listed above). Only one subject was randomized for Alpers/(POLG) and no subjects were randomized for MELAS. Therefore, the only strata in the ITT population will be Leigh syndrome and "Other" (Section 3.8).

Considering the patient population currently enrolled into the study, we have pre-specified two specific mitochondrial disease subtypes, in addition to Leigh syndrome, that are of interest and in whom we seek to understand treatment effect independent of those recorded in the overall population. These additional disease subtypes are pontocerebellar hypoplasia type 6 (PCH6) secondary to arginine-tRNA synthetase 2 (RARS2) mutation and mt-aaRS2 disorders (for all mitochondrial aminoacyl tRNA synthetase disorders which includes subjects with RARS-2 mutations associated with PCH6 mutation). All analyses planned for the overall population will be repeated for Leigh syndrome, PCH6, and mt-aaRS2 disorders.

In the protocol, the secondary end point related to the change in motor seizures and total seizures was planned to be analyze in proportion of subjects with ≥25%, ≥50%, ≥75%, and 100% reduction. This proportion of change does not capture disease progression and potential increase in seizures. Therefore, the analysis groupings were changed to reflect the proportion of subjects with >30%, 30% to -30%, < -30% to -60%, > -60% to -100% for motor seizure and total seizures will be summarized for double-blind period by treatment groups.

4. SUBJECT DATA

4.1. Participant Dispositions

The disposition of subjects in the double-blind period, including the number of subjects screened, the number of subject randomized, 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, number of subjects who complete the final follow up call and the reason the final call not completed will be tabulated for ITT population.

A similar table that includes number of subjects who received at least one dose of study drug, number of subjects who prematurely discontinue study drug as well as the reason for the premature termination, number of subjects who complete the final follow up call and the reason the final call was not completed will be produced for open-label extension period by treatment sequence for the ITT population.

Similar table will be tabulated for Safety population when ITT and Safety population are different.

4.2. Protocol Deviation

All protocol deviations will be summarized for the ITT population. A listing of all subjects with one or more important and non-important protocol deviations will be presented for the ITT population.

All-important protocol deviations will be identified and documented by PTC team prior to unblinding at soft lock.

4.3. Demographic and Baseline Characteristics

Demographics and baseline characteristics including age, sex, race, ethnicity, region, country, height, weight, body mass index (BMI), and disease subtype will be summarized by treatment for ITT Population.

4.4. Medical History

Medical history will be coded with the Medical Dictionary for Regulatory Activities (MedDRA, version 23.0, or later).

Medical history records will be summarized by treatment and by SOC and PT for ITT population.

A subject data listing of medical history will be provided.

4.5. Concomitant Medications and Non-Drug Treatments

All investigator terms for medications recorded on the case report form (CRF) will be coded using the World Health Organization (WHO) Drug Dictionary (Version MAR2020 or later).

Prior medications will be defined as medications started prior to the first dose of study drug. Concomitant medications will be defined as medications (other than the study drug) that (1) started before the first dose of study drug and were continuing at the time of the first dose of

study drug, or (2) started on or after the date of the first dose of study drug. Any medication started before the first dose of study drug and continued at the time of first dose will be considered as both prior and concomitant medications.

Any medication with partial or missing start date in which the prior medication status cannot be determined will be considered as a prior medication. Similarly, any medication with partial or missing end date in which the concomitant medication status cannot be determined will be considered as a concomitant medication.

Prior medication, concomitant medication during double-blind period, concomitant medication during on-vatiquinone period will be summarized as following for ITT population:

- Number and percentage of subjects with at least one prior/ concomitant medications.
- By Anatomical Therapeutic Chemical (ATC) level 2 and preferred term (PT).

A subject data listing of all prior and concomitant medications will be listed.

Prohibited medications will be identified prior to unblinding and will be listed separately.

4.6. Study Drug Compliance Based on Diary

Treatment compliance will be calculated as the percentage of the number of doses taken as recorded on diary out of what is expected to be taken in a period of interest,

$$compliance = \frac{number\ of\ doses\ taken}{3\ x\ number\ of\ days\ in\ the\ period\ of\ interest (except\ on\ the\ day\ start\ first\ dose\ of\ OL\ period)} \times 100\%$$

The number of days in the period of interest is defined as last dose date in the period of interest - first dose date in the period of interest + 1.

Last dose date during double-blind period is the same date as the start of the open-label period, and subject is expected to take 2 doses of the double-blind period medication and 1 dose of the open-label period medication. Denominator for the compliance calculation will be adjusted accordingly for subjects entered the open-label period.

The study drug compliance will be summarized by treatment for double-blind period, and by treatment sequence for open-label period, as well as for on-vatiquinone for Safety Population.

4.7. Seizure Diary Compliance Based on Diary

Seizure diary compliance is defined as the percentage of number of days with a valid seizure diary entry out of number of days during the period of interest.

$$compliance = \frac{number\ of\ days\ with\ \text{seizure}\ diary}{number\ of\ days\ in\ the\ period\ of\ interest} \times 100\%$$

The number of days in double-blind period is defined as last dose date in the double-blind period - first dose date in the double-blind period + 1. For subject who entered the open-label period, the duration for the double-blind period is defined as: last dose date in the double-blind period - first dose date in the double-blind period. The handling is because the last dose date during double-blind period is the same as during start of open-label period, and the seizure diary will

not record the time of the seizure, all the seizures that occur on that day will be considered as during open-label period.

The number of days in open-label period is defined as last dose date in the open-label period-first dose date in the open-label period+1.

The seizure diary compliance will be summarized by treatment for double-blind period for ITT population.

5. EFFICACY ANALYSIS

Efficacy analysis will be based on the ITT population, unless otherwise specified.

5.1. Primary Endpoint(s) Analysis

5.1.1. Definition of Endpoint(s)

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

Motor seizure frequency will be counted across the entire 24 weeks (168 days) of the double-blind treatment period. Seizure frequency will be normalized to 28 days to allow for comparison of seizure frequency in the double-blind period to the seizure frequency observed during the 28-day run-in period. The 28-day run-in period will be used to establish the baseline seizure frequency.

During the study, subjects (and/or their parent/legal guardian) were required to record occurrence of seizures in a seizure diary on a daily basis. A blank entry in the seizure diary would be considered an invalid day; it is not considered to be an absence of seizures. Only days that have seizure diary information completed are considered to be valid entry days.

For calculation of the primary endpoint, the total number of motor seizures collected on valid entry days will be divided by the number of valid entry days and then multiplied by 28 to provide the average number of seizures normalized "per" 28 days. The formula to calculate this is shown below:

$$\text{Seizure frequency per 28 days during DB period} = \frac{\text{Total number of reported motor seizures during DB period}}{\text{Number of "valid" days with reported motor seizures during DB period}} \times 28$$

5.1.2. Primary Analysis

For primary analysis, both the motor seizure frequencies per 28 days in the pre-randomization period as well as the percentage change in motor seizure frequencies per 28 days in the double-blind period will be rank transformed separately.

The analysis of covariance (ANCOVA) will then be conducted on this rank transformed data with treatment, and stratifying factor and the ranked pre-randomization phase seizure frequency per 28 days as a covariate. P-values will be computed 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. The difference in median of the percent change from baseline between the two treatment groups will be estimated using Hodges-Lehmann estimator along with 95% CI for understanding the treatment effect size.

The stepwise approach to estimate the median difference of percent change using Hodges-Lehmann estimator is*:*

a. derive the seizure frequency per 28 days for baseline (BL) and double-blind (DB) period.
b. calculate the percent change from baseline as 100 × (DB – BL)/BL.
c. apply the below SAS code of PROC NPAR1WAY nonparametric model to generate Hodges Lehmann Estimator:

```
*** Hodges Lehmann Estimator;
 proc npar1way data=<dataset name> HL ;
   class <treatment variable name>;
   var <percent change from baseline variable name>;
   ods select HodgesLehmann;
 run;
```

5.1.3. Sensitivity Analysis for Primary Endpoint

The following sensitivity analyses are planned.

1. The similar analysis as described for the primary analysis will be repeated using the PP population.
2. An analysis using the same model as described for primary analysis excluding all subjects with <80% of valid days entered in the diary in run-in period and/or the double-blind period.
3. An analysis using the same model as described for primary analysis excluding all subjects who change anti-seizure medication or increase dose of anti-seizure medication not related to weight or begin/change in ketogenic diet during the study.
4. An analysis using the same model as described for primary analysis excluding all subjects who discontinued due to withdrawal, noncompliance, and death during the double-blind period.
5. Analysis as described in the primary analysis will be performed for percentage change from baseline in the motor seizure frequency per 28 days during the double-blind period completers ie, all subjects that took medication until the end of the double-blind period as specified by the protocol and have >80% compliance for seizure diary, for ITT analysis sets.
6. Change from baseline of number of motor seizure per 28 days in the double-blind phase will be performed using the same model as described for primary analysis.
7. Analysis as described in the primary analysis will be performed for percentage change from baseline in the motor seizure frequency per 28 days during the last 12 weeks in the double-blind phase for ITT.
8. Analysis as described in the primary analysis will be performed for percentage change from baseline in the motor seizure frequency per 28 days during the last 4 weeks in the

double-blind phase for ITT. The last 4 weeks is defined as start 141st day post randomization until last dose date during the double-blind phase.

9. Descriptive summary will be performed for percentage change from baseline in the motor seizure frequency per 28 days during the double-blind phase for ITT population excluding subjects that had coronavirus disease of 2019 (COVID-19) during the double-blind phase.
10. Descriptive summary will be performed for percentage change from baseline in the motor seizure frequency per 28 days during the double-blind phase for ITT population excluding subjects with a do not resuscitate (DNR) order.
11. The median and 95% CI of percent change from baseline in motor seizure frequency per 28 days will also be summarized for the ITT population during
    - Week 4 (day 1 – 28)
    - Week 8 (day 29 – 56)
    - Week 12 (day 57 – 84)
    - Week 16 (day 85 – 112)
    - Week 20 (day 113 – 140)
    - Week 24 (day 141 – 168)

5.1.4. Subgroup Analysis

Median and corresponding 95% CI of percent change from baseline of motor seizure frequency per 28 days will be summarized for subgroups below for double-blind period for ITT population:

- Age group (<8 years old vs ≥8 years old)
- Sex (Male vs Female)
- Race (White vs Other)
- Region (United States vs Rest of the World)

5.2. Secondary Endpoint(s) Analysis

5.2.1. Key/Confirmatory Secondary Endpoint(s)

The key secondary efficacy endpoints of the study are as follows:

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

For the key secondary endpoints, if subject is without any events at baseline, ie, no hospitalization or status epilepticus at baseline, then subject will be considered as having 0 events at baseline.

5.2.1.1. Number of Disease-Related Hospitalization Days

Number of disease-related hospitalization days per 28 days will be calculated as the total number of disease-related hospitalization days multiplied by 28 and divided by the total number of days in the interval.

Disease related hospitalization is defined as any hospitalizations occurring as the consequence of an AE or SAE during the study period that is related to the subject's disease state or baseline conditions of the subject and lack of alternative explanation for the event. All the hospitalization events will be reviewed, classified as disease-related or not and agreed upon prior to the study unblinding.

The change from baseline in number of disease-related hospitalization days per 28 days during the double-blind period will be compared between the treatment groups using Wilcoxon rank-sum test for ITT population.

The number and the change from baseline in number of disease-related hospitalization days per 28 days during the double-blind period will be summarized descriptively for ITT population. Number of disease-related hospitalization days will be tabulated by frequency and percent as well for double-blind period, respectively, for ITT.

In addition, the change from baseline in number of disease-related hospitalization days per 28 days will also be summarized descriptively for the following subgroups for double-blind period for ITT population.

- Age group (<8 years old vs ≥8 years old)
- Sex (Male vs Female)
- Race (White vs Other)
- Region (United States vs Rest of the World)

5.2.1.2. Number of Occurrence/Recurrence of Status Epilepticus

Number of occurrence/recurrence of status epilepticus per 28 days will be calculated as the total number of status epilepticus multiplied by 28 and divided by the total number of days in the interval.

The change from baseline in the number of occurrence/recurrence of status epilepticus per 28 days during double-blind period will be compared between the treatment groups using Wilcoxon rank-sum test for ITT.

The number of subjects who had status epilepticus and the number and the change from baseline in the number of occurrence/recurrence of status epilepticus per 28 days will be summarized for double-blind period for ITT population. Number of occurrence/recurrence of status epilepticus will be tabulated by frequency and percent as well for double-blind period, for ITT population.

In addition, the number and the change from baseline in the number of occurrence/recurrence of status epilepticus per 28 days will also be summarized descriptively for the following subgroups for double-blind period, for ITT population.

- Age group (<8 years old vs ≥8 years old)
- Sex (Male vs Female)

- Race (White vs Other)
- Region (United States vs Rest of the World)

5.2.2. Supportive Secondary Endpoint(s)

5.2.2.1. Total Seizure

Percent change from baseline in total seizure frequency per 28 days will be analyzed in a similar way to the primary endpoint for double-blind period for ITT population.

Change from baseline and percent change from baseline in total seizure frequency per 28 days will also be summarized descriptively for double-blind period for ITT population.

Change from baseline and percent change from baseline in total seizure frequency per 28 days, will also be summarized descriptively by disease subtype and age group for double-blind period for ITT population.

- Age group (<8 years old vs ≥8 years old)

In addition, the seizure frequency per 28 days will be summarized by type: motor seizure or non-motor seizure during double-blind.

5.2.2.2. Responder Rate

Response rate of x% is defined as the proportion of subjects whose seizure frequency change per 28 days is more than x% compared to baseline. Response rates (>30%, 30% to -30%, < -30% to -60%, > -60% to -100%) for motor seizure and total seizure will be summarized for double-blind period by treatment groups. Where negative values indicate a decrease in seizures from baseline and positive values indicate an increase in the number of seizures from baseline.

Response rates during double-blind period will be compared between two treatment groups by Cochran-Mantel-Haenszel test adjusting for stratifying factor for ITT population.

In addition, the response rate of -50% in both motor seizure and in total seizure in last 12 weeks of double-blind period will be compared similarly for ITT population.

Responder rates for motor seizure and total seizure will also be summarized descriptively for double-blind period for ITT population.

- Age group (<8 years old vs ≥8 years old)

5.2.2.3. Disease-Related Emergency Room Visits and In-Patient Hospitalizations

Change from baseline in the total number of disease-related emergency room visits and in-patient hospitalizations per 28 days in double-blind period will be summarized descriptively. In addition, the number of subjects with disease-related emergency room visits and in-patient hospitalizations and the number of disease-related emergency room visits and in-patient hospitalizations, will be tabulated by frequency and percent for double-blind period for ITT population.

- Age group (<8 years old vs ≥8 years old)

A similar analysis will be performed for change from baseline in the total number of disease-related emergency room visits and in-patient hospitalizations per 28 days during the double-blind period for ITT population. This analysis will exclude subjects that have a DNR order or had COVID-19 during the double-blind period.

5.2.2.4. Rescue Medications for Seizures

Number of rescue medication uses and the number of subjects taking rescue medications will be summarized descriptively and tabulated by frequency and percent separately for double-blind period for ITT population. In addition, number of rescue medication uses and the number of subjects taking rescue medications will be tabulated by frequency and percent for double-blind period for ITT population.

- Age group (<8 years old vs ≥8 years old)

5.2.2.5. Seizure Clusters

Motor Seizure cluster data are collected on diary. The number of subjects with motor seizure clusters, number of days with motor seizure clusters and change from baseline in total number of motor seizure clusters per 28 days will be summarized by frequency and percent for double-blind period for ITT population.

Change from baseline in total number of motor seizure clusters per 28 days will be summarized descriptively for double-blind period for ITT population.

- Age group (<8 years old vs ≥8 years old)

5.2.2.6. Quality of Life

Quality of life assessment includes CarerQoL-7D and visual analogue scale (VAS). The description of these two instruments and weighted sum derivation are from Hoefman et al, 2013.

The CarerQolL-7D comprises five negative and two positive dimensions of lending informal care derived from a literature review of subjective burden measures. The five negative dimensions are (i) relational problems, (ii) mental health problems, (iii) problems combining daily activities with care, (iv) financial problems, and (v) physical health problems. The two positive dimensions are (i) fulfilment from caregiving and (ii) support with lending care. Respondents are asked to indicate whether an item applies to them with three possible responses: (i) no, (ii) some, and (iii) a lot of. The combination of items and answering categories yields 2187 (= 3^7) caregiving situations.

Answers on the negative dimensions of the CarerQol-7D receive value of 0 (a lot), 1 (some) and 2 (no); answers on the positive dimensions receive a value of 0 (no), 1 (some), and 2 (a lot). Summing the values for the seven dimensions, a score of 0 thus translates to the worst informal care situation (a lot of problems and no support or fulfilment); the higher the score, the better the situation.

Recently, a tariff has become available for the CarerQoLl (Hoefman et al. 2013), which enables researchers to calculate a weighted sum score of the CarerQoLl-7D, taking the severity of problems into account (Table 2).The tariff is based on Dutch preferences for different caregiving situations and therefore concern Dutch national tariffs. Using the weighted sum score, the worst

caregiving situation receives a score of 0, while the best now has a score of 100. The scores between 0 and 100 can be calculated using the tariffs in Table 3.

Table 3: National tariff CarerQoL-7D

Dimension	**Tariff for score**		
	no	some	a lot
Fulfilment	0	13.6	19.7
Relational problems	14.7	10.6	0
Mental health problems	13.3	9.3	0
Problems combining daily activities	10	6.4	0
Financial problems	14.3	10.6	0
Support	0	4.7	6.5
Physical health problems	15.1	15.1	0
Plus: a 'bonus' for:	no	yes	
No mental health problems and no physical health problems	0	6.6	

The CarerQoL-VAS, a valuation component, is a horizontal visual-analogue scale (VAS) measuring well-being of the informal caregiver in terms of general happiness, ranging from completely unhappy (=0) to completely happy (=10) (Brouwer et al. 2006).

The response to each of the CarerQoLl dimensions will be summarized descriptively by visit for double-blind period for ITT population.

The weighted sum (based on the table above) of the answers will be derived for each subject. Change from baseline in the weighted sum and VAS will be summarized descriptively by visit for double-blind period respectively, for ITT population.

CarerQoL-VAS and the weighted sum data will be listed.

5.3. Tertiary/Exploratory Endpoint(s) Analysis

5.3.1. PedsQL

Child and Parent Reports of the PedsQL™ 3.0 Neuromuscular Module (https://www.pedsql.org/score.html) are composed of 25 items comprising 3 dimensions as shown in Table 4.

Table 4: Description of the Neuromuscular Module

Dimension	**Number of Items**	**Cluster of Items**	**Reversed Scoring**
About My Neuromuscular	17	1-17	1-17
Communication	3	1-3	1-3
About Our Family Resources	5	1-5	1-5

The scores are transformed on a scale from 0 to 100, higher scores indicate lower problems. The scoring procedure are:

- Step 1 transform Score. Items are reversed scored and linearly transformed to a 0-100 scale as follows: 0=100, 1=75, 2=50, 3=24, 4=0.
- Step 2: calculate scores by dimension.
    - If more than 50% of the items in the scale are missing, the scale scores will not be computed.
    - Mean score=sum of the items over the number of items answered.

Total score: sum of all the items over the number of items answered on all the scales. If more than 50% of the items in the scale are missing, the total score will not be computed.

Change from baseline in PedsQL score for each dimension and total score will be summarized descriptively by visit for double-blind period for ITT population.

PedsQL data and the score for each dimension along with the total score will be listed.

6. SAFETY ANALYSES

All safety analysis will be based on the Safety population. Descriptive summary will be based on nominal visits by treatment group and overall for double-blind period, and by treatment sequences and overall for on-vatiquinone period.

6.1. Duration of Treatment with Study Drug

Duration of treatment with study drug in double-blind phase will be calculated as number of days from the date of the first dose of double-blind study drug to the date of the last dose of the double-blind study drug, inclusive.

Duration of treatment with study drug in open-label period will be calculated similarly for open-label study drug.

In addition, total duration of vatiquinone will be calculated as number of the days from the date of the first dose of vatiquinone to the date of the last dose of vatiquinone, inclusive.

Duration of treatment will be summarized by treatment for double-blinded period for Safety Population, by treatment sequence for open-label period for Safety Populations, and for total duration for on-vatiquinone for Safety Population.

Duration of treatment will also be summarized by the following categories.

- Double-blind period
  - ≤28 days (≤4 weeks)
  - 29-56 days (4-8 weeks)
  - 57-84 days (8-12 weeks)
  - 85 – 112 days (12-16 weeks)
  - 113 – 140 days (16-20 weeks)
  - 141 – 168 days (20-24 weeks)
- Open-label period
  - every 84 days (12 weeks)
- On-vatiquinone period
  - every 84 days (12 weeks)

6.2. Adverse Events

The AE verbatim descriptions (investigator terms from the CRF) will be classified into medical terminology using the MedDRA. AEs will be coded by System Organ Class (SOC) and PT using MedDRA, Version 23.0 or later.

AE summaries will be presented by treatment for double-blind period, and by treatment sequence and overall for on-vatiquinone period, unless specified otherwise.

Only treatment-emergent AEs (TEAEs) will be included in summary tables. All AEs will be presented in subject data listings.

6.2.1. Treatment Emergent Adverse Event

A TEAE is defined as an AE that had an onset date on or after the first dose of study drug but prior to 30 days after last dose or it occurs prior to first dose of study drug and worsens in severity after first dose of study drug.

AEs with missing or partial onset date and cannot be determined if it occurred prior to the first dose of study or which treatment period it occurred will be counted as TEAE under that treatment period.

A TEAE will be considered occurring during double-blind period if the AE had an onset date or date of worsening on or after the date of first dose of double-blind treatment and:

- Prior to the date of the first dose of open-label treatment if subject enrolled into open-label period
- Within 30 days of the date of the last dose of double-blind treatment if the subject does not enroll into open-label period

A TEAE will be considered occurring during the on-vatiquinone period if the AE had an onset date or date of worsening on or after the date of the first dose of vatiquinone treatment and within 30 days of the date of the last dose of vatiquinone treatment.

Overview TEAE table, including number and percentage of subjects with TEAEs, treatment related TEAE, treatment related serious TEAEs, TEAEs leading to study discontinuation, TEAEs by severity, and treatment related AE by severity will be provided.

Summary information (the number and percentage of subjects by treatment group or by treatment sequence, where appropriate) will be tabulated for:

- Incidence of TEAEs by SOC and PT
- Incidence of TEAEs by PT in descending order
- Incidence of treatment-related TEAEs by SOC and PT
- Incidence of TEAEs by SOC, PT, and maximum CTCAE grade
- Incidence of TEAEs by SOC, PT, and relationship to study drug

AE tables will be sorted by SOC and then PT in decreasing frequency of the number and percentage of subjects in the Vatiquinone group.

By-subject listing will be provided for AEs.

6.2.2. Deaths, serious AE, and AE leading to discontinuation

The following summaries will be presented:

- Incidence of treatment-emergent SAEs by SOC and PT
- Incidence of treatment-related, treatment-emergent SAEs by SOC and PT
- TEAEs leading to discontinuation by SOC and PT

Subjects who die, who have SAE and AE leading to discontinuation during the study will be listed in three separate listings.

6.2.3. Exposure Adjusted TEAE

The exposure-adjusted TEAEs will be summarized by treatment and by treatment sequences during on-vatiquinone for safety population as follows:

- Number of subjects and incidence rate per patient-years TEAE by SOC and PT
- Number of events and event rate per patient-years TEAE by SOC and PT

For incidence rate, subjects will be counted only once for a particular event. For event rate, repeated events of a particular type will be counted only once. Total patient years of exposure will be calculated as total duration in days divided by 365.25 days.

6.3. Clinical Laboratory Parameters

Number of subjects with clinically significant value for selected laboratory parameters as defined in Table 5 at any post-baseline visit, including unscheduled visits, will be summarized for double-blind period and for on-vatiquinone period.

Table 5: Criteria for Clinically Significant for Selected Laboratory Parameters

Category	Laboratory Parameters	Criteria
Elevated AST or ALT	AST and ALT	ALT or AST ≥3xULN
Elevated INR	INR	INR ≥ ULN INR ≥ 1.5xULN
Elevated serum creatinine	Serum creatinine	Serum creatinine ≥1.5xULN

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; ULN, upper limit of normal.

Lab parameters values and change from baseline will be summarized by treatment groups at each visit for double-blind period, and by treatment sequence at each visit for on-vatiquinone period.

A subject level listing of Laboratory results will be provided for Hematology, Coagulation, Serum Chemistry, and Urinalysis, respectively.

6.4. Vital Signs

Vital sign (temperature, respiratory rate, systolic blood pressure, diastolic blood pressure, heart rate, oxygen saturation, weight, and BMI) and change from baseline will be summarized by treatment groups at each visit for double-blind period, and by treatment sequence at each visit for on-vatiquinone period.

A subject level listing of vital signs will be provided.

6.5. Electrocardiogram (ECG)/Electroencephalogram (EEG)

ECG results and change from baseline results (heart rate, PR interval, RR interval, QRS interval, QTc interval) along with ECG interpretations will be summarized at each visit by each assessment timepoints by treatment groups for double-blind period, and by treatment sequence for on-vatiquinone period.

Subject level listings of ECG and EEG will be provided.

6.6. Physical examinations

A subject level listing of physical examination will be provided.

6.7. Other Analyses

6.7.1. Pharmacokinetics analysis

Vatiquinone concentration will be summarized by visit and timepoint for vatiquinone subjects. Pharmacokinetics analysis will be specified in a separate document.

7. MOCK TABLES, LISTINGS, AND GRAPHS

Include a sentence that references the current protocol with the date; for SAP version 1 include Table 1 with only the first row. For example:

The tables, listings, and graphs shells for the study will be provided in a separate document.

8. VERSION HISTORY

This Statistical Analysis Plan (SAP) for study PTC743-MIT-001-EP is based on the protocol Version 8.0 dated 27May2022.

SAP Version	Approval Date	Change	Rationale
1.0	23FEB2023	Not Applicable	Original version
2.0	15JUN2023	2.1.3 Per-Protocol Population: Population exclusion criteria updated. 3.1 Definition of Estimand: Details of intercurrent events added to Table 1 3.1.1 Intercurrent Events – new section added to provide details added to Table 1 5.1 Primary Endpoint(s) Analysis: Details of primary endpoint definition, sensitivity analysis, and analysis methods added.	To address FDA comments

9. REFERENCES

Hoefman, R.J., N.J.A. Van Exel, J.M. Rose, E.J. Lawerman-van de Wetering and W.B.F. Brouwer (2013) 'A Discrete Choice Experiment to obtain a tariff for valuing informal care situations measured with the CarerQol instrument'*, Medical Decision Making*.

Hoefman RJ, Van Exel NJA, Brouwer WBF (May 2013). iVICQ, iMTA Valuation of Informal Care Questionnaire, Version 1.1. *Institute of Health Policy & Management /Institute for Medical Technology Assessment*

Brouwer WBF, van Exel NJA, van Grop B, Redekp WK. The CarerQol instrument: a new instrument to measure care-related quality of life of informal caregivers for use in economic evaluations. Qual Life Res. 2006;15(6):1005-1021.

The PedsQL Scoring Algorithm: Scoring the pediatric quality of life inventory. Found at: https://pedsql.org/score.html. Last accessed 23 February 2023.

APPENDIX 1: Schedule of Events

Procedures	**Screening[a]**		**Baseline**	**Double-Blind 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																
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														
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	

Procedures	**Screening[a]**		**Baseline**	**Double-Blind 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
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; PO, orally; 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 period 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 post dose 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. 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 double-blind 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.